Claims

1. Optically pure compounds c h a r a c t e r i z e d in that the compounds are Na^+, Mg^{2+}, Li^+, K^+, Ca^{2+} and $N^+(R)_4$ salts of (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole and (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole, wherein R is an alkyl with 1-4 carbon atoms.

2. Compounds according to claim 1 c h a r a c t e r i z e d in that the compounds are (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole sodium salt, (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole sodium salt, (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole magnesium salt, (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole magnesium salt, (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole calcium salt and (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole calcium salt.

3. Compounds according to claims 1 and 2 c h a r a c t e r i z e d in that the compounds are (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole sodium salt, (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole sodium salt, (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole magnesium salt and (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole magnesium salt.

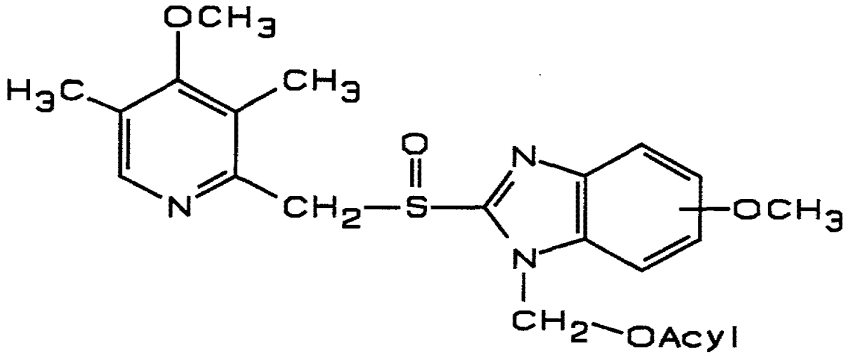
4. Compounds according to claims 1 and 2 c h a r a c t e r i z e d in that the compounds are (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-sulfinyl-1H-benzimidazole sodium salt and (-)-5-methoxy-2-[[(4-methoxy-3,5-

dimethyl-2-pyridinyl)methyl]sulfinyl-1H-benzimidazole sodium salt in their crystalline forms.

5. Compounds according to claims 1 and 2 characterized in that the compound is (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-sulfinyl-1H-benzimidazole sodium salt in its crystalline form.

6. Compounds according to claims 1 and 2 characterized in that the compound is (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-sulfinyl-1H-benzimidazole sodium salt in its crystalline form.

7. Process for the preparation of a compound according to claim 1 characterized in that a diastereomeric ester of formula IV



(IV)

wherein Acyl designates a chiral acyl group such as mandeloyl, having either R or S configuration, is separated, and each of the separated diastereomers is dissolved in an alkaline solution where the acyloxymethyl group is hydrolyzed to give the optically pure compound.

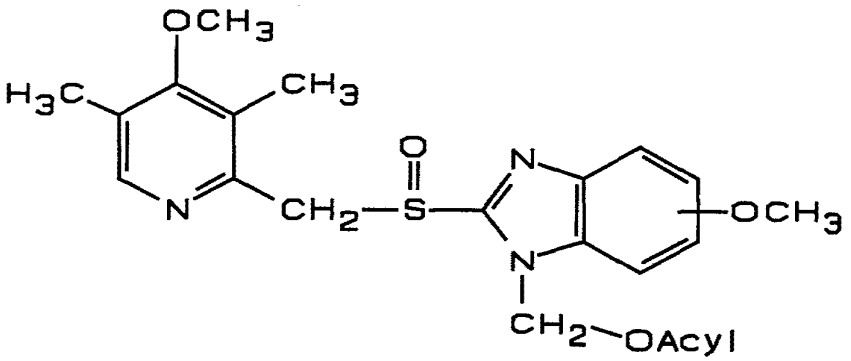
8. Process according to claim 7 characterized in that the diastereomers are separated by chromatography or fractional crystallization.

9. Process according to claim 7 c h a r a c t e r i z e d in that the solvolysis is performed in alkaline solution consisting of a base in a protic solvent, such as alcohols or water; or a base in an aprotic solvent, such as dimethylsulfoxide or dimethylformamide.

10. Process for the preparation of a compound according to claim 1 in crystalline form c h a r a c t e r i z e d in that a product from the process in claim 7 is neutralized with a neutralizing agent which can be an acid or an ester such as methyl formate, followed by treatment with a base in non-aqueous solution.

11. Process for preparation of (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-sulfinyl-1H-benzimidazole sodium salt and (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl-1H-benzimidazole sodium salt in their crystalline forms c h a r a c t e r i z e d in that (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-sulfinyl]-1H-benzimidazole sodium salt and (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl-1H-benzimidazole sodium salt crude product respectively is neutralized followed by treatment with NaOH in a non-aqueous medium.

12. Process for the preparation of (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole and (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole c h a r a c t e r i z e d in that a diastereomeric ester of formula IV



(IV)

wherein Acyl designates a chiral acyl group such as mandeloyl, having either R or S configuration, is separated, and each of the separated diastereomers is dissolved in an alkaline solution where the acyloxymethyl group is hydrolyzed off to give the optically pure compound after neutralization with a neutralizing agent which can be an acid or an ester.

13. Process according to claim 12 c h a r a c t e r i z e d in that the diastereomers are separated by chromatography or fractional crystallization.

14. Process according to claim 12 c h a r a c t e r i z e d in that the solvolysis is performed in alkaline solution consisting of a base in a protic solvent, such as alcohols or water; or a base in an aprotic solvent, such as dimethylsulfoxide or dimethylformamide.

15. The compound (+)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-sulfinyl]-1H-benzimidazole obtained by the process defined in claim 12.

16. The compound (-)-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]-sulfinyl]-1H-benzimidazole obtained by the process defined in claim 12.

17. Pharmaceutical preparation containing an optically pure compound according to any of claims 1-6 as active ingredient.

18. Optically pure compounds according to any of claims 1-6 for use in therapy.

19. Use of an optically pure compound according to any of claims 1-6 in the preparation of a pharmaceutical formulation for inhibiting gastric acid secretion.

20. Use of an optically pure compound according to any of claims 1-6 for the preparation of a pharmaceutical formulation for the treatment of gastrointestinal inflammatory diseases.

21. A method for inhibiting gastric acid secretion comprising administration to a mammal including man in need of such treatment an effective amount of an optically pure compound according to any of claims 1-6.

22. A method for the treatment of gastrointestinal inflammatory diseases comprising administration to a mammal including man in need of such treatment an effective amount of an optically pure compound according to any of claims 1-6.

23. The compound 6-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]-1-[mandeloyloxymethyl]-1H-benzimidazole.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 94/00509

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C07D 401/12, A61K 31/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP, A2, 0124495 (AKTIEBOLAGET HÄSSLE), 7 November 1984 (07.11.84) --	1-6,11,15-20
Y	DE, A1, 4035455 (BYK GULDEN LOMBERG CHEMISCHE FABRIK GMBH), 14 May 1992 (14.05.92)	1-6,11,15-20
X	-- --------	7-10,12-14, 23

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search	Date of mailing of the international search report
29 July 1994	17 -08- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Göran Karlsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 94/00509

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 21-22
because they relate to subject matter not required to be searched by this Authority, namely:

A method for treatment of the human or animal body by therapy, see rule 39.1(iv).

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/07/94

International application No.
PCT/SE 94/00509

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0124495	07/11/84	SE-T3- 0124495	
		AU-B- 563842	23/07/87
		AU-A- 2525784	06/09/84
		CA-A- 1264751	23/01/90
		GB-A,B- 2137616	10/10/84
		JP-C- 1651336	30/03/92
		JP-B- 3013233	22/02/91
		JP-A- 59167587	21/09/84
		SU-A- 1314953	30/05/87
		US-A- 4738974	19/04/88
DE-A1- 4035455	14/05/92	AU-A- 8840691	11/06/92
		WO-A- 9208716	29/05/92

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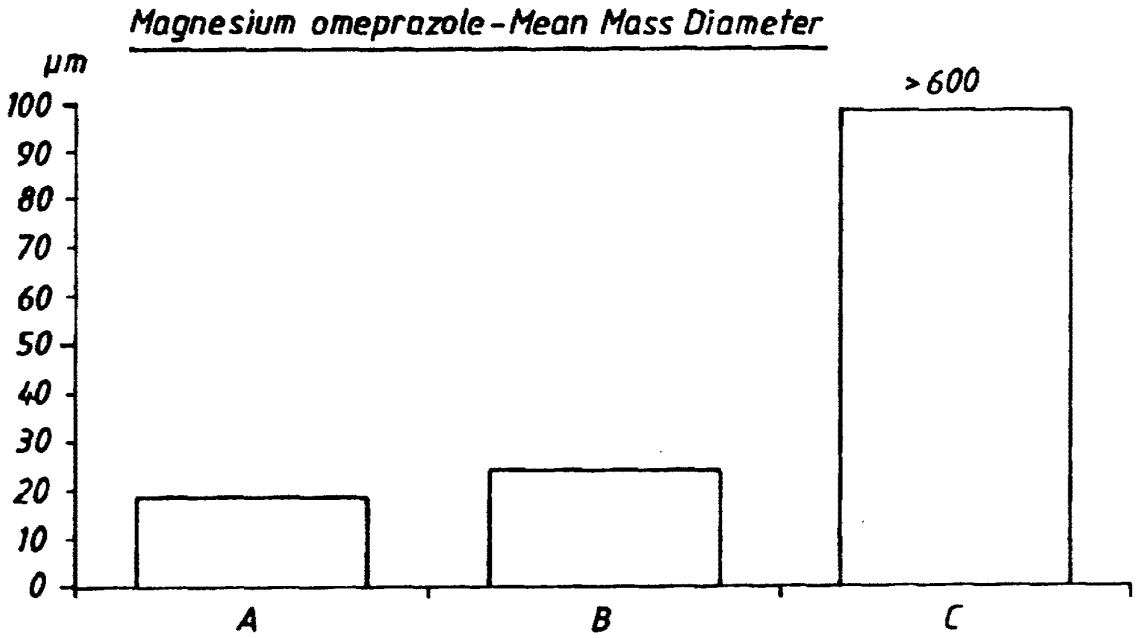
Published
With international search report.

(54) Title: MAGNESIUM OMEPRAZOLE



(57) Abstract

A novel compound form of magnesium omeprazole useful in the manufacture of pharmaceutical formulations, the use of the product and the process for its production are described.

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MAGNESIUM OMEPRAZOLE

Field of the invention

The present invention relates to a novel process for manufacturing the magnesium salt of omeprazole; the magnesium salt of omeprazole in a novel physical form, especially the magnesium salt as a product of the novel process; the use of the novel form of the magnesium salt of omeprazole in the manufacture of pharmaceutical formulations; and to the use of the novel form of the magnesium salt of omeprazole in medicine.

Background of the invention

The compound known under the generic name omeprazole is described i.a. in European patent specification 0005129.

Omeprazole is useful for inhibiting gastric acid secretion and has gastric mucosa protective activity in mammals and man. In a more general sense, omeprazole may be used for prevention and treatment of gastric acid related disorders and gastrointestinal inflammatory diseases in mammal and man, including e.g. gastritis, gastric ulcer and duodenal ulcer.

The term "omeprazole" as used in this specification designates the neutral form of the compound, that is the form without a salt forming cation present.

Certain salts of omeprazole are described in European patent specification 0124495. In said patent specification the requirements and importance regarding storage stability of pharmaceutical preparations are emphasized. Salts possessing superior properties with regard i.a. to storage stability are described in the said European patent specification.

In EP 0124495; examples 5 and 6 disclose the synthesis of a magnesium salt of omeprazole.

The isolation and purification in full manufacturing scale of the described magnesium omeprazole salts presents one major problem in that magnesium omeprazole salt crystals are very fragile making processes utilising such crystals less attractive in full scale production. Performing the process without crystallization of the magnesium omeprazole gives a product which is less suitable as a pharmaceutical substance.

In order to use the magnesium salt of omeprazole, in this specification denoted magnesium omeprazole, in full manufacturing scale in preparing pharmaceutical formulations primarily for oral administration, such as tablets, it is necessary that said magnesium omeprazole possesses a combination of properties which makes such full scale manufacturing feasible. One object of the present invention is to provide a process for full scale production of magnesium omeprazole. A further object of the present invention is to provide a novel form of the magnesium salt of omeprazole which can be used in full scale manufacturing of pharmaceutical formulations, such as tablets.

The combination of physical properties of the novel magnesium omeprazole product of the present invention with respect to the degree of crystallinity, particle diameter, density, hygroscopicity, water content and content of other solvents are favorable and permit the manufacture of magnesium omeprazole in a form which possesses the desired properties.

The novel form of magnesium omeprazole can also be formulated into other forms for oral administration and other types of administration such as rectal administration. Examples of formulations are tablets, pellets, granules, capsules, suspensions and suppositories.

The invention

We now provide a novel form of the magnesium salt of omeprazole exhibiting the desired combination of physical properties. This makes full scale production of magnesium omeprazole as well as full scale production of pharmaceutical formulations thereof feasible.

The novel process for the manufacture of magnesium omeprazole also circumvents the above described manufacturing problems and renders possible the recovery and work-up of the magnesium omeprazole substance in traditional chemical process equipment.

It has been found that the following property is significant to obtain such product:

a) Crystalline form, with a degree of crystallinity of not less than 70%, preferably higher than 75% as determined by X-ray powder diffraction

It is desirable that the product also exhibits the following properties;

b) Particle size measured as mean mass diameter (MMD) less than 30μm, preferably less than 20 μm as determined by laser diffraction technique.

c) Density between 1.33 g/cm^3 and 1.35 g/cm^3 as determined by powder pycnometer.

d) Hygroscopicity not exceeding 2% increase of weight upon storage for one month up to 94% relative atmospheric humidity as determined gravimetrically.

e) A content of water of between 5% and 10% by weight as determined by titration according to Karl Fischer.

f) A content of methanol less than 0.1% preferably less than 0.05% by weight as determined by gas chromatography, in case methanol is used as solvent.

In a further aspect, the invention also relates to a process for manufacturing the novel form of magnesium omeprazole. This process is described in more detail below.

The invention relates to all of the aspects given under Field of the invention.

The process for producing the novel form of magnesium omeprazole is characterized by the following consecutive steps

a) treating omeprazole or a salt thereof with magnesium alcoholate in a solution

b) separating inorganic salts from the reaction mixture

c) crystallizing magnesium omeprazole

d) isolating the obtained crystalline magnesium omeprazole and, optionally,

e) purifying and drying the crystalline magnesium omeprazole using conventional methods.

The process for manufacturing the new product can be described in the following way.

A lower alcohol, such as methanol, ethanol, n-propanol or iso-propanol, preferably methanol, is treated in a solution of polar solvents with a weighed amount of magnesium at temperatures between $0^{o}C$ and reflux temperature. The temperature should preferably be between 10 and $30^{o}C$. After addition of the magnesium to the solution the temperature can, in a second step be raised further to between $0^{o}C$ and reflux temperature, preferably 20-$50^{o}C$. After termination of the reaction the

temperature is reduced to 0-40°C, preferably 10-25°C. Omeprazole or a salt of omeprazole is then added to the solution and after termination of the reaction the mixture is cooled to -10°C to +20°C, preferably -5°C to +5°C. The solvent is then evaporated to 40-60% of the initial volume, which makes the inorganic salts precipitate. The precipitate is separated from the reaction solution for example by centrifugation or filtration and the solution is heated to 5°C to 30°C whereafter the solution is seeded with magnesium omeprazole crystals. An amount of water, which is approximately equal to the volume of the solution, is added to start the crystallization. The solution is cooled to -10 to +20°C, preferably 0-10°C to complete the crystallization. The crystals are then separated from the mother liquid for example by centrifugation or filtration and washed with polar solvents preferably an aqueous lower alcohol such as aqueous methanol. Finally, the produced crystals are dried preferably under reduced pressure and heating.

The process for manufacturing the new form of magnesium omeprazole differs from the earlier known processes in that the product is recovered after a controlled crystallization step in aqueous alcohol, preferably methanol by, first, separating the inorganic salts from the mother liquour. The crystallinity resulting from this step is, unexpectedly, higher and the product possesses a higher degree of purity and is more stable to decomposition from uptake of moisture. The drying step can be performed without caking. The new process is possible to perform in conventional chemical process equipment and gives a product with a higher yield than the processes hitherto known.

The following detailed Example 1 will serve to more fully illustrate the process for manufacturing magnesium omeprazole in full scale according to the present invention. In Figures 1 and 2 sample A is manufactured according to this example.

Example 1

A reactor was filled with 2026 litres of methanol. The stirrer was started and the temperature was adjusted to 20°C. 3,90 kg of magnesium was added to the vessel and immediately thereafter 1,0 litre of CH_2Cl_2. The reactor was heated to 40°C and kept at this temperature for 60 min. It was then cooled to 15°C before the addition of 99,9 kg of omeprazole. The reactor was kept at this temperature for 60 min and then cooled to 0°C. The temperature was kept at this level for 30 minutes before 1000 l of methanol were evaporated under vacuum and the inorganic solid salt was separated from the liquid first by centrifugation and then by filtration. The liquid was heated to 10°C and the liquid was seeded with magnesium omeprazole crystals whereafter the magnesium omeprazole salt was precipitated by addition of 900 l of water. The mixture was then cooled to 5°C. After the crystallization had been completed the magnesium omeprazole crystals were centrifuged off and then washed with a mixture of 50 l of methanol and 150 l of water. The produced magnesium omeprazole was dried under reduced pressure finally producing 92,5 kg of crystalline product corresponding to a yield of 81,4%.

The novel form of the magnesium salt of omeprazole according to Example 1 possesses the following properties:

a) Crystalline form, with a degree of crystallinity of 76%, as determined by X-ray powder diffraction.

b) Particle size measured as mean mass diameter (MMD) of 19µm as determined by laser diffraction technique.

c) Density of 1.342 g/cm^3 as determined by powder pycnometer.

d) Hygroscopicity of 1.62% increase of weight upon storage for one month at 94% relative atmospheric humidity as determined gravimetrically.

e) Content of moisture water of 7.6 by weight as determined by titration according to Karl Fischer.

f) Content of methanol of 0.006% by weight as determined by gas chromatography.

A comparison between two different samples of the novel form of magnesium omeprazole of the present invention obtained from two laboratory scale experiments by prior art methods and magnesium omeprazole goes forth from diagrams 1 and 2. In these diagrams sample A represents the novel form of the present invention as manufactured in full scale process equipment. Sample B represents the product of preparation via synthesis by treatment of omeprazole with $Mg(OCH_3)_2$. Sample C represents the product of preparation via treatment of sodium omeprazole with $MgCl_2$.

Figure 1 shows in diagram 1 that the particle size measured as mean mass diameter of the product of method A is 19 µm which is smaller than the corresponding particle size for the products of method B which is 25 µm and of method C which is greater than 600 µm.

Figure 2 shows in diagram 2 that the degree of crystallinity of the particles of the product of method A is 76% which is higher than the corresponding figure for the product of sample B, which is 0% and also higher than the corresponding figure of sample C, which 67%.

CLAIMS

1. Magnesium omeprazole characterized in having a degree of crystallinity which is higher than 70% as determined by X-ray powder diffraction.

2. Magnesium omeprazole according to claim 1 wherein the degree of crystallinity is higher than 75%.

3. Magnesium omeprazole according to claim 1 wherein the mean particle diameter as determined by laser diffraction technique is less than 30µm, and preferably less than 20µm.

4. Magnesium omeprazole according to claim 1 wherein the density is between 1.33 g/cm^3 and 1.35 g/cm^3 as determined by powder pycnometer.

5. Magnesium omeprazole according to claim 1 wherein the hygroscopicity is less than 2% increase of weight upon storage for one month at up to 94% relative atmospheric humidity as determined by gravimetry.

6. Magnesium omeprazole according to claim 1 wherein the water content is between 5% and 10% by weight as determined by titration according to Karl Fischer.

7. Magnesium omeprazole according to claim 1 containing less than 0.1% by weight of solvent as determined by gas chromatography.

8. Magnesium omeprazole according to claim 1 containing less than 0.05% by weight of solvent as determined by gas chromatography.

9. A process for the manufacture of magnesium omeprazole according to any of claims 1 to 8 characterized by in consecutive steps

a) treating omeprazole or a salt thereof with magnesium alcoholate in a solution,

b) separating inorganic salts from the reaction mixture,

c) crystallizing magnesium omeprazole,

d) isolating the obtained crystalline magnesium omeprazole and, optionally,

e) purifying and drying the crystalline magnesium omeprazole using conventional methods.

10. A process according to Claim 9 wherein the magnesium alcoholate is magnesium methyl alcoholate.

11. A process according to claim 9 wherein the solvent is methanol.

12. A process according to claim 9 wherein the crystallization is accomplished by addition of water.

13. A process according to claim 9 wherein the isolation of the magnesium omeprazole is performed by centrifugal separation of the crystals.

14. A process according to claim 9 wherein the isolation of the magnesium omeprazole is performed by crystallization followed by filtration of the crystals.

15. A process according to claim 9 wherein the purification of the magnesium omeprazole crystals is performed by washing the crystals with a solution of polar solvents.

16. A process according to claim 9 wherein the magnesium omeprazole crystals are dried preferably under reduced pressure.

17. A process according to claim 9 wherein the drying of the magnesium omeprazole crystals is performed by evaporating the remaining solvent by heating.

18. Magnesium omeprazole obtained by a process according to any of claims 9 to 17.

19. A pharmaceutical composition containing magnesium omeprazole according to any of claims 1 to 8 as an active ingredient.

20. A pharmaceutical formulation for oral administration containing magnesium omeprazole according to any of claims 1 to 8 as an active ingredient.

21. A tablet formulation containing magnesium omeprazole according to any of claims 1 to 8 as an active ingredient.

22. Magnesium omeprazole according to any of claims 1 to 8 for use in therapy.

23. Magnesium omeprazole according to any of claims 1 to 8 for use in inhibiting gastric acid secretion in mammals and man.

24. Magnesium omeprazole according to any of claims 1 to 8 for use as an agent with gastric mucosa protective activity in mammals and man.

25. Magnesium omeprazole according to any of claims 1 to 8 for use in the treatment of gastric acid related diseases in mammals and man.

26. The use of magnesium omeprazole according to any of claims 1 to 8 in the manufacture of a medicament for inhibiting gastric acid secretion.

27. The use of magnesium omeprazole according to any of claims 1 to 8 in the manufacture of a medicament for obtaining gastric mucosa protective activity.

28. The use of magnesium omeprazole according to any of claims 1 to 8 in the manufacture of a medicament for the treatment of gastric acid related diseases.

29. A method for inhibiting gastric acid secretion in mammals and man by administering to a host in need thereof a therapeutically effective dose of magnesium omeprazole according to any of claims 1 to 8.

30. A method for the treatment of gastric acid related diseases in mammals and man by administering to a host in need thereof a therapeutically effective dose of magnesium omeprazole according to any of claims 1 to 8.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 94/00680

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07D 401/12, A61K 31/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A2, 0124495 (AKTIEBOLAGET HÄSSLE), 7 November 1984 (07.11.84), see example 5 and 6	1-28

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 October 1994	24-10-1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Göran Karlsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 94/00680

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 29-30
because they relate to subject matter not required to be searched by this Authority, namely:

A method for treatment of the human or animal body by therapy, see rule 39.1.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/08/94

International application No.
PCT/SE 94/00680

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0124495	07/11/84	SE-T3- 0124495	
		AU-B- 563842	23/07/87
		AU-A- 2525784	06/09/84
		CA-A- 1264751	23/01/90
		GB-A,B- 2137616	10/10/84
		JP-C- 1651336	30/03/92
		JP-B- 3013233	22/02/91
		JP-A- 59167587	21/09/84
		SU-A- 1314953	30/05/87
		US-A- 4738974	19/04/88

CORRECTED VERSION*

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INTERNATIONAL APPLICATION PUBLISHED U

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(51) International Patent Classification 6 :
C07D 401/12, A61K 31/44 | A1

(43) International Publication Date: 19 January 1995 (19.01.95)

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(30) Priority Data:
9302396-8 9 July 1993 (09.07.93) SE

(71) Applicant *(for all designated States except US)*: ASTRA AKTIEBOLAG [SE/SE]; S-151 85 Södertälje (SE).

(72) Inventors; and
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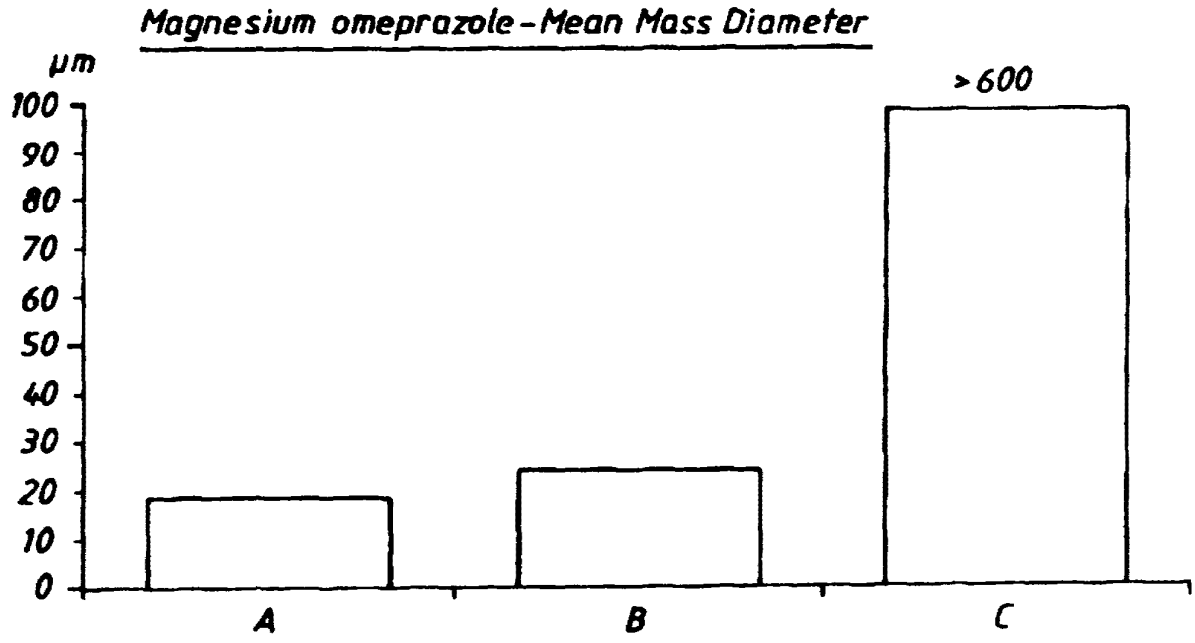
(74) Agent: LARSSON, Birgitta; Astra Aktiebolag, Patent Dept., S-151 85 Södertälje (SE).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD).

Published
With international search report.

(54) Title: MAGNESIUM OMEPRAZOLE



(57) Abstract

A novel compound form of magnesium omeprazole useful in the manufacture of pharmaceutical formulations, the use of the product and the process for its production are described.

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Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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MAGNESIUM OMEPRAZOLE

Field of the invention

The present invention relates to a novel process for manufacturing the magnesium salt of omeprazole; the magnesium salt of omeprazole in a novel physical form, especially the magnesium salt as a product of the novel process; the use of the novel form of the magnesium salt of omeprazole in the manufacture of pharmaceutical formulations; and to the use of the novel form of the magnesium salt of omeprazole in medicine.

Background of the invention

The compound known under the generic name omeprazole is described i.a. in European patent specification 0005129.

Omeprazole is useful for inhibiting gastric acid secretion and has gastric mucosa protective activity in mammals and man. In a more general sense, omeprazole may be used for prevention and treatment of gastric acid related disorders and gastrointestinal inflammatory diseases in mammal and man, including e.g. gastritis, gastric ulcer and duodenal ulcer.

The term "omeprazole" as used in this specification designates the neutral form of the compound, that is the form without a salt forming cation present.

Certain salts of omeprazole are described in European patent specification 0124495. In said patent specification the requirements and importance regarding storage stability of pharmaceutical preparations are emphasized. Salts possessing superior properties with regard i.a. to storage stability are described in the said European patent specification.

In EP 0124495; examples 5 and 6 disclose the synthesis of a magnesium salt of omeprazole.

The isolation and purification in full manufacturing scale of the described magnesium omeprazole salts presents one major problem in that magnesium omeprazole salt crystals are very fragile making processes utilising such crystals less attractive in full scale production. Performing the process without crystallization of the magnesium omeprazole gives a product which is less suitable as a pharmaceutical substance.

In order to use the magnesium salt of omeprazole, in this specification denoted magnesium omeprazole, in full manufacturing scale in preparing pharmaceutical formulations primarily for oral administration, such as tablets, it is necessary that said magnesium omeprazole possesses a combination of properties which makes such full scale manufacturing feasible. One object of the present invention is to provide a process for full scale production of magnesium omeprazole. A further object of the present invention is to provide a novel form of the magnesium salt of omeprazole which can be used in full scale manufacturing of pharmaceutical formulations, such as tablets.

The combination of physical properties of the novel magnesium omeprazole product of the present invention with respect to the degree of crystallinity, particle diameter, density, hygroscopicity, water content and content of other solvents are favorable and permit the manufacture of magnesium omeprazole in a form which possesses the desired properties.

The novel form of magnesium omeprazole can also be formulated into other forms for oral administration and other types of administration such as rectal administration. Examples of formulations are tablets, pellets, granules, capsules, suspensions and suppositories.

The invention

We now provide a novel form of the magnesium salt of omeprazole exhibiting the desired combination of physical properties. This makes full scale production of magnesium omeprazole as well as full scale production of pharmaceutical formulations thereof feasible.

The novel process for the manufacture of magnesium omeprazole also circumvents the above described manufacturing problems and renders possible the recovery and work-up of the magnesium omeprazole substance in traditional chemical process equipment.

It has been found that the following property is significant to obtain such product:

a) Crystalline form, with a degree of crystallinity of not less than 70%, preferably higher than 75% as determined by X-ray powder diffraction

It is desirable that the product also exhibits the following properties;

b) Particle size measured as mean mass diameter (MMD) less than 30μm, preferably less than 20 μm as determined by laser diffraction technique.

c) Density between 1.33 g/cm^3 and 1.35 g/cm^3 as determined by powder pycnometer.

d) Hygroscopicity not exceeding 2% increase of weight upon storage for one month up to 94% relative atmospheric humidity as determined gravimetrically.

e) A content of water of between 5% and 10% by weight as determined by titration according to Karl Fischer.

f) A content of methanol less than 0.1% preferably less than 0.05% by weight as determined by gas chromatography, in case methanol is used as solvent.

In a further aspect, the invention also relates to a process for manufacturing the novel form of magnesium omeprazole. This process is described in more detail below.

The invention relates to all of the aspects given under Field of the invention.

The process for producing the novel form of magnesium omeprazole is characterized by the following consecutive steps

a) treating omeprazole or a salt thereof with magnesium alcoholate in a solution

b) separating inorganic salts from the reaction mixture

c) crystallizing magnesium omeprazole

d) isolating the obtained crystalline magnesium omeprazole and, optionally,

e) purifying and drying the crystalline magnesium omeprazole using conventional methods.

The process for manufacturing the new product can be described in the following way.

A lower alcohol, such as methanol, ethanol, n-propanol or iso-propanol, preferably methanol, is treated in a solution of polar solvents with a weighed amount of magnesium at temperatures between 0°C and reflux temperature. The temperature should preferably be between 10 and 30°C. After addition of the magnesium to the solution the temperature can, in a second step be raised further to between 0°C and reflux temperature, preferably 20-50°C. After termination of the reaction the

temperature is reduced to 0-40°C, preferably 10-25°C. Omeprazole or a salt of omeprazole is then added to the solution and after termination of the reaction the mixture is cooled to -10°C to +20°C, preferably -5°C to +5°C. The solvent is then evaporated to 40-60% of the initial volume, which makes the inorganic salts precipitate. The precipitate is separated from the reaction solution for example by centrifugation or filtration and the solution is heated to 5°C to 30°C whereafter the solution is seeded with magnesium omeprazole crystals. An amount of water, which is approximately equal to the volume of the solution, is added to start the crystallization. The solution is cooled to -10 to +20°C, preferably 0-10°C to complete the crystallization. The crystals are then separated from the mother liquid for example by centrifugation or filtration and washed with polar solvents preferably an aqueous lower alcohol such as aqueous methanol. Finally, the produced crystals are dried preferably under reduced pressure and heating.

The process for manufacturing the new form of magnesium omeprazole differs from the earlier known processes in that the product is recovered after a controlled crystallization step in aqueous alcohol, preferably methanol by, first, separating the inorganic salts from the mother liquour. The crystallinity resulting from this step is, unexpectedly, higher and the product possesses a higher degree of purity and is more stable to decomposition from uptake of moisture. The drying step can be performed without caking. The new process is possible to perform in conventional chemical process equipment and gives a product with a higher yield than the processes hitherto known.

The following detailed Example 1 will serve to more fully illustrate the process for manufacturing magnesium omeprazole in full scale according to the present invention. In Figures 1 and 2 sample A is manufactured according to this example.

Example 1

A reactor was filled with 2026 litres of methanol. The stirrer was started and the temperature was adjusted to 20°C. 3,90 kg of magnesium was added to the vessel and immediately thereafter 1,0 litre of CH_2Cl_2. The reactor was heated to 40°C and kept at this temperature for 60 min. It was then cooled to 15°C before the addition of 99,9 kg of omeprazole. The reactor was kept at this temperature for 60 min and then cooled to 0°C. The temperature was kept at this level for 30 minutes before 1000 l of methanol were evaporated under vacuum and the inorganic solid salt was separated from the liquid first by centrifugation and then by filtration. The liquid was heated to 10°C and the liquid was seeded with magnesium omeprazole crystals whereafter the magnesium omeprazole salt was precipitated by addition of 900 l of water. The mixture was then cooled to 5°C. After the crystallization had been completed the magnesium omeprazole crystals were centrifuged off and then washed with a mixture of 50 l of methanol and 150 l of water. The produced magnesium omeprazole was dried under reduced pressure finally producing 92,5 kg of crystalline product corresponding to a yield of 81,4%.

The novel form of the magnesium salt of omeprazole according to Example 1 possesses the following properties:

a) Crystalline form, with a degree of crystallinity of 76%, as determined by X-ray powder diffraction.

b) Particle size measured as mean mass diameter (MMD) of 19µm as determined by laser diffraction technique.

c) Density of 1.342 g/cm^3 as determined by powder pycnometer.

d) Hygroscopicity of 1.62% increase of weight upon storage for one month at 94% relative atmospheric humidity as determined gravimetrically.

e) Content of moisture water of 7.6 by weight as determined by titration according to Karl Fischer.

f) Content of methanol of 0.006% by weight as determined by gas chromatography.

A comparison between two different samples of the novel form of magnesium omeprazole of the present invention obtained from two laboratory scale experiments by prior art methods and magnesium omeprazole goes forth from diagrams 1 and 2. In these diagrams sample A represents the novel form of the present invention as manufactured in full scale process equipment. Sample B represents the product of preparation via synthesis by treatment of omeprazole with $Mg(OCH_3)_2$. Sample C represents the product of preparation via treatment of sodium omeprazole with $MgCl_2$.

Figure 1 shows in diagram 1 that the particle size measured as mean mass diameter of the product of method A is 19 µm which is smaller than the corresponding particle size for the products of method B which is 25 µm and of method C which is greater than 600 µm.

Figure 2 shows in diagram 2 that the degree of crystallinity of the particles of the product of method A is 76% which is higher than the corresponding figure for the product of sample B, which is 0% and also higher than the corresponding figure of sample C, which 67%.

CLAIMS

1. Magnesium omeprazole characterized in having a degree of crystallinity which is higher than 70% as determined by X-ray powder diffraction.

2. Magnesium omeprazole according to claim 1 wherein the degree of crystallinity is higher than 75%.

3. Magnesium omeprazole according to claim 1 wherein the mean particle diameter as determined by laser diffraction technique is less than 30µm, and preferably less than 20µm.

4. Magnesium omeprazole according to claim 1 wherein the density is between 1.33 g/cm^3 and 1.35 g/cm^3 as determined by powder pycnometer.

5. Magnesium omeprazole according to claim 1 wherein the hygroscopicity is less than 2% increase of weight upon storage for one month at up to 94% relative atmospheric humidity as determined by gravimetry.

6. Magnesium omeprazole according to claim 1 wherein the water content is between 5% and 10% by weight as determined by titration according to Karl Fischer.

7. Magnesium omeprazole according to claim 1 containing less than 0.1% by weight of solvent as determined by gas chromatography.

8. Magnesium omeprazole according to claim 1 containing less than 0.05% by weight of solvent as determined by gas chromatography.

9. A process for the manufacture of magnesium omeprazole according to any of claims 1 to 8 characterized by in consecutive steps

a) treating omeprazole or a salt thereof with magnesium alcoholate in a solution,

b) separating inorganic salts from the reaction mixture,

c) crystallizing magnesium omeprazole,

d) isolating the obtained crystalline magnesium omeprazole and, optionally,

e) purifying and drying the crystalline magnesium omeprazole using conventional methods.

10. A process according to Claim 9 wherein the magnesium alcoholate is magnesium methyl alcoholate.

11. A process according to claim 9 wherein the solvent is methanol.

12. A process according to claim 9 wherein the crystallization is accomplished by addition of water.

13. A process according to claim 9 wherein the isolation of the magnesium omeprazole is performed by centrifugal separation of the crystals.

14. A process according to claim 9 wherein the isolation of the magnesium omeprazole is performed by crystallization followed by filtration of the crystals.

15. A process according to claim 9 wherein the purification of the magnesium omeprazole crystals is performed by washing the crystals with a solution of polar solvents.

16. A process according to claim 9 wherein the magnesium omeprazole crystals are dried preferably under reduced pressure.

17. A process according to claim 9 wherein the drying of the magnesium omeprazole crystals is performed by evaporating the remaining solvent by heating.

18. Magnesium omeprazole obtained by a process according to any of claims 9 to 17.

19. A pharmaceutical composition containing magnesium omeprazole according to any of claims 1 to 8 as an active ingredient.

20. A pharmaceutical formulation for oral administration containing magnesium omeprazole according to any of claims 1 to 8 as an active ingredient.

21. A tablet formulation containing magnesium omeprazole according to any of claims 1 to 8 as an active ingredient.

22. Magnesium omeprazole according to any of claims 1 to 8 for use in therapy.

23. Magnesium omeprazole according to any of claims 1 to 8 for use in inhibiting gastric acid secretion in mammals and man.

24. Magnesium omeprazole according to any of claims 1 to 8 for use as an agent with gastric mucosa protective activity in mammals and man.

25. Magnesium omeprazole according to any of claims 1 to 8 for use in the treatment of gastric acid related diseases in mammals and man.

26. The use of magnesium omeprazole according to any of claims 1 to 8 in the manufacture of a medicament for inhibiting gastric acid secretion.

27. The use of magnesium omeprazole according to any of claims 1 to 8 in the manufacture of a medicament for obtaining gastric mucosa protective activity.

28. The use of magnesium omeprazole according to any of claims 1 to 8 in the manufacture of a medicament for the treatment of gastric acid related diseases.

29. A method for inhibiting gastric acid secretion in mammals and man by administering to a host in need thereof a therapeutically effective dose of magnesium omeprazole according to any of claims 1 to 8.

30. A method for the treatment of gastric acid related diseases in mammals and man by administering to a host in need thereof a therapeutically effective dose of magnesium omeprazole according to any of claims 1 to 8.

Fig. 1

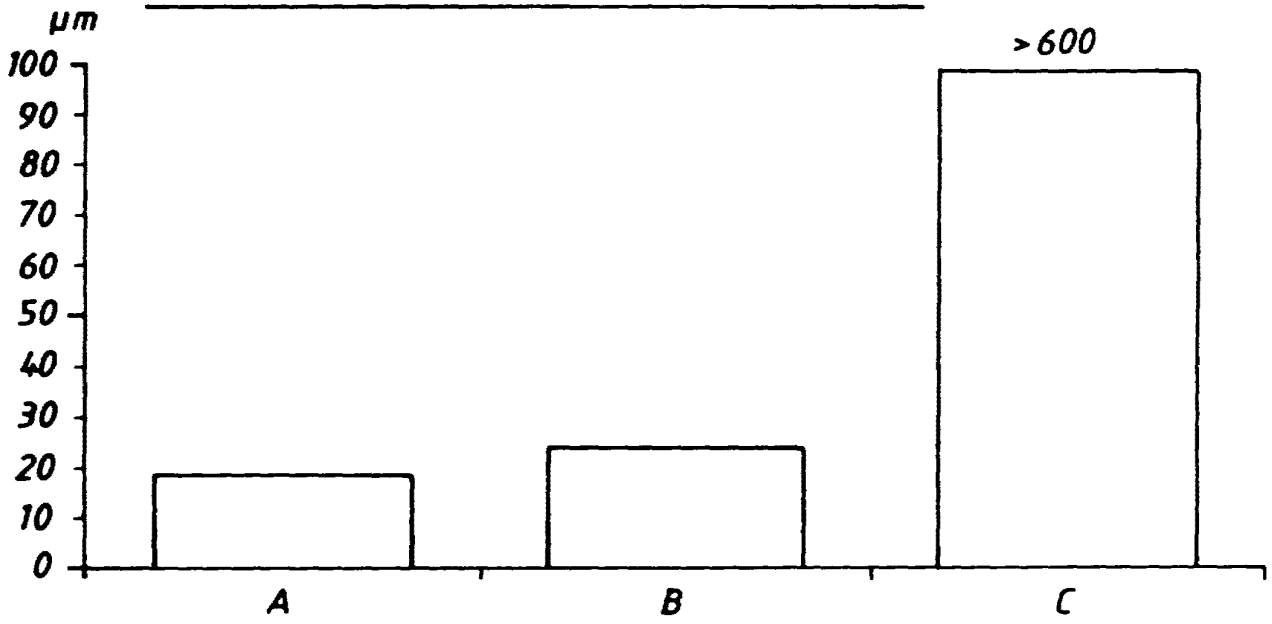
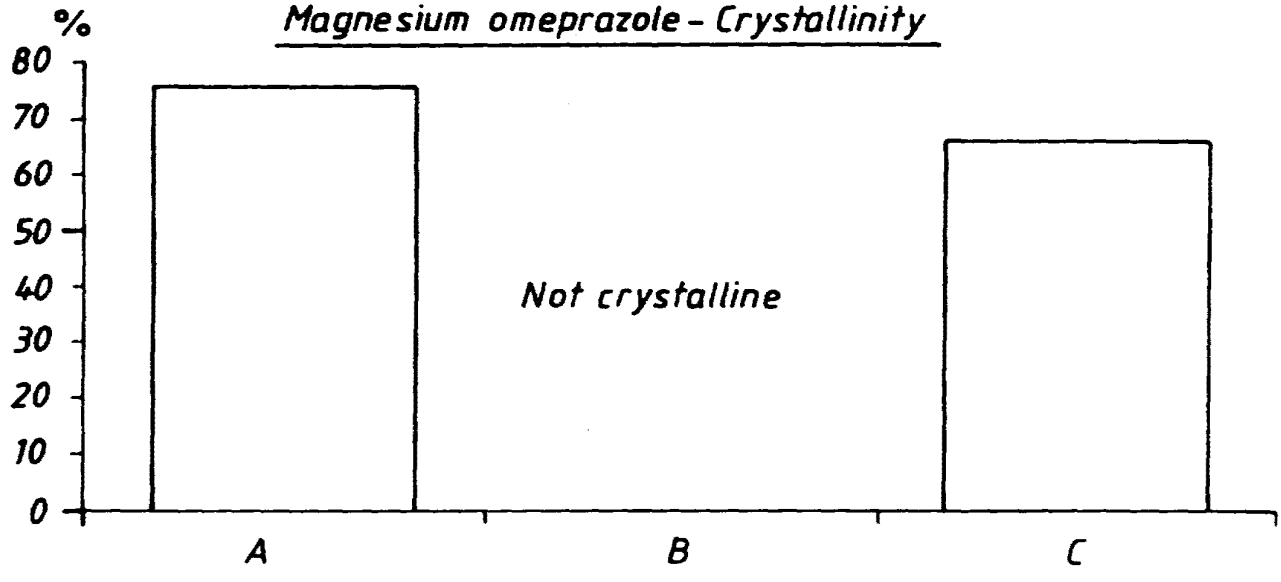

Magnesium omeprazole - Mean Mass Diameter
µm
100
90
80
70
60
50
40
30
20
10
0
>600
A
B
C

Fig. 2


Magnesium omeprazole - Crystallinity
%
80
70
60
50
40
30
20
10
0
Not crystalline
A
B
C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 94/00680

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07D 401/12, A61K 31/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A2, 0124495 (AKTIEBOLAGET HÄSSLE), 7 November 1984 (07.11.84), see example 5 and 6 -- --------	1-28

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 October 1994	24 -10- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Göran Karlsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 94/00680

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 29-30
because they relate to subject matter not required to be searched by this Authority, namely:

A method for treatment of the human or animal body by therapy, see rule 39.1.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/08/94

International application No.

PCT/SE 94/00680

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0124495	07/11/84	SE-T3- 0124495	
		AU-B- 563842	23/07/87
		AU-A- 2525784	06/09/84
		CA-A- 1264751	23/01/90
		GB-A,B- 2137616	10/10/84
		JP-C- 1651336	30/03/92
		JP-B- 3013233	22/02/91
		JP-A- 59167587	21/09/84
		SU-A- 1314953	30/05/87
		US-A- 4738974	19/04/88

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

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(21) International Application Number: PCT/SE95/00519

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(30) Priority Data:
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(34) Countries for which the regional or international application was filed: AT et al.

(60) Parent Application or Grant
(63) Related by Continuation
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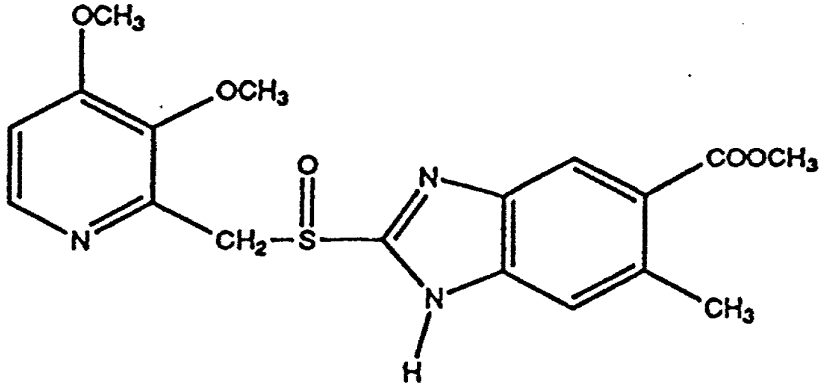
(54) **Title:** NOVEL DIALKOXY-PYRIDINYL-BENZIMIDAZOLE DERIVATIVES

(57) **Abstract**

The novel optically pure compounds, i.e. the single enantiomeric compounds, (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole and (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole or a therapeutically acceptable salt thereof, such as Na^+, Mg^{2+}, Li^+, K^+, Ca^{2+} and $N^+(R)_4$ salts, where R is an alkyl group with 1-4 carbon atoms, processes for the preparation thereof and pharmaceutical preparations containing the compounds as active ingredients, as well as the use of the compounds in pharmaceutical preparations and intermediates obtained by preparing the compounds.



(Ia,Ib)

Ia (+)-enantiomer

Ib (-)-enantiomer

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NOVEL DIALKOXY-PYRIDINYL-BENZIMIDAZOLE DERIVATIVES

Field of the invention

The present invention is directed to new compounds with high optical purity, their use in medicine, a process for their preparation and their use in the manufacture of pharmaceutical preparation. The invention also relates to novel intermediates in the preparation of the compounds of the invention.

Background of the invention

The compound 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole, and therapeutically acceptable salts thereof are described in application number EP 91911618.6. This compound and its therapeutically acceptable salts are effective gastric acid secretion inhibitors, and are useful as antiulcer agents. The compounds, being sulfoxides, have an asymmetric center in the sulfur atom, i.e. exist as two optical isomers (enantiomers). It is desirable to obtain compounds with improved pharmacokinetic and metabolic properties which will give an improved therapeutic profile. The present invention provides such compounds, which are novel salts of single enantiomers of 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole as well as the novel single enantiomers of the neutral form of said compound.

The separation of the enantiomers of therapeutically active sulfoxides, such as substituted benzimidazoles, for example omeprazole (5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole) in analytical scale is described in e.g. J. Chromatography, 532 (1990), 305-19. The isolation of single enantiomers of the sulfoxide agent Ro 18-5364 is described in Euro. J. Biochem. 166 (1987) 453-459. Furthermore, separation of the enantiomers of omeprazole in a preparative scale is described in DE 4035455. The latter has been done by using a diastereomeric ether which is separated and thereafter hydrolysed in an acidic solution. Under the acidic conditions needed for hydrolysis of the attached group, the active compound, omeprazole, is quite sensitive and the acid has to be quickly

neutralized with a base to avoid degradation of the acid-sensitive compound. In the above mentioned application this is done by adding the reaction mixture containing concentrated sulfuric acid to a concentrated solution of NaOH. This is disadvantageous because there is a great risk of locally reaching pH values between 1-6, which would be devastating for the substance. Moreover, instantaneous neutralization will create heat which will be difficult to handle in large scale production.

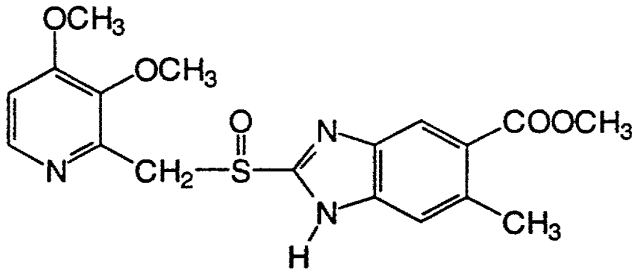
The present invention in a further aspect provides a novel method for preparing the novel compounds of the invention in large scale. Thus, this novel method can be used in large scale to obtain single enantiomers of the compound of the invention in neutral form, as well as in the form of the therapeutically acceptable salts.

These novel compounds of the invention, being sulfoxides, could be expected to undergo racemization in neutral pH as well as in basic pH. See for example Brändström et al, Acta Chemica Scandinavia 43 (1989) p.536-547. Surprisingly, the inventors now found that the novel single enantiomers of 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole as well as its therapeutically acceptable salts are stable towards racemization.

There is no example known in the prior art of any isolated or characterized single enantiomers of the compound of the invention. Furthermore, the inventors are not aware of any description in the scientific literature of any isolated salt of a single enantiomer of the claimed type.

Detailed description of the invention

The present invention refers to the new single enantiomers of 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole according to compounds Ia and Ib



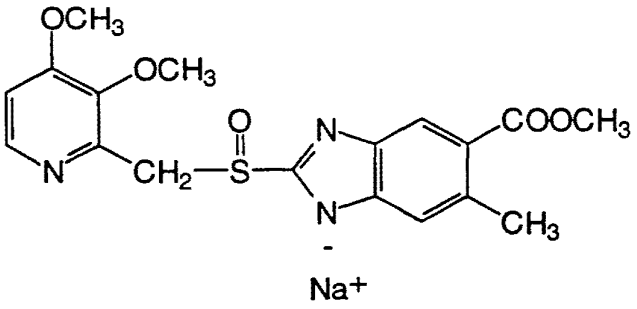
(Ia, Ib)

Ia (+)-enantiomer
Ib (-)-enantiomer

as well as therapeutically acceptable salts thereof. Such salts are for example the Na^+, Mg^{2+}, Ca^{2+}, Li^+, K^+ and $N^+(R)_4$ salts of the single enantiomers of said compound, where R is an alkyl group with 1-4 carbon atoms, i.e. (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole and (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole as well as Na^+, Mg^{2+}, Ca^{2+}, Li^+, K^+ and $N^+(R)_4$ salts of the single enantiomers, where R is an alkyl group with 1-4 carbon atoms.

Particularly preferred salts of the compound of the invention are the Na^+, Mg^{2+} and Ca^{2+} salts of the single enantiomers of 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole.

The most preferred compounds of the invention are the optically pure 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole according to the above formulas Ia and Ib. Further preferred compounds are the optically pure Na^+ salts of 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole according to compounds IIa and IIb



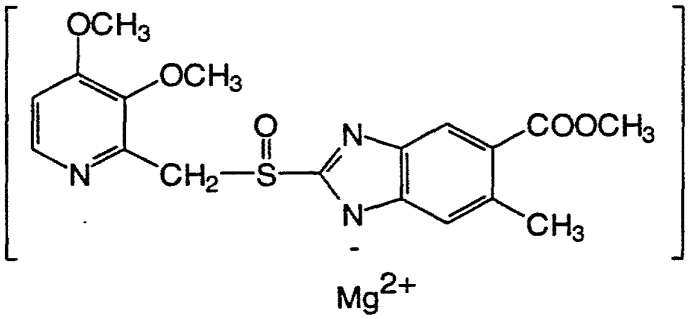
(IIa, IIb)

Na^+

IIa (+)-enantiomer

IIb (-)-enantiomer

and the optically pure magnesium salts of said compounds having the formulas IIIa and IIIb.



(IIIa, IIIb)

Mg^{2+}

IIIa (+)-enantiomer

IIIb (-)-enantiomer

With the expression "optically pure compound of the invention" is meant the (+)-enantiomer of said compound essentially free from the corresponding (-)-enantiomer and the (-)-enantiomer essentially free from the corresponding (+)-enantiomer, respectively. Thus, every single compound of the invention is obtained in high optical purity. By means of the novel specific method according to one aspect of the invention of preparing the single enantiomers, the compounds of the invention are easy to obtain. Moreover, as mentioned above the novel optically pure compounds are stable towards racemization in neutral pH as well as basic pH. The former was surprising since the mechanism of the degradation reactions at neutral pH of these kind of sulfoxides (omeprazole analogues) contains reversible reactions via achiral intermediates (see *e.g.* Brändström *et al.* Acta Chemica Scandinavica 43 (1989) 536-547, especially p.538). It is obvious that such reversible reactions from achiral intermediates back to a sulfoxide would

cause a racemic product. Further, the novel optically pure compounds are stable towards racemization in basic pH, which was surprising since the known deprotonation at the carbon atom between the pyridine ring and the chiral sulphur atom was expected to cause racemization under alkaline conditions. This high stability towards racemization, both in neutral pH and basic pH, makes it possible to use a single enantiomeric compound of the invention in the neutral form as well as salts thereof in therapy.

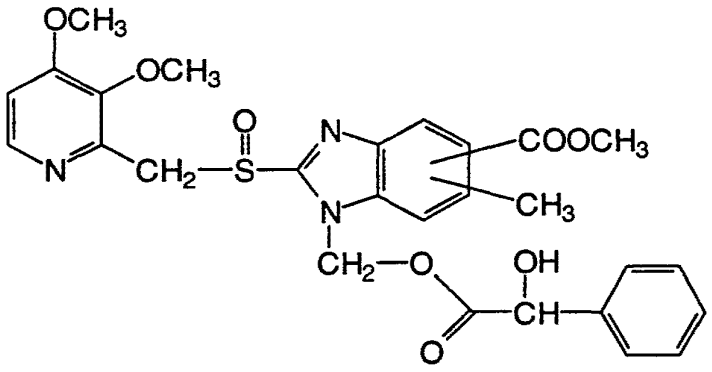
The specific method of preparation of the single enantiomers of the compound of the invention is a further aspect of the invention as mentioned above and it can be used to obtain the single enantiomeric compounds in the neutral form as well as the salts thereof.

The single enantiomeric compounds of the invention as well as the racemate have a high level of bioavailability, and does not block the uptake of iodine into the thyroid gland, and still said compounds are very effective as inhibitors of gastric acid secretion and exhibit high stability properties at neutral pH.

The compounds according to the invention may be used for inhibiting gastric acid secretion in mammals and man. In a more general sense, the single enantiomeric compounds of the invention may be used for the treatment of gastric acid-related diseases and gastrointestinal inflammatory diseases in mammals and man, such as gastric ulcer, duodenal ulcer, reflux esophagitis, and gastritis. Furthermore, the compounds may be used for treatment of other gastrointestinal disorders where gastric antisecretory effect is desirable e.g. in patients on NSAID therapy, in patients with gastrinomas, and in patients with accute upper gastrointestinal bleeding. They may also be used in patients in intensive care situations, and pre- and postoperatively to prevent acid aspiration and stress ulceration. The compound of the invention may also be used for treatment or prophylaxis of inflammatory conditions in mammals, including man, especially those involving lysozymal enzymes. Conditions that may be sepcifically mentioned are rheumatoid arthritis and gout. The compound of the invention may also be useful in the treatment of psoriasis as well as in the treatment of Helicobacter infections.

Yet a further aspect of the invention is the diasteromeric mixture of a regioisomeric mixture having the formula IV, which is an intermediate used in the

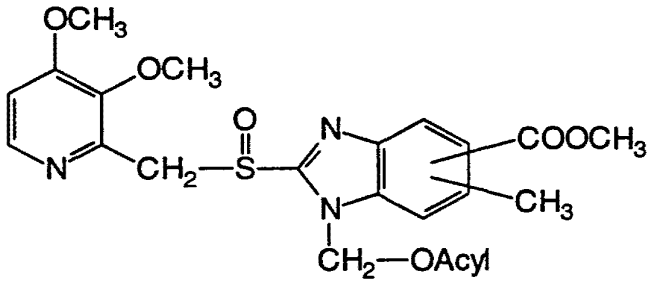
specific method of preparation, wherein the carbomethoxy and methyl substituents in the benzimidazole moiety are in the 5 or 6 position, respectively.



(IV)

Preparation

The optically pure compounds of the invention, i.e. the single enantiomers, are prepared by separating the stereoisomers of a diastereomeric mixture of the regioisomeric mixture of the following type, 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]-(R/S)-sulfinyl]-1-[(R)-acyloxymethyl-1H-benzimidazole and 6-carbomethoxy-5-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]-(R/S)-sulfinyl]-1-[(R)-acyloxymethyl-1H-benzimidazole formula V



(V)

wherein the carbomethoxy and methyl substituents in the benzimidazole moiety are in position 5 or 6, respectively, and wherein the Acyl radical is as defined below, followed by a solvolysis of each separated diastereomer in an alkaline solution. The formed single enantiomeric compounds of the invention in neutral form are then isolated by neutralizing aqueous solutions of the salts of said

compounds with a neutralizing agent which can be an acid or an ester such as methyl formate.

The Acyl moiety in the diastereomeric ester may be a chiral acyl group such as mandeloyl, and the asymmetric center in the chiral acyl group can have either R or S configuration.

The diastereomeric esters can be separated either by chromatography or fractional crystallization.

The solvolysis usually takes place together with a base in a protic solvent such as alcohols or water; or with a base in a mixture of acetonitrile and water, but the acyl group may also be hydrolysed off by a base in an aprotic solvent such as dimethylsulfoxide or dimethylformamide. The reacting base may be OH^- or R^1O^- where R^1 can be any alkyl or aryl group.

To obtain the optically pure Na^+ salts of the invention, i.e. Na^+ salts of the single enantiomeric compound of the invention, the resulting compound in neutral form is treated with a base, such as NaOH, in an aqueous or nonaqueous medium, or with $NaOR^2$ wherein R^2 is an alkyl group containing 1-4 carbon atoms, or with $NaNH_2$. Also alkaline salts wherein the cation is Li^+ or K^+ may be prepared using lithium or potassium salts of the above mentioned bases. In order to obtain the crystalline form of the single enantiomers of the Na^+ salts, to the optically pure Na^+ salts as a syrup are added a mixture of 2-butanone and toluene, but the crystalline form of the single enantiomers of the Na^+ salt may also be prepared by adding NaOH to a mixture of the single enantiomeric compound of invention and a non-aqueous medium, such as a mixture of 2-butanone and toluene.

To obtain the optically pure Mg^{2+} salts of the invention, optically pure Na^+ salts are treated with an aqueous solution of an inorganic magnesium salt such as $MgCl_2$, whereupon the Mg^{2+} salts are precipitated. The optically pure Mg^{2+} salts may also be prepared by treating single enantiomeric compound of the invention with a base, such as $Mg(OR^3)_2$, wherein R^3 is an alkyl group containing 1-4 carbon atoms, in a non-aqueous solvent such as alcohol (only for alcoholates), e.g. ROH, or in an ether such as tetrahydrofuran. In an analogous way, also alkaline salts wherein the cation is Ca^{2+} can be prepared, using an aqueous solution of an inorganic calcium salt such as $CaCl_2$.

Alkaline salts of the single enantiomers of the invention are, as mentioned above, beside the sodium salts (compounds IIa and IIb) and the magnesium salts (compound IIIa and IIIb), exemplified by their salts with Li^+, K^+, Ca^{2+} and $N^+(R)_4$, where R is an alkyl group with 1-4 C-atoms.

For clinical use the single enantiomers, i.e. the optically pure compounds, of the invention are formulated into pharmaceutical formulations for oral, rectal, parenteral or other modes of administrations. The pharmaceutical formulations contain the single enantiomers of the invention normally in combination with a pharmaceutically acceptable carrier. The carrier may be in form of a solid, semi-solid or liquid diluent, or capsule. These pharmaceutical preparations are a further object of the invention. Usually the amount of active compound is between 0.1-95% by weight of the preparation, between 0.2-20% by weight in preparations for parenteral use and between 1-50% by weight in preparations for oral administration. An active compound in a form with high solubility in water is requested for parenteral preparations, for some oral preparations an active compound in a form with low solubility is suitable.

In the preparation of pharmaceutical formulations in form of dosage units for oral administration the single enantiomeric compound may be mixed with a solid, powdered carrier, such as lactose, saccharose, sorbitol, mannitol, starch, amylopectin, cellulose derivates, gelatin or another suitable carrier, stabilizing substances such as alkaline compounds e.g. carbonates, hydroxides and oxides of sodium, potassium, calcium, magnesium and the like as well as with lubricating agents such as magnesium stearate, calcium stearate, sodium stearyl fumarate and polyethylenglycol waxes. The mixture is then processed into granules or pressed into tablets. Granules and tablets may be coated with an enteric coating which protects the active compound from acid catalysed degradation as long as the dosage form remains in the stomach. The enteric coating is chosen among pharmaceutically acceptable enteric-coating materials e.g. beeswax, shellac or anionic film-forming polymers and the like, if preferred in combination with a suitable plasticizer. To the coating various dyes may be added in order to distinguish among tablets or granules with different amounts of the active compound present.

Soft gelatine capsules may be prepared with capsules containing a mixture of the active compound, vegetable oil, fat, or other suitable vehicle for soft gelatine capsules. Soft gelatine capsules may also be enteric-coated as described above.

Hard gelatine capsules may contain granules or enteric-coated granules of the active compound. Hard gelatine capsules may also contain the active compound in combination with a solid powdered carrier such as lactose, saccharose, sorbitol, mannitol, potato starch, amylopectin, cellulose derivates or gelatin. The capsules may be enteric-coated as described above.

Dosage units for rectal administration may be prepared in the form of suppositories which contain the active substance mixed with a neutral fat base, or they may be prepared in the form of a gelatine rectal capsule which contains the active substance in a mixture with a vegetable oil, paraffin oil or other suitable vehicle for gelatine rectal capsules, or they may be prepared in the form of a ready-made micro enema, or they may be prepared in the form of a dry micro enema formulation to be reconstituted in a suitable solvent just prior to administration.

Liquid preparation for oral administration may be prepared in the form of syrups or suspensions, e.g. solutions or suspensions containing from 0.2% to 20% by weight of the active ingredient and the remainder consisting of sugar or sugar alcohols and a mixture of ethanol, water, glycerol, propylene glycol and/or polyethylene glycol. If desired, such liquid preparations may contain colouring agents, flavouring agents, saccharine and carboxymethyl cellulose or other thickening agents. Liquid preparations for oral administration may also be prepared in the form of dry powder to be reconstituted with a suitable solvent prior to use.

Solutions for parenteral administrations may be prepared as solutions of the single enantiomeric compounds of the invention in pharmaceutically acceptable solvents, preferably in a concentration from 0.1 to 10% by weight. These soultions may also contain stabilizing agents and/or buffering agents and may be manufactured in different unit dose ampoules or vials. Solutions for parenteral administration may also be prepared as dry preparations to be reconsituted with a suitable solvent extemporaneously before use.

The typical daily dose of the active compound will depend on various factors such as for example the individual requirement of each patient, the route of

administration and the disease. In general, oral and parenteral dosages will be in the range of 5 to 500 mg per day of active substance.

The invention is illustrated by the following examples.

Example 1. Preparation of (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole

The crude product of the diastereomers of a mixture of two regioisomeric mandelic esters, namely 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]-(R/S)-sulfinyl]-1-[(R)-mandeloyloxymethyl]-1H-benzimidazole and 6-carbomethoxy-5-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]-(R/S)-sulfinyl]-1-[(R)-mandeloyloxymethyl]-1H-benzimidazole (1.8 g, 3.3 mmol) was divided into three parts. Each part was chromatographed on a reversed phase column (HPLC, Kromasil C8) in order to separate the diastereomers. The stereoisomers were easily separated by elution with a mixture of aqueous 0.1 M ammonium acetate and acetonitrile (70/30), but each separated diastereomer consisted of a mixture of the two regioisomers. These intermediates were used directly in their solutions during the hydrolyses; To the acetonitrile/aqueous solutions of the more lipophilic diastereomer were added 1 M aqueous solutions of NaOH until the pH was around 12-13. After 5 minutes the solutions were neutralized with 3.0 M aqueous solutions of NH_4Cl. The solutions from each preparation were combined and extracted with methylenechloride whereupon the organic phases were dried over Na_2SO_4. Removal of the solvents and flash chromatography of the residue (silica gel, methanol-methylenechloride gradient 1-8%) yielded 250 mg of a yellow oil. The product was crystallised by adding acetonitrile (3 ml) and after filtration there was obtained 210 mg (32%) of the title compound as white crystals m.p. 171-173° C. $[a]^{20}_D$= +153.1° (c=0.5%, chloroform).

NMR data are given below.

Example 2. Preparation of (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole

To the acetonitrile/aqueous solutions of the less lipophilic diastereomer of 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]-(R/S)-sulfinyl]-1-[(R)-mandeloyloxymethyl]-1H-benzimidazole and 6-carbomethoxy-5-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]-(R/S)-sulfinyl]-1-[(R)-mandeloyloxymethyl]-1H-benzimidazole (obtained from the very same reversed phase chromatographic preparations described in Example 1) were added 1.0 M NaOH until the pH was around 12-13. After 5 minutes the solutions were neutralized with 3.0 M aqueous solutions of NH_4Cl. The solutions from each preparation were combined and extracted with methylenechloride whereupon the organic phases were dried over Na_2SO_4. Removal of the solvents and flash chromatography of the residue (silica gel, methanol-methylenechloride gradient 1-8%) yielded 270 mg of a yellow oil. The product was crystallized by adding acetonitrile (3 ml) and after filtration there was obtained 210 mg (32%) of the title compound as white crystals m.p. 173-174° C. $[a]^{20}_D$= -150.0° (c=0.5%, chloroform).

NMR data are given below.

Example 3. Preparation of (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole sodium salt

To a mixture of (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)-methyl]sulfinyl]-1-H-benzimidazole (200 mg, 0.51 mmol) and ethanol (10 ml) was added an aqueous solution of 2.0 M NaOH (0.26 ml, 0.51 mmol). The solvent was removed by film evaporation whereupon the residue was dissolved in 2-butanone (1 ml). Toluene (5 ml) was added dropwise while stirring. The formed precipitate was removed by centrifugation and washed with diethyl ether. There was obtained 170 mg (81%) of the title compound as white crystals m. p. (decomp.) 170°-173°C. $[a]^{20}_D$= +93.6°(c=1%, methanol).

NMR data are given below

Example 4. Preparation of (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole sodium salt

To a mixture of (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)-methyl]sulfinyl]-1-H-benzimidazole (200 mg, 0.51 mmol) and ethanol (10 ml) was added an aqueous solution of 2.0 M NaOH (0.26 ml, 0.51 mmol). The solvent was removed by film evaporation whereupon the residue was dissolved in 2-butanone (2 ml). Toluene (5 ml) was added dropwise while stirring. The formed precipitate was isolated by filtration and washed with diethyl ether. There was obtained 200 mg (96%) of the title compound as white crystals m. p. (decomp.) 172°-175°C. $[a]^{20}_D$= -93.8° (c=1%, methanol).

NMR data are given below

Example 5. Preparation of (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole magnesium salt

(+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole sodium salt (100 mg, 0.24 mmol) obtained as in Example 3 was dissolved in water (2 ml) and $MgCl_2x6H_2O$ (25 mg, 0.12 mmol) dissolved in water (1 ml) was added dropwise. The formed precipitate was isolated by centrifugation and washed with water. The product was dried in a desiccator and there was obtained 84 mg (87%) of a white powder. $[a]^{20}_D$= + 170° (c=0.5%, DMSO).

Example 6. Preparation of (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole magnesium salt

(-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole sodium salt (100 mg, 0.24 mmol) obtained as in Example 4 was dissolved in water (2 ml) and $MgCl_2x6H_2O$ (25 mg, 0.12 mmol) dissolved in water (1 ml) was added dropwise. The formed precipitate was isolated by centrifugation and washed with water. The product was dried in a desiccator and there was obtained 84 mg (87%) of a white powder.$[a]^{20}_D$= -178.8° (c=0.5%, DMSO).

Table 1.

Ex.	Solvent	NMR data d ppm
1.	DMSO-d_6 300 MHz	2.62 (s, 3H), 3.75 (s, 3H), 3.84 (s, 3H), 3.88 (s, 3H), 4.68 (s, 2H), 7.09 (d, 1H), 7.53 (s, 1H), 8.11 (s, 1H), 8.12 (d, 1H), 13.75 (b, 1H).
2.	DMSO-d_6 300 MHz	2.62 (s, 3H), 3.75 (s, 3H), 3.84 (s, 3H), 3.88 (s, 3H), 4.68 (s, 2H), 7.09 (d, 1H), 7.53 (s, 1H), 8.11 (s, 1H), 8.12 (d, 1H), 13.75 (b, 1H).
3.	DMSO-d_6 300 MHz	2.58 (s, 3H), 3.77 (s, 3H), 3.79 (s, 3H), 3.89 (s, 3H), 4.36 (d, 1H), 4.74 (d, 1H), 7.07 (d, 1H), 7.31 (s, 1H), 8.10 (s, 1H), 8.21 (d, 1H).
4.	DMSO-d_6 300 MHz.	2.58 (s, 3H), 3.77 (s, 3H), 3.79 (s, 3H), 3.89 (s, 3H), 4.34 (d, 1H), 4.74 (d, 1H), 7.07 (d, 1H), 7.29 (s, 1H), 8.11 (s, 1H), 8.22 (d, 1H).

Preparation of the synthetic intermediates according to the invention will be described in the following example.

Example 7. Preparation of 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]-(R/S)-sulfinyl]-1-[(R)-mandeloyloxymethyl]-1H-benzimidazole and 6-carbomethoxy-5-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]-(R/S)-sulfinyl]-1-[(R)-mandeloyloxymethyl]-1H-benzimidazole

A solution of 0.33 g (8.2 mmol) sodium hydroxide in 1.6 ml water was added to a mixture of 1.4 g (4.1 mmol) tetrabutylammonium hydrogen sulfate and 0.62 g (4.1 mmol) of (R)-(-)-mandelic acid. Chloroform (50 ml) and a mixture of 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]-sulfinyl]-1-(chloromethyl)-1H-benzimidazole and 6-carbomethoxy-5-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-(chloromethyl)-1H-benzimidazole (as racemates) were added and the mixture was refluxed for 3 hours. The reaction mixture was chilled and then partitioned between ethyl acetate and water. The

layers were separated and the organic phase was washed with water and dried over Na_2SO_4. Removal of solvents yielded a diastereomeric mixture of the two regioisomeric mandelic esters. The crude product was used directly in the chromatographic step where the diastereomers were separated (Example 1 and 2). Yield: 2.4 g, 62%.

NMR data are given below.

Table 2.

Ex.	Solvent	NMR data d ppm
7.	$CDCl_3$ 500 MHz	2.6-2.8 (m, 3H), 3.8-4.1 (m, 9H), 4.75-4.95 (m, 1H), 5.00-5.15 (m, 1H), 5.3-5.4 (m, 1H), 6.45-6.70 (m, 2H), 6.70-6.80 (m, 1H), 7.1-8.4 (m, 8H).

The best mode of carrying out the invention known at present is to use the magnesium salts of the optically pure compounds of the invention, thus the compounds described in Examples 5 and 6.

Pharmaceutical preparations containing the compounds of the invention as active ingredient are illustrated in the following formulations.

Syrup

A syrup containing 1% (weight per volume) of active substance was prepared from the following ingredients:

Compound according to Example 1	1.0 g
Sugar, powder	30.0 g
Saccharine	0.6 g
Glycerol	5.0 g
Flavouring agent	0.05 g
Ethanol 96%	5.0 g
Distilled water q.s. to a final volume of	100 ml

Sugar and saccharine were dissolved in 60 g of warm water. After cooling the active compound was added to the sugar solution and glycerol and a solution of flavouring agents dissolved in ethanol were added. The mixture was diluted with water to a final volume of 100 ml.

Enteric-coated tablets

An enteric coated tablet containing 50 mg of active compound was prepared from the following ingredients:

I	Compound according to Example 2		500 g
	Lactose		700 g
	Methyl cellulose		6 g
	Polyvinylpyrrolidone cross-linked		50 g
	Magnesium stearate		15 g
	Sodium carbonate		6 g
	Distilled water	q.s.	
II	Cellulose acetate phthalate		200 g
	Cetyl alcohol		15 g
	Isopropanol		2000 g
	Methylene chloride		2000 g

I Compound according to Example 2, powder, was mixed with lactose and granulated with a water solution of methyl cellulose and sodium carbonate. The wet mass was forced through a sieve and the granulate dried in an oven. After drying the granulate was mixed with polyvinylpyrrolidone and magnesium stearate. The dry mixture was pressed into tablet cores (10 000 tablets), each tablet containing 50 mg of active substance, in a tabletting machine using 7 mm diameter punches.

II A solution of cellulose acetate phthalate and cetyl alcolhol in isopropanol/methylene chloride was sprayed onto the tablets I in an Accela Cota^{R}, Manesty coating equipment. A final tablet weight of 110 mg was obtained.

Solution for intravenous administration

A parenteral formulation for intravenous use, containing 4 mg of active compound per ml, was prepared from the following ingredients:

Compound according to Example 3	4 g
Sterile water to a final volume of	1000 ml

The active compound was dissolved in water to a final volume of 1000 ml. The solution was filtered through a 0.22 µm filter and immediately dispensed into 10 ml sterile ampoules. The ampoules were sealed.

Capsules

Capsules containing 30 mg of active compound were prepared from the following ingredients:

Compound according to Example 6	300 g
Lactose	700 g
Microcrystalline cellulose	40 g
Hydroxypropyl cellulose low-substituted	62 g
Disodium hydrogen phosphate	2 g
Purified water	q.s.

The active compound was mixed with the dry ingredients and granulated with a solution of disodium hydrogen phosphate. The wet mass was forced through an extruder and spheronized and dried in a fluidized bed dryer.

500 g of the pellets above were first coated with a solution of hydroxypropyl methylcellulose, 30 g, in water, 750 g, using a fluidized bed coater. After drying, the pellets were coated with a second coating as given below:

Coating solution:

Hydroxypropyl methylcellulose phthalate	70 g
Cetyl alcohol	4 g
Acetone	200 g
Ethanol	600 g

The final coated pellets were filled into capsules.

Suppositories

Suppositories were prepared from the following ingredients using a welding procedure. Each suppository contained 40 mg of active compound.

Compound according to Example 2	4 g
Witepsol H-15	180 g

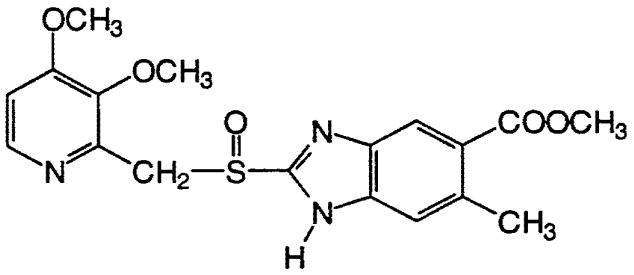
The active compound was homogenously mixed with Witepsol H-15 at a temperature of 41° C. The molten mass was volume filled into pre-fabricated suppository packages to a net weight of 1.84 g. After cooling the packages were heat sealed. Each suppository contained 40 mg of active compound.

Stability towards racemization at different pH:es

The stability of the optically pure compounds of the invention towards racemization has been measured at low concentrations (10^{-5} M) at 37°C in aqueous buffer solutions at pH 7 and pH 11. The stereo chemical stability was measured by comparing the optical purity for the (-)-enantiomer of 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole in buffer solution immediately after dissolving and after several hours. The surprising high stereo chemical stability in neutral conditions as well as in alkaline conditions for the compounds of invention is exemplified by the fact that no racemization for the test compound was obtained neither at pH 7 nor at pH 11, even after 24 hours. At pH 7, however, the chemical degradation of the compound is much apparent after 28 hours.

Claims

1. Single enantiomeric compounds having the formula Ia and Ib



(Ia, Ib)

Ia (+)-enantiomer
Ib (-)-enantiomer

and the therapeutically acceptable salts thereof.

2. Compounds according to claim 1 c h a r a c t e r i z e d in that the compound is (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole or a therapeutically acceptable salt thereof, substantially free of its (-)-enantiomer.

3. Compounds according to to claim 1 c h a r a c t e r i z e d in that the compound is (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole, or a therapeutically acceptable salt thereof, substantially free of its (+)-enantiomer.

4. Compounds according to any of claims 1-3 c h a r a c t e r i z e d in that the therapeutically acceptable salts are Na^+, Mg^{2+}, Ca^{2+}, Li^+, K^+ and $N^+(R)_4$ salts wherein R is an alkyl group with 1-4 carbon atoms.

5. Compounds according to any of claims 1-4 c h a r a c t e r i z e d in that the compounds are (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole sodium salt, (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole sodium salt, (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole magnesium salt and (-)-5-

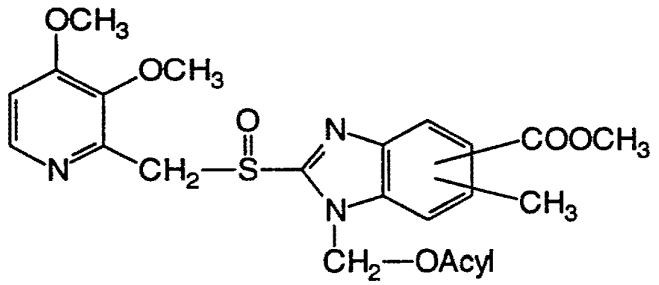
carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole magnesium salt.

6. Compounds according to any of claims 1-3 characterized in that the compounds are (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole or its magnesium salt and (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole or its magnesium salt, in their crystalline forms.

7. Compounds according to claims 1 and 2 characterized in that the compound is (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole or the magnesium salt thereof, respectively, in its crystalline form substantially free of its (-)-enantiomer.

8. Compounds according to claims 1 and 3 characterized in that the compound is (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole or the magnesium salt thereof, respectively, in its crystalline form substantially free of its (+)-enantiomer.

9. Process for the preparation of a compound according to claim 1 characterized in that a diastereomeric ester of formula V



(V)

wherein the carbomethoxy and methyl substituents in the benzimidazole moiety are in the 5 or 6 position, respectively, and wherein Acyl designates a chiral acyl group such as mandeloyl, having either R or S configuration, is separated, and each of the separated diastereomers is subjected to solvolysis with an alkaline solution where the acyloxymethyl group is hydrolyzed off to give the enantiomeric compound in neutral form after neutralization with a neutralizing

agent whereupon the enantiomeric compound in neutral form optionally is converted into a therapeutically acceptable salt.

10. Process according to claim 9 c h a r a c t e r i z e d in that the diastereomers are separated by chromatography or fractional crystallization.

11. Process according to claim 9 c h a r a c t e r i z e d in that the solvolysis is performed in an alkaline solution consisting of a base in a protic solvent, such as alcohols or water; or a base in an aprotic solvent, such as dimethylsulfoxide or dimethylformamide; or a base in a mixture of protic and aprotic solvents, such as water and acetonitrile.

12. Process for the preparation of a compound according to any of claims 1-4 in crystalline form c h a r a c t e r i z e d in that a product obtained in claim 9 either neutral form or in the form of a therapeutically salt is treated with a non-aqueous solvent to precipitate the product.

13. Process for preparation of (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole or its sodium salt and (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole or its sodium salt in their crystalline forms c h a r a c t e r i z e d in that (+)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole or its sodium salt and (-)-5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1-H-benzimidazole or its sodium salt crude product, respectively is treated with a non-aqueous medium, such as 2-butanone and toluene.

14. Pharmaceutical preparation comprising single enantiomeric compound according to any of claims 1-8 as active ingredient.

15. Single enantiomeric compounds according to any of claims 1-8 for use in therapy.

16. Use of a single enantiomeric compound according to any of claims 1-8 in the manufacture of a pharmaceutical formulation for inhibiting gastric acid secretion.

17. Use of a single enantiomeric compound according to any of claims 1-8 for the manufacture of a pharmaceutical formulation for the treatment of gastrointestinal. inflammatory diseases.

18. A method for inhibiting gastric acid secretion comprising administration to a mammal including man in need of such treatment an effective amount of an enantiomeric compound according to any of claims 1-8.

19. A method for the treatment of gastrointestinal inflammatory diseases comprising administration to a mammal including man in need of such treatment an effective amount of an enantiomeric compound according to any of claims 1-8.

20. The compounds 5-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-[mandeloyloxymethyl]-1H-benzimidazole and 6-carbomethoxy-6-methyl-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-[mandeloyloxymethyl]-1H-benzimidazole.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/00519

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07D 401/12, A61K 31/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	WO 9119712 A1 (AKTIEBOLAGET ASTRA), 26 December 1991 (26.12.91) --	1-17,20
Y	DE 4035455 A1 (BYK GULDEN LOMBERG CHEMISCHE FABRIK GMBH), 14 May 1992 (14.05.92) -- --------	9-13,20

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 Sept 1995	19.09.1995
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Göran Karlsson Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/00519

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 18-19
because they relate to subject matter not required to be searched by this Authority, namely:
A method for treatment of the human or animal body by therapy, see Rule 39.1.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

31/07/95

International application No.
PCT/SE 95/00519

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9119712	26/12/91	AU-B- 649453	26/05/94
		AU-B- 649456	26/05/94
		AU-A- 8009791	07/01/92
		AU-A- 8061791	07/01/92
		CA-A- 2083606	21/12/91
		CA-A- 2083714	21/12/91
		CN-A- 1058212	29/01/92
		CN-A- 1058213	29/01/92
		CZ-B- 279434	12/04/95
		EP-A- 0535081	07/04/93
		EP-A- 0593463	27/04/94
		HU-D- 9204033	00/00/00
		HU-D- 9204034	00/00/00
		NZ-A- 238546	25/03/94
		OA-A- 9682	15/05/93
		OA-A- 9683	15/05/93
		PL-B- 165898	28/02/95
		PL-B- 166209	28/04/95
		US-A- 5430042	04/07/95
		WO-A- 9119711	26/12/91
DE-A1- 4035455	14/05/92	AU-A- 8840691	11/06/92
		WO-A- 9208716	29/05/92

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(74) Agent: JANG, Seong, Ku; 275, Yangjae-dong, Seocho-gu, Seoul 137-130 (KR).

(81) Designated States: AU, CA, CN, JP, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

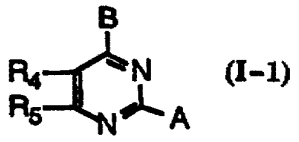
Published

With international search report.

(54) Title: NOVEL PYRIMIDINE DERIVATIVES AND PROCESSES FOR THE PREPARATION THEREOF

(57) Abstract

The present invention relates to novel pyrimidine derivatives of formulae (I-1) and (I-2) and pharmaceutically acceptable salts thereof which possess an excellent anti-secretory activity, pharmaceutical compositions containing same as an active ingredient, their novel intermediates, and processes for the preparation thereof. In said formulae, R_4 and R_5, which may be the same or different, are independently hydrogen or a C_1-C_3 alkyl group, or jointly form a cyclopentyl or cyclohexyl ring; A is a group of formula (II) wherein R_1 and R_2 are, independently of each other, hydrogen or a C_1-C_3 alkyl group, and R_3 is hydrogen, a C_1-C_3 alkyl group or a halogen; and B is 1-(substituted)-1,2,3,4-tetrahydroisoquinolin-2-yl of formula (III-1) or 7-(substituted)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl of formula (III-2) wherein R_6 is hydrogen or a C_1-C_3 alkyl group.



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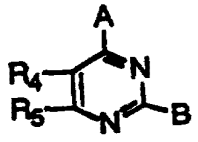

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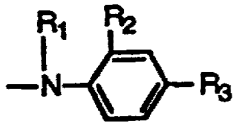

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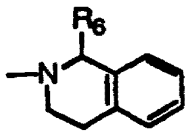

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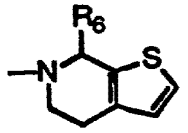

(III-2)

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GA	Gabon				

NOVEL PYRIMIDINE DERIVATIVES AND PROCESSES FOR THE PREPARATION THEREOF

Field of the Invention

The present invention relates to novel pyrimidine derivatives and pharmaceutically acceptable salts thereof which possess an excellent anti-secretory activity, pharmaceutical compositions containing same as an active ingredient, their novel intermediates, and processes for the preparation thereof.

Background of the Invention

For the treatment of peptic ulcer disease, various drugs such as antacid, anticholinergic agent, H_2-receptor antagonist and proton pump inhibitor have been used. Recently, the advent of omeprazole useful as a proton pump inhibitor has rekindled research activities in this field.

However, it has been pointed out that the proton pump inhibition by omeprazole is irreversible, which may induce side effects. Accordingly, various attempts to develop a reversible proton pump inhibitor are being actively made. For example, European Patent Nos. 322133 and 404322 disclose quinazoline derivatives, European Patent No. 259174 describes quinoline derivatives, and WO 91/13337 offers pyrimidine derivatives, as a reversible proton pump inhibitor. Further, the present inventors have also reported quinazoline derivatives in WO 94/14795.

Summary of the Invention

The present inventors have carried out extensive research to develop a reversible proton pump inhibitor with

improved efficacy; and, as a result, have discovered that pyrimidine derivatives having a tetrahydroisoquinoline group at the 2- or 4-position of the pyrimidine nucleus exhibit excellent proton pump inhibition effects and possess the ability to attain a reversible proton pump inhibition.

Accordingly, it is a primary object of the present invention to provide novel pyrimidine derivatives having a tetrahydroisoquinoline group at the 2- or 4-position of the pyrimidine nucleus, and pharmaceutically acceptable salts thereof.

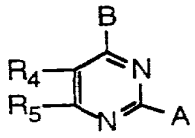
It is another object of the present invention to provide processes for preparing said compounds.

It is a further object of the present invention to provide pharmaceutical compositions containing same as active ingredients.

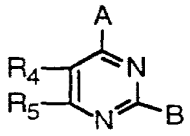
It is still another object of the invention to provide novel intermediate compounds useful for the preparation of the novel pyrimidine derivatives.

Detailed Description of the Invention

In accordance with the present invention, there are provided novel pyrimidine derivative compounds of formulae (I-1) and (I-2) inclusive of pharmaceutically acceptable salts thereof:



(I-1)

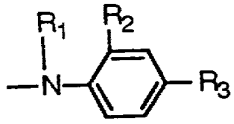


(I-2)

wherein:

R_4 and R_5, which may be the same or different, are independently hydrogen or a C_1-C_3 alkyl group, or jointly form a cyclopentyl or cyclohexyl ring;

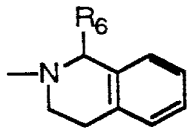
A is a group of formula(II):



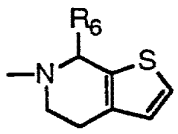
(II)

wherein R_1 and R_2 are, independently of each other, hydrogen or a C_1-C_3 alkyl group, and R_3 is hydrogen, a C_1-C_3 alkyl group or a halogen; and

B is 1-(substituted)-1,2,3,4-tetrahydroisoquinolin-2-yl of formula (III-1) or 7-(substituted)-4,5,6,7-tetrahydro thieno[2,3-c]pyridin-6-yl of formula (III-2)



(III-1)



(III-2)

wherein R_6 is hydrogen or a C_1-C_3 alkyl group.

Among the compounds of the present invention, preferred are those wherein: R_1, R_2 and R_6 are independently hydrogen or a methyl group; R_3 is hydrogen or a fluorine; and R_4 and R_5, which may be the same or different, are independently hydrogen or a C_1-C_3 alkyl group, or jointly form a cyclopentyl or cyclohexyl ring.

Particularly, preferred compounds of the present invention are:

2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

6-methyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

6-methyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-(4-fluorophenylamino)pyrimidine hydrochloride;

6-methyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetra-

hydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(2-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propylpyrimidine hydrochloride;
4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propyl-2-(4-fluorophenylamino)pyrimidine hydrochloride;
2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propylpyrimidine hydrochloride;
5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(phenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

(R)-5,6-dimethyl-2-(4-phenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(2-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(4-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride;
2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride;
2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride;
2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinoline-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride;
6-methyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-methyl-2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinoline-2-yl)pyrimidine hydrochloride;
6-methyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroiso-

quinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(2-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazolinehydrochloride;
2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride;
2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride;
2-(2-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride;
6-methyl-2-(2-methyl-4-fluorophenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;
6-methyl-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]-pyridin-6-yl)-2-(4-fluorophenylamino)pyrimidine hydrochloride;
6-methyl-2-(N-methylphenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;
5-methyl-2-(2-methyl-4-fluorophenylamino)-4-(7-methyl-4,5,

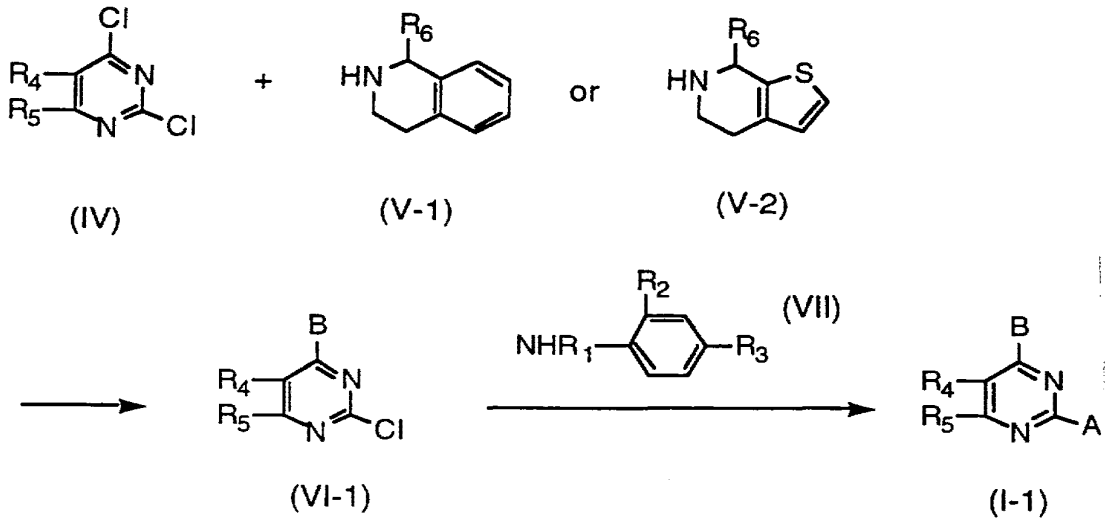
6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-6-ethylpyrimidine hydrochloride;
6-methyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-methyl-4-(4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-methyl-4-(2-methyl-4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;
6-methyl-4-(4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;
6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-4-(2-methyl-4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-4-(4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-4-(N-methylphenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl- 1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-4-(4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-4-(4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(N-methylphenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-4-(N-methylphenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

(S)-5,6-dimethyl-4-(N-methylphenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(N-methylphenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]-pyridin-6-yl)-4-(4-fluorophenylamino)pyrimidine hydrochloride;
5,6-dimethyl-4-(N-methylphenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride; and
4-(2-methyl-4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride.

The pyrimidine derivatives of formulae (I-1) and (I-2) in the present invention may exist in the form of an optical isomer, (R) or (S), or a mixture thereof. Both types of the isomeric compounds are found to exhibit excellent anti-secretory activity.

The compounds of formulae (I-1) and (I-2) may be prepared in accordance with Scheme 1 and Scheme 2, respectively, described below.

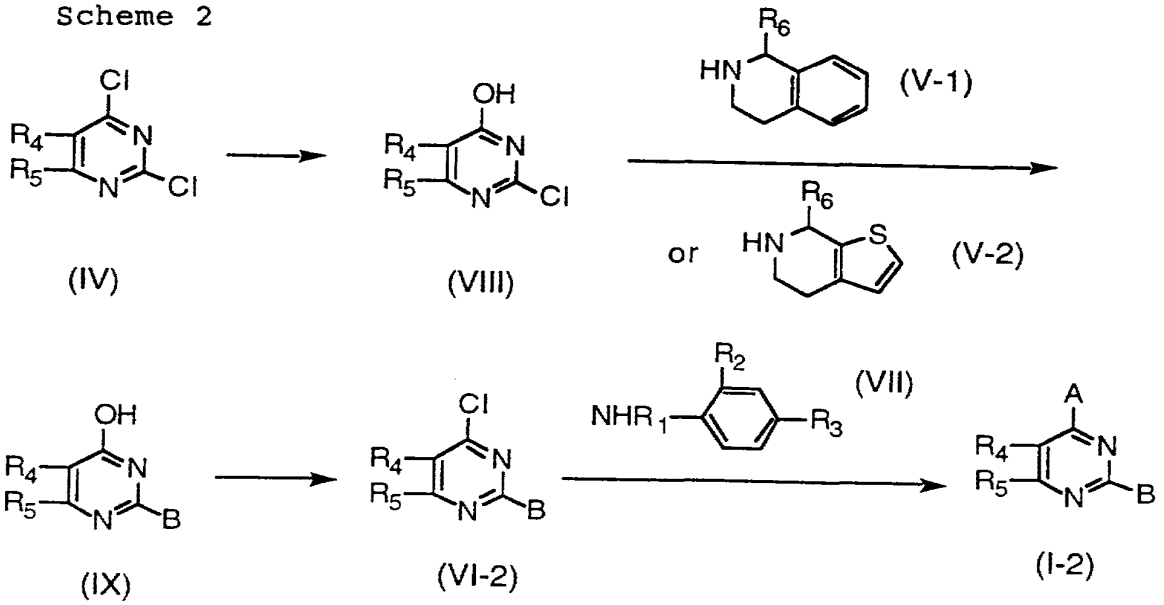
Scheme 1



wherein A, B, R_1, R_2, R_3, R_4, R_5 and R_6 are the same as defined as above.

Specifically, the compound of formula (I-1) may be prepared by a process which comprises: reacting a compound of formula (IV) with a compound of formula (V-1) or (V-2) to give a compound of formula(VI-1); and reacting the compound of formula(VI-1) with a compound of formula(VII).

Scheme 2



wherein A, B, R_1, R_2, R_3, R_4, R_5 and R_6 are the same as defined as above.

Further, the compound of formula(I-2) may be prepared by a process which comprises: hydrolyzing a compound of formula(IV) at its 4-position to give a compound of formula(VIII); reacting the compound of formula(VIII) with a compound of formula (V-1) or (V-2) to give a compound of formula(IX); chlorinating the compound of formula(IX) at its 4-position to give a compound of formula (VI-2); and then reacting the compound of formula (VI-2) with a compound of formula (VII).

In the processes of Scheme 1 and Scheme 2, the compound of formula(IV) may be prepared by using a known process [see, e.g., J. Heterocyclic Chem., 28, 231(1991); and Org. Synth., Coll. Vol, 638], and the compounds of formula (V-1) and (V-2) may be prepared in accordance with the process disclosed in European Patent No. 230871. The compound of formula(VII) is commercially available(for example from Aldrich Co. in U.S.A.)

As shown in Scheme 1 and Scheme 2, the pyrimidine compounds (IV) and (VIII) are reacted with the compounds of formula (V-1) or (V-2) in the presence of an appropriate solvent and a base for 1 to 24 hours to give the compounds of formula (VI-1) or (VI-2), respectively. Suitable solvents for this reaction may include dichloromethane, acetone, acetonitrile and dimethylformamide. The reaction temperature preferably ranges from a room temperature to 150°C. Suitable bases for this reaction may include triethylamine, N,N-dimethylaniline and pyridine.

The substituted pyrimidine compounds of formula(VI-1) and (VI-2) so obtained are then reacted with the compounds of formula(VII) in an appropriate solvent for 2 to 5 hours to give the present compounds of formula (I-1) and (I-2), respectively. Suitable solvents for this reaction may include dimethylformamide, p-dioxane, dimethylsulfoxide and the like. The reaction temperature preferably ranges from

80°C and 140°C.

In the process of Scheme 2, prior to the reaction with the compound of formula (V-1) or (V-2), the 4-position of the compound of formula (IV) may be hydrolyzed selectively using NaOH solution in an appropriate solvent. Suitable solvents for this reaction may include acetone, acetonitrile and tetrahydrofurane.

The compound of formula (VI-2) is prepared from the compound of formula(IX) by using a chlorinating agent such as phosphorous oxychloride.

The compounds of formula (VI-1) and (VI-2) prepared as above are novel and useful as intermediates for the preparation of the pyrimidine compounds of formula(I-1) or (I-2). Therefore, the present invention encompasses, within its scope, the novel compounds of formula (VI-1) or (VI-2) and processes for the preparation thereof.

The compounds of the present invention may be administered, either orally or intraperitoneally, in an effective amount ranging from 0.1 to 500 mg/kg, preferably from 1.0 to 100mg/kg into a subject patient per day.

The present invention further includes, within its scope, pharmaceutically acceptable salts of the compounds of formula(I-1) and (I-2). The non-toxic salts which fall within the scope of the present invention may include inorganic acid salts such as hydrochloride, sulfate, phosphate and nitrate, and organic acid salts such as tartrate, fumarate, citrate, mesylate and acetate.

The pharmaceutically acceptable salts may be prepared in accordance with a known method, e.g., by reacting the compounds of formula (I-1) or (I-2) with the acids mentioned above in the presence of a solvent, e.g., ethyl alcohol, dichloromethane, ethyl acetate and diethyl ether.

The present invention also includes within its scope pharmaceutical compositions comprising one or more of the inventive compounds as an active ingredient, in association with a pharmaceutically acceptable carrier, excipient and/or other additives, if necessary. The active ingredient

present in the composition may range from 0.1% to 99.9% by weight thereof.

The following Examples are given for the purpose of illustration only, and are not intended to limit the scope of the invention. 1-Methyl-1,2,3,4-tetrahydroisoquinoline, (R)-1-methyl-1,2,3,4-tetrahydroisoquinoline and (S)-1-methyl-1,2,3,4-tetrahydroisoquinoline were prepared by the same method as described in Preparation of WO 94/14795.

Preparation 1: Preparation of 7-methyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine

Step 1: 2-(3-thienyl)chloroethane

Thionyl chloride(17ml, 0.23mol) was added dropwise to a mixture solution of 2-(3-thienyl)ethanol(22.4ml, 0.2mol) and chloroform(60ml) while maintaining the temperature of the reaction system below 10°C, followed by stirring at room temperature for 1 hour. Then the reaction mixture was concentrated under a reduced pressure and distilled in vacuo to give 24g of the titled compound. (Yield : 81.5 %)

Step 2: 7-methyl-6,7-dihydrothieno[2,3-c]pyridine

To a solution of 2-(3-thienyl)chloroethane(20g, 0.136mol) prepared in the above Step 1 and anhydrous acetonitrile(350ml) was added tin(IV) chloride(20ml, 0.17mol) at room temperature. The reaction mixture was heated to reflux for 16 hours and cooled, to which water was added to remove excess tin(IV) chloride. And then the reaction mixture was washed by dichloromethane. The water layer was separated and basified with aqueous K_2CO_3 solution under ice-cooling and then extracted with dichloromethane. The combined dichloromethane layers were dried over magnesium sulfate and concentrated to give 10.56g of the titled compound. (Yield : 51%)

Step 3: 7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine

Sodium borohydride(4.4g, 116mmol) was added portionwise at room temperature to a mixture solution of 7-methyl-6,7-dihydrothieno[2,3-c]pyridine(10.5g, 69.4mmol) prepared in the above Step 2 and ethanol(100ml). After stirring for 1 hour, the reaction mixture was diluted with water, and extracted with dichloromethane. The combined dichloromethane layers were dried over magnesium sulfate and concentrated to give 10.34g of the titled compound. (Yield: 97%)

Preparation 2: Preparation of 2,4-dichloro-6-ethylpyrimidine

Step 1: 2-mercapto-6-ethylpyrimidine-4-one

To a solution of sodium methoxide(24g, 0.44mol) and ethanol(180ml) were added thiourea(15.22g, 0.2mol) and methyl propionylacetate(25.1ml, 0.2mol). After distillating solvent slowly, water(200ml) was added to the reaction mixture, which was then heated to reflux for 30 minutes. Active carbon was added to the reaction mixture, which was then stirred for 5 minutes and filtered. The filtrate was cooled to a room temperature and acidified by glacial acetic acid and the resulting solid was filtered and dried to give 29g of the titled compound. (Yield : 93%)

Step 2: 2,4-dihydroxy-6-ethylpyrimidine

A mixture solution of chloroacetic acid(33.3g, 0.352mol), water(400ml) and 2-mercapto-6-ethylpyrimidine-4-one(29g, 0.186mol) prepared in the above Step 1 was heated to reflux for 14 hours and cooled to a room temperature. To the reaction mixture was added conc. HCl(95ml) and the mixture was heated to reflux for 1 day. After the reaction mixture was cooled to a room temperature and concentrated under a reduced pressure, the residue was diluted with

water. After stirring for 2 hours, the resulting solid was filtered and dried to give 11.16g of the titled compound. (Yield : 43%)

Step 3: 2,4-dichloro-6-ethylpyrimidine

A mixture of phosphorous oxychloride(43ml), N,N-dimethyl aniline(6.6ml) and 2,4-dihydroxy-6-ethyl pyrimidine(11.12g, 79.3mmol) prepared in the above Step 2 was heated to reflux for 6 hours. The reaction mixture was cooled to a room temperature and diluted with dichloromethane. The diluted solution was added slowly to ice water, while maintaining the temperature of the reaction system below 10°C and the mixture was extracted with dichloromethane. The combined dichloromethane layers were dried over magnesium sulfate and concentrated to give 13.10g of the titled compound as an oily form. (Yield : 93.3%)

Preparation 3: Preparation of 2,4-dichloro-6-propyl-pyrimidine

In accordance with the same procedure as in Preparation 2, except that sodium methoxide(24g, 0.44mol), thiourea (15.22g, 0.2mol), ethyl butyrylacetate(31.6ml, 0.2mol) and ethanol(180ml) were used as starting materials, 10.5g of the titled compound was prepared as an oily form.

Preparation 4: Preparation of 2,4-dichloro-5-methyl-6-ethyl-pyrimidine

In accordance with the same procedure as in Preparation 2, except that sodium methoxide(24g, 0.44mol), thiourea (15.22g, 0.2mol), ethyl 2-propionyl propionate(31.6g, 0.2mol) and ethanol(180ml) were used as starting materials, 16.5g of the title compound was prepared as an oily form.

Example 1: Synthesis of 2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

Step 1: 4-(1-Methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine

A mixture solution of 2,4-dichloropyrimidine(3.0g, 20mmol), 1-methyl-1,2,3,4-tetrahydroisoquinoline(3.3g, 22mmol), triethylamine(3.4ml, 24.4mmol) and N,N-dimethyl formamide(20ml) was stirred for 5 hours, diluted with dichloromethane, washed with water several times. The dichloromethane layer was separated, dried over anhydrous sodium sulfate and then concentrated under a reduced pressure. The resulting residue was crystallized by silica gel column chromatography to give 1.5g of the titled compound. (Yield: 28.9%)

Step 2: 2-(2-Methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

2-Methyl-4-fluoroaniline(1.1ml, 10.2mmol) was added to a mixture solution of 4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.5g, 5.8mmol) and dimethylformamide(10ml). The reaction mixture was stirred for 3 hours at 110-120°C, cooled to a room temperature, diluted with dichloromethane, and then washed with water. The dichloromethane layer was separated, basified with aqueous sodium hydroxide, washed with water, dried and concentrated. The resulting residue was crystallized by silica gel column chromatography to give free base form of the titled compound. To a mixture solution of the free base form of the titled compound and ethyl ether was added aqueous hydrochloric acid and the resulting titled compound was filtered and dried in vacuo. Recrystallization from ethanol afforded 1.2g of the titled compound as a white crystalline solid.

Yield: 58.6%
M.P.: 160-163°C
^{1}H-NMR(DMSO-d_6): δ 1.49(d, 3H), 2.30(s, 3H), 2.90(m, 2H), 3.45(m, 1H), 4.20(bs, 1H), 5.40(bs, 1H), 6.05(d, 1H), 6.45(s, 1H), 6.90(m, 2H), 7.18(m, 4H), 7.88(m, 4H), 7.95(d, 1H).

Example 2: Synthesis of 6-methyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

Step 1: 6-methyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 6-methyl-2,4-dichloropyrimidine (6.52g, 40mmol), 1-methyl-1,2,3,4-tetrahydroisoquinoline (6.6g, 44mmol), triethylamine(6.8ml, 48.8mmol) and N,N-dimethylformamide(30ml) were used as starting materials, 5.5g of the titled compound was prepared. (Yield: 50.2%)

Step 2: 6-methyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(1.1ml, 10.2mmol) was added to a mixture solution of 6-methyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.5g, 5.5mmol) and dimethylformamide(10ml), 1.2g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.
Yield: 51.7%
M.P.: 177-179°C
^{1}H-NMR(DMSO-d_6): δ 1.42(d, 3H), 2.30(s, 3H), 2.32(s, 3H), 2.90(m, 2H), 3.50(qq, 1H), 4.22(qq, 1H), 5.42(qq, 1H), 6.70(s, 1H), 7.18(m, 6H), 7.63(m, 1H), 9.80(s, 1H), 13.30(bs, 1H).

Example 3: Synthesis of 6-methyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-(4-fluorophenylamino)pyrimidine hydrochloride

After 4-fluoroaniline(0.8ml, 8.4mmol) was added to a mixture solution of 6-methyl-4-(1-methyl-1,2,3,4-tetrahydro isoquinolin-2-yl)-2-chloropyrimidine(1.5g, 5.5mmol) and dimethylformamide (10ml), 1.5g of the title compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 70.7%

M.P.: 194-196°C

^{1}H-NMR(DMSO-d_6): δ 1.50(d, 3H), 2.38(s, 3H), 2.92(bs, 2H), 3.50(m, 1H), 4.30(qq, 1H), 5.58(qq, 1H), 6.70(s, 1H), 7.1-7.40(m, 6H), 7.60(m, 2H), 10.50(s, 1H), 13.10(bs, 1H).

Example 4: Synthesis of 6-methyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After N-methylaniline(0.9ml, 8.4mmol) was added to a mixture solution of 6-methyl-4-(1-methyl-1,2,3,4-tetrahydro isoquinolin-2-yl)-2-chloropyrimidine(1.5g, 5.5mmol) and dimethylformamide(10ml), 1.2g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1

Yield: 57.3%

M.P.: 170-172°C

^{1}H-NMR(DMSO-d_6): δ 1.40(d, 3H), 2.38(s, 3H), 2.95(m, 2H), 3.58(s, 3H), 3.60(bs, 1H), 4.30(qq, 1H), 5.50(qq, 1H), 6.70(s, 1H), 7.10-7.38(m, 4H), 7.40-7.60(m, 5H), 12.00(s, 1H).

Example 5: Synthesis of 6-ethyl-2-(2-methyl-4-fluorophenyl-amino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

Step 1: 6-ethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 1-methyl-1,2,3,4-tetrahydroisoquinoline(4.1g, 27.8mmol), triethylamine(4.7ml, 33.7mmol), N,N-dimethylformamide(20ml) and 6-ethyl-2,4-dichloropyrimidine(4.9g, 27.7mmol) obtained in Preparation 2 were used as starting materials, 5.58g of the titled compound was prepared. (Yield: 70%)

Step 2: 6-ethyl-2-(2-methyl-4-fluorphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.77ml, 6.93mmol) was added to a mixture solution of 6-ethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.0g, 3.47mmol) and dimethylformamide(5ml), 0.92g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 64%

M.P.: 172-174°C

^{1}H-NMR($CDCl_3$): δ 1.38-1.60(tt+dd, 6H), 2.43(ss, 3H), 2.68-3.06(m, 4H), 3.76(m, 1H), 3.94(m, 1H), 5.33(qq, 1H), 6.01(ss, 1H), 6.85-7.30(m, 6H), 7.58(t, 1H), 9.83(s, 1H), 14.00(s, 1H).

Example 6: Synthesis of 6-ethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 4-fluoroaniline(0.38ml, 4.01mmol) was added to a mixture solution of 6-ethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine (0.57g, 1.98mmol) and dimethylformamide (5ml), 0.17g of the titled compound was obtained in accordance with the same procedure as in Step 2

of Example 1
Yield: 22%
M.P.: 156-158°C
^{1}H-NMR(DMSO-d_6): δ 1.29(t, 3H), 1.49(d, 3H), 2.65(q, 2H), 2.93-2.96(m, 2H), 3.70(m, 1H), 4.05-4.60(m, 1H), 5.60(qq, 1H), 7.10-7.55(m, 6H), 7.60-7.65(m, 2H), 10.60(s, 1H), 10.90(s, 1H).

Example 7: Synthesis of 6-ethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After N-methylaniline(0.46ml, 4.25mmol) was added to a mixture solution of 6-ethyl-4-(1-methyl-1,2,3,4-tetrahydro isoquinolin-2-yl)-2-chloropyrimidine(0.61g, 2.12mmol) and dimethylformamide(5ml), 0.50g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.
Yield: 60%
M.P.: 109-111°C
^{1}H-NMR(DMSO-d_6): δ 1.22(t, 3H), 1.43(dd, 3H), 2.78(q, 2H), 2.95(s, 1H), 3.30(m, 1H), 3.62(s, 3H), 4.37(mm, 1H), 5.70(q, 1H), 6.70(s, 1H), 7.06-7.58(m, 9H), 12.15(s, 1H).

Example 8: Synthesis of 6-ethyl-2-(2-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methylaniline(0.46ml, 4.31mmol) was added to a mixture solution of 6-ethyl-4-(1-methyl-1,2,3,4-tetrahydro isoquinolin-2-yl)-2-chloropyrimidine(0.61g, 2.12mmol) and dimethylformamide(5ml), 0.52g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.
Yield: 62%
M.P.: 78-81°C
^{1}H-NMR(DMSO-d_6): δ 1.80-2.20(m, 6H), 2.90(s, 3H), 3.07(s,

1H), 3.24(q, 2H), 3.43(s, 1H), 3.96(s, 3H), 4.16(mm, 1H), 4.88(mm, 1H), 6.08(qq, 1H), 7.23(ss, 1H), 7.64-7.90(m, 7H), 8.32(t, 1H), 10.50(s, 1H), 14.10(s, 1H).

Example 9: Synthesis of 2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propyl-pyrimidine hydrochloride

Step 1: 4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propyl-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 1-methyl-1,2,3,4-tetrahydroiso quinoline(1.6g, 10.9mmol), triethylamine(1.6ml, 11.5mmol), N,N-dimethylformamide(20ml) and 2,4-dichloro-6-propyl pyrimidine(1.8g, 9.4mmol) obtained in Preparation 3 were used as starting materials, 1.6g of the titled compound was prepared. (Yield: 56.4%)

Step 2: 2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propylpyrimidine hydrochloride

After 4-fluoro-2-methylaniline(0.35ml, 3.15mmol) was added to a mixture solution of 4-(1-methyl-1,2,3,4 -tetra hydroisoquinolin-2-yl)-6-propyl-2-chloropyrimidine(0.5g, 1.66mmol) and dimethylformamide(5ml), 0.2g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 28.2%

M.P.: 95-97°C

^{1}H-NMR(DMSO-d_6): δ 1.00(t, 3H), 1.50(dd, 3H), 1.81(q, 2H), 2.35(s, 3H), 2.70(t, 2H), 2.94(bd, 2H), 3.60(mm, 1H), 4.30(dd, 1H), 5.55(dd, 1H), 6.70(s, 1H), 7.22(bs, 6H), 7.75(bs, 1H), 9.90(s, 1H), 13.30(bs, 1H).

Example 10: Synthesis of 4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propyl-2-(4-fluorophenylamino)pyrimidine hydrochloride

After 4-fluoroaniline(0.27ml, 2.85mmol) was added to a mixture solution of 4-(1-methyl-1,2,3,4-tetrahydroiso quinolin-2-yl)-6-propyl-2-chloropyrimidine(0.5g, 1.66mmol) and dimethylformamide(5ml), 0.3g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 43.8%

M.P.: 100-105°C

^{1}H-NMR(DMSO-d6): δ 0.96(t, 3H), 1.54(m, 3H), 1.75(q, 2H), 2.60(t, 2H), 2.96(m, 2H), 3.62(mm, 1H), 4.35(qq, 1H), 5.60(qq, 1H), 6.70(d, 1H), 7.00-7.40(m, 6H), 7.62(m, 2H).

Example 11: 2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propylpyrimidine hydrochloride

After N-methylaniline(0.27ml, 2.49mmol) was added to a mixture solution of 4-(1-methyl-1,2,3,4-tetrahydroiso quinolin-2-yl)-6-propyl-2-chloropyrimidine(0.5g, 1.66mmol) and dimethylformamide (5ml), 0.5g of the title compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 73.6%

M.P.: 92-94°C

^{1}H-NMR(DMSO-d_6): δ 0.96(t, 3H), 1.46(dd, 3H), 1.59(q, 2H), 2.57(t, 2H), 2.90(bd, 2H), 3.50(mm+d, 4H), 4.35(qq, 1H), 5.56(qq, 1H), 6.65(d, 1H), 7.00-7.70(m, 9H).

Example 12: Synthesis of 5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

Step 1: 5,6-Dimethyl-2,4-dichloropyrimidine

A mixture solution of 5,6-dimethyl-2,4-dihydroxy pyrimidine(72g, 0.51mol), phosphorus oxychloride(250ml) and N,N-dimethylaniline(41ml) was heated to reflux for 3 hours. After cooling to room temperature, the reaction mixture was added slowly to ice water. The resulting solid was filtered and recrystallized from dichloromethane to give 54.3g of the titled compound. (Yield: 60%)

Step 2: 5,6-Dimethyl-4-(1-methyl-1,2,3,4-tetrahydroiso quinolin-2-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 1-methyl-1,2,3,4-tetrahydroiso quinoline(3.9g, 26.4mmol) and 5,6-dimethyl-2,4-dichloro pyrimidine(4.3g, 24mmol) prepared in the above Step 1 were used as starting materials, 4.17g of the titled compound was prepared. (Yield: 60.4%)

Step 3: 5,6-Dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(1.1ml, 9.9mmol) was added to a mixture solution of 5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.4g, 4.8mmol) prepared in the above Step 2 and dimethylformamide (10ml), 1.35g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 68%

M.P.: 201-205°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.17(s, 3H), 2.36(s, 3H), 2.89(bd, 1H), 3.08(m, 1H), 3.59(m, 1H), 4.19(bd, 1H), 5.38(q, 1H), 7.34(m, 6H), 7.60(m, 2H), 10.40(s, 1H).

Example 13: Synthesis of (R)-5,6-Dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroiso-

quinolin-2-yl)pyrimidine hydrochloride

Step 1: (R)-5,6-Dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that (R)-1-methyl-1,2,3,4-tetrahydroisoquinoline(3.9g, 26.4mmol) and 5,6-dimethyl-2,4-dichloropyridine(4.3g, 24mmol) were used as starting materials, 4.35g of the titled compound was prepared. (Yield: 63%)

Step 2: (R)-5,6-Dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(1.1ml, 9.9mmol) was added to a mixture solution of (R)-5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.4g, 4.8mmol) obtained in the above Step 1 and dimethylformamide(10ml), 1.10g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 55.5%

M.P.: 203-205°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.17(s, 3H), 2.36(s, 3H), 2.89(bd, 1H), 3.08(m, 1H), 3.59(m, 1H), 4.19(bd, 1H), 5.38(q, 1H), 7.34(m, 6H), 7.60(m, 2H), 10.40(s, 1H).

Example 14: Synthesis of (S)-5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

Step 1: (S)-5,6-Dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that (S)-1-methyl-1,2,3,4-tetrahydroiso-

quinoline(3.9g, 26.4mmol) and 5,6-dimethyl-2,4-dichloropyridine(4.3g, 24mmol) were used as starting materials, 4.2g of the titled compound was prepared. (Yield: 60.8%)

Step 2: (S)-5,6-Dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(1.1ml, 9.9mmol) was added to a mixture solution of (S)-5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.4g, 4.8mmol) obtained in the above Step 1 and dimethylformamide (10ml), 0.90g of the title compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 45.5%

M.P.: 202-204°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.17(s, 3H), 2.36(s, 3H), 2.89(bd, 1H), 3.08(m, 1H), 3.59(m, 1H), 4.19(bd, 1H), 5.38(q, 1H), 7.34(m, 6H), 7.60(m, 2H), 10.40(s, 1H).

Example 15: Synthesis of 5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After 4-fluoroaniline(1.0ml, 10mmol) was added to a mixture solution of 5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.4g, 4.8mmol) and dimethylformamide(10ml), 1.32g of the title compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 69%

M.P.: 205-208°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.17(s, 3H), 2.36(s, 3H), 2.89(bd, 1H), 3.08(m, 1H), 3.59(m, 1H), 4.19(bd, 1H), 5.38(q, 1H), 7.34(m, 6H), 7.60(m, 2H), 10.40(s, 1H).

Example 16: Synthesis of (R)-5,6-dimethyl-2-(4-fluorophenyl-amino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After 4-fluoroaniline(1ml, 10mmol) was added to a mixture solution of (R)-5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.4g, 4.8mmol) and dimethylformamide(10ml), 1.20g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 62.7%

M.P.: 205-207°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.17(s, 3H), 2.36(s, 3H), 2.89(bd, 1H), 3.08(m, 1H), 3.59(m, 1H), 4.19(bd, 1H), 5.38(q, 1H), 7.34(m, 6H), 7.60(m, 2H), 10.40(s, 1H).

Example 17: Synthesis of (S)-5,6-dimethyl-2-(4-fluorophenyl-amino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After 4-fluoroaniline(1ml, 10mmol) was added to a mixture solution of (S)-5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.4g, 4.8mmol) and dimethylformamide(10ml), 1.50g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 78.3%

M.P.: 204-206°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.17(s, 3H), 2.36(s, 3H), 2.89(bd, 1H), 3.08(m, 1H), 3.59(m, 1H), 4.19(bd, 1H), 5.38(q, 1H), 7.34(m, 6H), 7.60(m, 2H), 10.40(s, 1H).

Example 18: Synthesis of 5,6-dimethyl-2-(N-methylphenyl-amino)-4-(1- methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After N-methylaniline(1.5ml, 14mmol) was added to a

mixture solution of 5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.9g, 6.6mmol) and dimethylformamide(10ml), 0.25g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 9%

M.P.: 220-222°C

^{1}H-NMR($CDCl_3$): δ 1.34(d, 3H), 2.19(s, 3H), 2.77(s, 3H), 2.93(bd, 2H), 3.48(m, 1H), 3.98(s, 3H), 4.04(bd, 1H), 5.02(m, 1H), 6.88(m, 1H), 7.16-7.42(m, 5H), 7.58(m, 3H), 13.42(bd, 1H).

Example 19: Synthesis of (R)-5,6-Dimethyl-2-(N-methylphenyl-amino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After N-methylaniline(1.04ml, 9.6mmol) was added to a mixture solution of (R)-5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.4g, 4.8mmol) and dimethyl formamide(10ml), 0.55g of the title compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 29%

M.P.: 221-223°C

^{1}H-NMR($CDCl_3$): δ 1.34(d, 3H), 2.19(s, 3H), 2.77(s, 3H), 2.93(bd, 2H), 3.48(m, 1H), 3.98(s, 3H), 4.04(bd, 1H), 5.02(m, 1H), 6.88(m, 1H), 7.16-7.42(m, 5H), 7.58(m, 3H), 13.42(bd, 1H).

Example 20: Synthesis of (S)-5,6-dimethyl-2-(N-methylphenyl-amino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After N-methylaniline(1.04ml, 9.6mmol) was added to a mixture solution of (S)-5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(1.4g, 4.8mmol) and dimethylformamide(10ml), 0.70g of the titled

compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 37%

M.P.: 220-223°C

^{1}H-NMR($CDCl_3$): δ 1.34(d, 3H), 2.19(s, 3H), 2.77(s, 3H), 2.93(bd, 2H), 3.48(m, 1H), 3.98(s, 3H), 4.04(bd, 1H), 5.02(m, 1H), 6.88(m, 1H), 7.16-7.42(m, 5H), 7.58(m, 3H), 13.42(bd, 1H).

Example 21: Synthesis of 5,6-dimethyl-2-(phenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After aniline(0.53ml, 5.5mmol) was added to a mixture solution of 5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroiso quinolin-2-yl)-2-chloropyrimidine(0.72g, 2.5mmol) and dimethylformamide(5ml), 0.21g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 22%

M.P.: 243-245°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.15(s, 3H), 2.34(s, 3H), 2.90(bd, 1H), 3.12(m, 1H), 3.64(m, 1H), 4.25(m, 1H), 5.42(q, 1H), 7.21(m, 5H), 7.43(m, 2H), 7.56(m, 2H), 10.30(s, 1H), 13.35(bd, 1H).

Example 22: Synthesis of (R)-5,6-Dimethyl-2-(4-phenyl-amino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After aniline(0.53ml, 5.5mmol) was added to a mixture solution of (R)-5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydro isoquinolin-2-yl)-2-chloropyrimidine(0.72g, 2.5mmol) and dimethylformamide(5ml), 0.25g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 26%

M.P.: 243-246°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.15(s, 3H), 2.35(s, 3H), 2.89(bd, 1H), 3.12(m, 1H), 3.64(m, 1H), 4.25(m, 1H), 5.42(q, 1H), 7.20(m, 5H), 7.43(m, 2H), 7.56(m, 2H), 10.30(s, 1H), 13.35(bd, 1H).

Example 23: Synthesis of (S)-5,6-Dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After aniline(0.53ml, 5.5mmol) was added to a mixture solution of (S)-5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(0.72g, 2.5mmol) and dimethylformamide(5ml), 0.20g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 21%

M.P.: 243-245°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.15(s, 3H), 2.34(s, 3H), 2.89(bd, 1H), 3.12(m, 1H), 3.64(m, 1H), 4.25(m, 1H), 5.42(q, 1H), 7.20(m, 5H), 7.43(m, 2H), 7.56(m, 2H), 10.30(s, 1H), 13.35(bd, 1H).

Example 24: Synthesis of 5,6-Dimethyl-2-(2-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After 2-methylaniline(1.0ml, 9.6mmol) was added to a mixture solution of 5,6-Dimethyl-4-(1-methyl-1,2,3,4-tetra hydroisoquinolin-2-yl)-2-chloropyrimidine(1.34g, 4.6mmol) and dimethylformamide(5ml), 0.65g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 36%

M.P.: 94-96°C

^{1}H-NMR(DMSO-d_6): δ 1.52(d, 3H), 2.17(s, 3H), 2.30(s, 3H), 2.37(s, 3H), 2.82(d, 1H), 3.01(m, 1H), 3.54(t, 1H), 4.15(bd,

1H), 5.31(t, 1H), 7.15(m, 5H), 7.30(m, 2H), 7.73(d, 1H), 9.55(s, 1H), 13.73(bd, 1H).

Example 25: Synthesis of 5,6-dimethyl-2-(4-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After p-toluidine(0.45g, 4.20mmol) was added to a mixture solution of 5,6-dimethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(0.80g, 2.78mmol) and dimethylformamide(5ml), 0.30g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 27%

M.P.: 243-245°C

^{1}H-NMR($CDCl_3$): δ 1.64(d, 3H), 2.18(s, 3H), 2.36(s, 3H), 2.44(s, 3H), 2.87(bd, 1H), 3.28(tt, 1H), 3.60(tt, 1H), 4.30(bd, 1H), 5.42(q, 1H), 7.08-7.23(m, 6H), 7.52(d, 2H), 10.20(s, 1H), 14.10(bs, 1H).

Example 26: Synthesis of 5-methyl-6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

Step 1: 5-Methyl-6-ethyl-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 1-methyl-1,2,3,4-tetrahydroisoquinoline (2.3g, 15.6mmol) and 2,4-dichloro-5-methyl-6-ethylpyrimidine (2.7g, 14.1mmol) prepared in Preparation 4 were used as starting materials, 2.3g of the titled compound was prepared. (Yield: 54%)

Step 2: 5-Methyl-6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 4-fluoro-2-methylaniline(0.55ml, 4.95mmol) was added to a mixture solution of 5-methyl-6-ethyl-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(0.80g, 2.65mmol) and dimethylformamide (5ml), 0.25g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 22.1%

M.P.: 171-173°C

^{1}H-NMR(DMSO-d_6): δ 1.20(t, 3H), 1.46(d, 3H), 2.16(s, 3H), 2.22(s, 3H), 2.68(q, 2H), 2.95(m, 1H), 3.48(t, 1H), 4.12(d, 2H), 5.20(q, 1H), 6.90-7.30(m, 6H), 7.58(m, 1H).

Example 27: Synthesis of 5-methyl-6-ethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl) pyrimidine hydrochloride

After 4-fluoroaniline(0.50ml, 5.28mmol) was added to a mixture solution of 5-methyl-6-ethyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine (0.80g, 2.65mmol) and dimethylformamide(5ml), 0.55g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 50.3%

M.P.: 198-200°C

^{1}H-NMR(DMSO-d_6): δ 1.20(t, 3H), 1.56(d, 3H), 2.18(s, 3H), 2.56(q,2H), 2.81(bd, 1H), 3.05(m, 1H), 3.58(t, 1H), 4.41(d, 1H), 5.38(q, 1H), 7.00-7.40(m, 6H), 7.58(m, 2H).

Example 28: Synthesis of 5-methyl-6-ethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After N-methylaniline(0.44ml, 4.06mmol) was added to a mixed solution of 5-methyl-6-ethyl-4-(1-methyl-1,2,3,4-tetra hydroisoquinolin-2-yl)-2-chloropyrimidine(0.80g, 2.65mmol) and dimethylformamide(5ml), 0.60g of the titled compound was obtained in accordance with the same procedure as in Step 2

of Example 1.
Yield: 55.4%
M.P.: 214-216°C
^{1}H-NMR(DMSO-d_6): δ 1.90(t, 3H), 1.46(d, 3H), 2.18(s, 3H), 2.67(q, 2H), 2.79(bs, 2H), 2.90-3.18(m, 1H), 3.40-3.60(s+m, 4H), 4.18(dd, 1H), 5.25(q, 1H), 7.05-7.20(s, 4H), 7.32-7.58(m, 5H).

Example 29: Synthesis of 2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta-[d]pyrimidine hydrochloride

Step 1: 2-Amino-4-hydroxycyclopenta[d]pyrimidine

A solution of 2-ethoxycarbonyl cyclopentanone (114ml, 0.77mol) and N,N-dimethylformamide (40ml) was added dropwise to a mixture solution of sodium methoxide(83.2g, 0.44mol) and N,N-dimethylformamide(80ml), while maintaining the temperature of the reaction system below 0°C. A solution of guanidine HCl salt(81g, 0.85mol) and methanol(127ml) was added to the above reaction mixture and then was heated to reflux for 14 hours. The reaction mixture was neutralized by conc. HCl and the resulting solid was filtered and dried under reduced pressure to give 20.69g of titled compound. (Yield: 18%)

Step 2: 2,4-Dihydroxycyclopenta[d]pyrimidine

To a mixture solution of 20% HCl(62ml) and 2-amino-4-hydroxycyclopenta[d]pyrimidine (20.6g, 0.136mol) prepared in the above Step 1 was added aqueous solution of sodium nitrite (19.4g) for 4 hours while keeping the temperature of the reaction system at 70°C. The reaction mixture was cooled to 0°C and the resulting solid was filtered, dried under reduced pressure to give 15.43g of titled compound. (Yield: 74.6%)

Step 3: 2,4-dichlorocyclopenta[d]pyrimidine

A mixture solution of phosphorous oxychloride(49ml), N,N-dimethylaniline(8.0ml) and 2,4-dihydroxycyclopenta[d] pyrimidine(15.4g, 0.1mol) prepared in the above Step 2 was heated to reflux for 3 hours and cooled to a room temperature. After the reaction mixture was diluted with dichloromethane, the diluted solution was added to ice water, while maintaining the temperature of the reaction system below 10°C. The reaction mixture was extracted with dichloromethane, dried over anhydrous sodium sulfate and concentrated in vacuo to give 2.8g of titled compound as an oily form. (Yield: 15%)

Step 4: 4-(1-Methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chlorocyclopenta[d]pyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 1-methyl-1,2,3,4-tetrahydroisoquinoline(1.7g, 11.55mmol) and 2,4-dichlorocyclopenta[d] pyrimidine(2.0g, 10.5mmol) prepared in the above Step 3 were used as starting materials, 1.95g of the title compound was prepared. (Yield: 62%)

Step 5: 2-(2-Methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride

After 4-fluoro-2-methylaniline(0.40ml, 3.60 mmol) was added to a mixture solution of 4-(1-methyl-1,2,3,4-tetra hydroisoquinolin-2-yl)-2-chlorocyclopenta[d]pyrimidine (0.50g, 1.70mmol) and dimethylformamide(5ml), 0.15g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 20.8%

M.P.: 110-112°C

^{1}H-NMR(DMSO-d_6): δ 1.50(t, 3H), 2.12(m, 2H), 2.25(s, 3H),

2.93(bd, 3H), 3.10(m, 2H), 3.42(bd, 2H), 3.70(bd, 1H), 4.40(bd, 1H), 5.78(bd, 1H), 7.22(m, 6H), 7.50(m, 5H), 7.60(m, 1H), 9.80(s, 1H), 13.32(bd, 1H).

Example 30: Synthesis of 2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]-pyrimidine hydrochloride

After 4-fluoroaniline(0.40ml, 4.2mmol) was added to a mixture solution of 4-(1-methyl-1,2,3,4-tetrahydroisoquinoline-2-yl)-2-chlorocyclopenta[d]pyrimidine(0.60g, 2.0mmol) and dimethylformamide(5ml), 0.11g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 13.4%

M.P.: 220-222°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.10(bd, 2H), 3.01(bd, 4H), 3.18(m, 2H), 3.60(bd, 1H), 4.45(bd, 1H), 5.64(bd, 1H), 7.30(m, 6H), 7.62(m, 2H), 10.42(s, 1H), 13.15(bd, 1H).

Example 31: Synthesis of 2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4- tetrahydroisoquinolin-2-yl)cyclopenta-[d]pyrimidine hydrochloride

After N-methylaniline(0.20ml, 1.90mmol) was added to a mixture solution of 4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chlorocyclopenta[d]pyrimidine (0.51g, 1.70mmol) and dimethylformamide(5ml), 0.20g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 29%

M.P.: 105-107°C

^{1}H-NMR(DMSO-d_6): δ 1.42(bd, 3H), 2.10(m, 2H), 2.87(m, 5H), 3.10(m, 2H), 3.58(s, 3H), 4.38(bd, 1H), 5.53(q, 1H), 7.21(m, 4H), 7.48(m, 5H), 12.62(bd, 1H).

Example 32: Synthesis of 2-(2-methyl-4-fluorophenylamino)-

4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

Step 1: 2,4-Dihydroxy-5,6,7,8-tetrahydroquinazoline

A mixture solution of 2,4-dihydroxyquinazoline(39.2g, 0.24mol), platinium oxide(4g) and trifluoroacetic acid(300ml) was hydrogenated by Parr reactor for 2 hours. Platinium was filtered and the filtrate was concentrated, diluted with water, and basified with 1N-NaOH solution. The resulting solid was filtered and dried to give 13.76g of the titled compound. (Yield: 34.5%)

Step 2: 2,4-Dichloro-5,6,7,8-tetrahydroquinazoline

2,4-Dihydroxy-5,6,7,8-tetrahydroquinazoline(3.4g, 20mmol) prepared in the above Step 1 was suspended in a mixture solution of phosphorous oxychloride(10mL) and N,N-dimethylaniline(0.8ml). The reaction mixture was heated to reflux for 3 hours and cooled to room temperature. The reaction mixture was added to ice water while maintaining the temperature of the reaction system below 10°C and the resulting solid was filtered, dried under reduced pressure to give 3.26g of the titled compound. (Yield: 80%)

Step 3: 4-(1-Methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloro-5,6,7,8-tetrahydroquinazoline

A mixture solution of 1-methyl-1,2,3,4-tetrahydroiso quinoline(2.6g, 17.4mmol), triethylamine(2.8mL), N,N-dimethylformamide (20ml) and 2,4-dichloro-5,6,7,8-tetra hydroquinazoline(3.2g, 15.8mmol) prepared in the above Step 2 were stirred at 80°C for 3 hours and cooled. The reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated. The residue was purified

with silica gel column chromatography to give 3.1g of the titled compound. (Yield: 62.5%)

Step 4: 2-(2-Methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

After 4-fluoro-2-methylaniline(0.60ml, 5.4mmol) was added to a mixture solution of 4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloro-5,6,7,8-tetrahydroquinazoline(0.75g, 2.40mmol) and dimethylformamide(5ml), 0.58g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.
Yield: 55%
M.P.: 190-193°C
^{1}H-NMR(DMSO-d_6): δ 1.53(d, 3H), 1.60-1.96(m, 3H), 2.34(s, 3H), 2.55(bd, 2H), 2.75(bd, 4H), 2.98(m, 1H), 3.54(m, 1H), 4.25(bd, 1H), 5.36(q, 1H), 7.12-7.31(m, 6H), 7.60(m, 1H), 9.69(s, 1H).

Example 33: Synthesis of 2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinoline-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

After N-methylaniline(0.50ml, 4.8mmol) was added to a mixture solution of 4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloro-5,6,7,8-tetrahydroquinazoline(0.75g, 2.40mmol) and dimethylformamide(5ml), 0.26g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.
Yield: 26%
M.P.: 207-210°C
^{1}H-NMR(DMSO-d_6): δ 1.42(d, 3H), 1.53-1.96(m, 3H), 2.57(bd, 1H), 2.80(m, 5H), 2.95(m, 1H), 3.45(bd, 1H), 3.60(s, 3H), 4.18(bd, 1H), 5.25(q, 1H), 7.16(m, 3H), 7.50(m, 6H), 12.10(s, 1H).

Example 34: Synthesis of 6-methyl-2-(2-methyl-4-fluoro-phenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

Step 1: 6-methyl-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 6-methyl-2,4-dichloropyrimidine (3.26g, 20mmol), 1,2,3,4-tetrahydroisoquinoline(2.6ml, 20.5mmol), triethylamine(3.4ml, 24.4mmol) and N,N-dimethyl formamide (10ml) were used as starting materials, 3.1g of the titled compound was prepared. (Yield: 59.7%)

Step 2: 6-Methyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.8ml, 7.2mmol) was added to a mixture solution of 6-methyl-4-(1,2,3,4-tetra hydroisoquinoline-2-yl)-2-chloropyrimidine(1.0g, 3.8mmol) and dimethylformamide(10ml), 0.85g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 58%

M.P.: 183-185°C

^{1}H-NMR($CDCl_3$): δ 2.41(s, 3H), 2.48(d, 3H), 2.88(t, 1H), 3.02(t, 1H), 3.75(t, 1H), 3.91(t, 1H),4.67(s, 1H), 4.78(s, 1H), 6.00(d, 1H), 6.90-7.30(m, 5H), 7.58(m, 1H), 9.75(s, 1H), 14.20(bs, 1H).

Example 35: Synthesis of 6-methyl-2-(4-fluorophenyl-amino)-4-(1,2,3,4-tetrahydroisoquinoline-2-yl)pyrimidine hydrochloride

After 4-fluoroaniline(0.7ml, 7.4mmol) was added to a mixture solution of 6-methyl-4-(1,2,3,4-tetrahydroiso quinoline-2-yl)-2-chloropyrimidine(1.0g, 3.8mmol) and

dimethylformamide(10ml), 0.6g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 42.6%

M.P.: 238-240°C

^{1}H-NMR(CDCl3): δ 2.45(d, 3H), 2.90-3.10(m, 2H), 3.78(t, 1H), 4.05(t, 1H), 4.70(s, 1H), 4.92(t, 1H), 6.05(d, 1H), 6.90-7.30(m, 6H), 7.60(m, 2H), 10.40(s, 1H), 13.80(bs, 1H).

Example 36: Synthesis of 6-methyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After N-methylaniline(0.61ml, 5.48mmol) was added to a mixture solution of 6-methyl-4-(1,2,3,4-tetrahydroiso quinolin-2-yl)-2-chloropyrimidine(0.95g, 3.65mmol) and dimethylformamide(10ml), 0.7g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 52.3%

M.P.: 85-95°C

^{1}H-NMR($CDCl_3$): δ 2.75(s, 3H), 2.99(s, 3H), 3.70(m, 2H), 3.87(s, 3H), 4.51(s, 1H), 4.65(s, 1H), 5.30(bs, 1H), 6.08(d, 1H), 6.88(d, 1H), 7.05-7.60(m, 8H), 13.05(s, 1H).

Example 37: Synthesis of 6-ethyl-2-(2-methyl-4-fluorophenyl-amino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

Step 1: 6-Ethyl-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 1,2,3,4-tetrahydroisoquinoline(3.5ml, 28mmol), triethylamine(3.9ml, 28mmol), N,N-dimethylformamide (20ml) and 6-ethyl-2,4-dichloropyrimidine(4.9g, 27.7mmol) prepared in Preparation 2 were used as starting materials,

5.0g of the titled compound was prepared. (Yield: 66%)

Step 2: 6-Ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.57ml, 5.13mmol) was added to a mixture solution of 6-ethyl-4-(1,2,3,4-tetra hydroisoquinoline-2-yl)-2-chloropyrimidine(0.7g, 2.56mmol) and dimethylformamide(5ml), 0.55g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 54%

M.P.: 223-225°C

^{1}H-NMR($CDCl_3$): δ 1.36(qq, 3H), 2.35(s, 3H), 2.69(tt, 2H), 2.90(tt, 2H), 3.77(tt, 2H), 4.66(ss, 2H), 5.93(d, 2H), 6.72-7.30(m, 6H), 7.50(dd, 1H), 9.80(s, 1H), 14.00(s, 1H).

Example 38: Synthesis of 6-ethyl-2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 4-fluoroaniline(0.50ml, 5.28mmol) was added to a mixture solution of 6-ethyl-4-(1,2,3,4-tetrahydroiso-quinoline-2-yl)-2-chloropyrimidine(0.7g, 2.56mmol) and dimethylformamide(5ml), 0.41g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 42%

M.P.: 203-206°C

^{1}H-NMR($CDCl_3$): δ 1.42(tt, 3H), 2.74(qq, 2H), 3.02(tt, 2H), 3.93(tt, 2H), 4.82(ss, 2H), 6.03(ss, 2H), 7.00-7.32(m, 6H), 7.54-7.64(m, 2H), 10.60(s, 1H), 13.80(s, 1H).

Example 39: Synthesis of 6-ethyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After N-methylaniline(0.54ml, 5.15mmol) was added to a

mixture solution of 6-ethyl-4-(1,2,3,4-tetrahydroiso quinoline-2-yl)-2-chloropyrimidine(0.7g, 2.56mmol) and dimethylformamide(5ml), 0.56g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 57%

M.P.: 98-100°C

^{1}H-NMR($CDCl_3$): δ 1.24-1.40(m, 3H), 2.83(tt, 2H), 3.16-3.24(m, 2H), 3.65(tt, 2H), 3.89(s, 3H), 4.53(ss, 2H), 6.00(ss, 1H), 6.85(d, 1H), 7.05-7.55(m, 8H), 13.40(s, 1H).

Example 40: Synthesis of 6-ethyl-2-(2-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methylaniline(0.55ml, 5.15mmol) was added to a mixture solution of 6-ethyl-4-(1,2,3,4-tetrahydroiso-quinoline-2-yl)-2-chloropyrimidine(0.7g, 2.56mmol) and dimethylformamide(5ml), 0.23g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 24%

M.P.: 153-155°C

^{1}H-NMR($CDCl_3$): δ 1.37-1.47(m, 3H), 2.50(s, 3H), 2.74-2.76(m, 2H), 2.97(tt, 2H), 3.87(tt, 2H), 4.76(ss, 2H), 5.98(ss, 1H), 7.10-7.28(m, 7H), 7.70(t, 1H), 9.82(s, 1H), 14.17(s, 1H).

Example 41: Synthesis of 5,6-dimethyl-2-(2-methyl-4-fluoro-phenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

Step 1: 5,6-dimethyl-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 1,2,3,4-tetrahydroisoquinoline(2.9g, 23mmol) and 5,6-dimethyl-2,4-dichloropyrimidine(3.8g, 21mmol) and 1,2,3,4-tetrahydroisoquinoline(2.9g, 23mmol)

prepared in Step 1 of Example 12 were used as starting materials, 3.95g of the titled compound was prepared. (Yield: 68.7%)

Step 2: 5,6-Dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 4-fluoro-2-methylaniline(0.8ml, 7mmol) was added to a mixture solution of 5,6-dimethyl-4-(1,2,3,4-tetrahydro isoquinoline-2-yl)-2-chloropyrimidine(1.0g, 3.6mmol) and dimethylformamide(10ml), 0.58g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 44%

M.P.: 190-193°C

^{1}H-NMR(DMSO-d_6): δ 2.17(s, 3H), 2.30(s, 3H), 2.36(s, 3H), 2.90(t, 2H), 3.80(t, 1H), 4.75(s, 2H), 7.08-7.19(m, 6H), 7.70(m, 1H), 9.63(s, 1H), 13.62(s, 1H).

Example 42: Synthesis of 5,6-dimethyl-2-(4-fluorophenyl-amino)-4-(1,2,3,4- tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 4-fluoroaniline(0.7ml, 7.4mmol) was added to a mixture solution of 5,6-dimethyl-4-(1,2,3,4-tetrahydroiso quinolin-2-yl)-2-chloropyrimidine(1.0g, 3.6mmol) and dimethylformamide(5ml), 0.67g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 48%

M.P.: 251-253°C

^{1}H-NMR(DMSO-d_6): δ 2.23(s, 3H), 2.41(s, 3H), 3.02(t, 2H), 3.94(t, 2H), 4.87(s, 2H), 7.35(m, 6H), 7.65(m, 2H), 10.39(s, 1H), 13.20(bd, 1H).

Example 43: Synthesis of 5,6-dimethyl-2-(N-methylphenyl-amino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine

hydrochloride

After N-methylaniline(0.84ml, 7.8mmol) was added to a mixture solution of 5,6-dimethyl-4-(1,2,3,4-tetrahydroiso quinolin-2-yl)-2-chloropyrimidine(1.0g, 3.6mmol) and dimethylformamide(5ml), 0.55g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.
Yield: 39%
M.P.: 58-60°C
^{1}H-NMR(DMSO-d_6): δ 2.14(s, 3H), 2.45(s, 3H), 2.83(t, 2H), 3.64(s, 3H), 3.71(t, 2H), 4.66(s, 2H), 7.07-7.15(m, 4H), 7.38-7.54(m, 5H), 12.40(s, 1H).

Example 44: Synthesis of 5-methyl-6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

Step 1: 5-Methyl-6-ethyl-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 1,2,3,4-tetrahydroisoquinoline(3.5ml, 28mmol) and 2,4-dichloro-5-methyl-6-ethylpyrimidine(4.9g, 27.7mmol) prepared in Preparation 4 were used as starting materials, 5.0g of the titled compound was prepared.
(Yield: 66%)

Step 2: 5-Methyl-6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 4-fluoro-2-methylaniline(0.5ml, 3.6mmol) was added to a mixture solution of 5-methyl-6-ethyl-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(0.7g, 2.4mmol) and dimethylformamide(5ml), 0.53g of the titled compound was obtained in accordance with the same procedure

as in Step 2 of Example 1.
Yield: 53.5%
M.P.: 192-194°C
^{1}H-NMR(DMSO-d_6): δ 1.25(t, 3H), 2.19(s, 3H), 2.28(s, 3H), 2.68(q, 2H), 2.88(t, 2H), 3.79(t, 2H), 4.75(s, 2H), 7.15(m, 6H), 7.70(m, 1H), 9.80(s, 1H).

Example 45: Synthesis of 5-methyl-6-ethyl-2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 4-fluoroaniline(0.45ml, 3.6mmol) was added to a mixture solution of 5-methyl-6-ethyl-4-(1,2,3,4-tetrahydro isoquinolin-2-yl)-2-chloropyrimidine(0.7g, 2.4mmol) and dimethylformamide(5ml), 0.50g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.
Yield: 52.2%
M.P.: 235-238°C
^{1}H-NMR($CDCl_3$): δ 1.42(t, 3H), 2.25(s, 3H), 2.76(q, 2H), 3.04(t, 2H), 3.90(t, 2H), 4.80(s, 2H), 6.95-7.35(m, 6H), 7.55(m, 2H), 10.50(s, 1H), 13.80(bd, 1H).

Example 46: Synthesis of 5-methyl-6-ethyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After N-methylaniline(0.40ml, 3.6mmol) was added to a mixture solution of 5-methyl-6-ethyl-4-(1,2,3,4-tetrahydro isoquinolin-2-yl)-2-chloropyrimidine(0.7g, 2.4mmol) and dimethylformamide(5ml), 0.50g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.
Yield: 52.7%
M.P.: 75-80°C
^{1}H-NMR($CDCl_3$): δ 1.32(t, 3H), 2.15(s, 3H), 2.80(t, 2H), 3.10(m, 2H), 3.60(m, 2H), 3.80(s, 3H), 4.48(s,2H), 6.95(m,

2H), 7.05-7.70(m, 7H).

Example 47: Synthesis of 2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]-pyrimidine hydrochloride

Step 1: 4-(1,2,3,4-Tetrahydroisoquinolin-2-yl)-2-chloro cyclopenta[d]pyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 1,2,3,4-tetrahydroisoquinoline(0.5ml, 4mmol) and 2,4-dichlorocyclopenta[d]pyrimidine(0.79g, 4mmol) prepared in Step 3 of Example 29 were used as starting materials, 0.58g of the titled compound was prepared. (Yield: 51%)

Step 2: 2-(2-Methyl-4-fluorophenylamino)-2-(1,2,3,4-tetra-hydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride

After 4-fluoro-2-methylaniline(0.25ml, 2.20mmol) was added to a mixture solution of 4-(1,2,3,4-tetrahydroiso quinolin-2-yl)-4-chlorocyclopenta[d]pyrimidine(0.58g, 2.0mmol) and dimethylformamide(5ml), 0.34g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 41.4%

M.P.: 170-172°C

^{1}H-NMR(DMSO-d_6): δ 2.06(m, 2H), 2.26(s, 3H), 2.90(m, 4H), 3.12(t, 2H), 3.97(t, 2H), 4.90(s, 2H), 7.11-7.21(m, 6H), 9.78(s, 1H), 13.25(bd, 1H).

Example 48: Synthesis of 2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydro-quinazoline hydrochloride

Step 1: 4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloro-5,6,7,8-tetrahydroquinazoline hydrochloride

In accordance with the same procedure as in Step 1 of Example 1, except that a mixture solution of 1,2,3,4-tetrahydroisoquinoline(2.8ml, 22mmol), triethylamine(3.4ml, 24mmol), N,N-dimethylformamide(10ml) and 2,4-dichloro-5,6,7,8-tetrahydroquinazoline(4.0g, 20mmol) prepared in Step 2 of Example 32 were used as starting materials, 4.7g of the titled compound was prepared. (Yield: 78.4%)

Step 2: 2-(2-Methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

After 4-fluoro-2-methylaniline(0.75ml, 6.6mmol) was added to a mixture solution of 4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloro-5,6,7,8-tetrahydroquinazoline(0.90g, 3.0mmol) and dimethylformamide(5ml), 0.36g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 28%

M.P.: 191-193°C

^{1}H-NMR(DMSO-d_6): δ 1.62-1.80(bd, 4H), 2.26(s, 3H), 2.65(bd, 4H), 2.88(t, 2H), 3.84(t, 2H), 4.78(s, 2H), 7.18(m, 6H), 7.67(m, 1H), 9.72(s, 1H), 13.40(bd, 1H).

Example 49: Synthesis of 2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

After 4-fluoroaniline(0.60ml, 6.3mmol) was added to a mixture solution of 4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloro-5,6,7,8-tetrahydroquinazoline(0.90g, 3.0mmol) and dimethylformamide(5ml), 0.62g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 50%

M.P.: 215-218°C

^{1}H-NMR(DMSO-d_6): δ 1.62-1.74(bd, 4H), 2.68(m, 4H), 2.95(t,

2H), 3.90(t, 2H), 4.86(s, 2H), 7.19-7.41(m, 6H), 7.57(m, 2H), 10.42(s, 1H), 11.40(bd, 1H).

Example 50: Synthesis of 2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

After N-methylaniline(0.70ml, 6.3mmol) was added to a mixture solution of 4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloro-5,6,7,8-tetrahydroquinazoline(0.90g, 3.0mmol) and dimethylformamide(5ml), 0.48g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 39%

M.P.: 164-167°C

^{1}H-NMR(DMSO-d_6): δ 1.59-1.74(bd, 4H), 2.64(t, 2H), 2.78(m, 4H), 3.51(s, 3H), 3.78(t, 2H), 4.72(s, 2H), 7.19-7.17(m, 4H), 7.38-7.50(m, 5H), 12.18(bd, 1H).

Example 51: Synthesis of 2-(2-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

After 2-methylaniline(0.30ml, 2.7mmol) was added to a mixture solution of 4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloro-5,6,7,8-tetrahydroquinazoline(0.75g, 2.5mmol) and dimethylformamide(5ml), 0.50g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 49%

M.P.: 173-175°C

^{1}H-NMR(DMSO-d_6): δ 1.63-1.77(bd, 4H), 2.32(s, 3H), 2.65(m, 4H), 2.88(t, 2H), 3.85(t, 2H), 4.80(s, 2H), 7.09-7.32(m, 7H), 7.72(m, 1H), 9.67(s, 1H), 13.43(bd, 1H).

Example 52: Synthesis of 6-methyl-2-(2-methyl-4-fluoro-phenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]-

pyridin-6-yl)pyrimidine hydrochloride

Step 1: 6-Methyl-4-(7-methyl-4,5,6,7-tetrahydrothieno [2,3-c]pyridin-6-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that 2,4-dichloro-6-methylpyrimidine(3.1g, 19mmol) and 7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine (2.9g, 19mmol) in Preparation 1 were used as starting materials, 2.2g of the titled compound was obtained as white crystal. (Yield: 41%)

Step 2: 6-Methyl-2-(2-methyl-4-fluorophenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-pyrimidine hydrochloride

After 4-fluoro-2-methylaniline(0.5ml, 4.6mmol) was added to a mixture solution of 6-methyl-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-2-chloropyrimidine(0.7g, 2.5mmol) and dimethylformamide(10ml), 0.45g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.
Yield: 44.4%
M.P.: 120-121°C
^{1}H-NMR($CDCl_3$): δ 1.54(dd, 3H), 2.40(s, 3H), 2.48(s, 3H), 2.68(m, 1H), 2.80(m, 1H), 3.30(mm, 1H), 4.45(dd, 1H), 5.48(qq, 1H), 6.02(d, 1H), 6.78(m, 1H), 6.95(m, 2H), 7.20(t, 1H), 7.50(m, 1H), 9.80(s, 1H), 14.20(bs, 1H).

Example 53: Synthesis of 6-methyl-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-2-(4-fluorophenylamino)-pyrimidine hydrochloride

After 4-fluoroaniline(0.4ml, 3.7mmol) was added to a mixture solution of 6-methyl-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-2-chloropyrimidine(0.7g, 2.5mmol) and dimethylformamide(10ml), 0.7g of the titled compound was

obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 71.6%

M.P.: 210-212°C

^{1}H-NMR($CDCl_3$): δ 1.80(dd, 3H), 2.42(s, 3H), 2.80(m, 2H), 3.40(mm, 1H), 4.60(dd, 1H), 5.60(mm, 1H), 6.08(d, 1H), 6.80(m, 1H), 7.08(t, 2H), 7.21(m, 1H), 7.55(m, 2H), 10.40(s, 1H), 13.80(s, 1H).

Example 54: Synthesis of 6-methyl-2-(N-methylphenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-pyrimidine hydrochloride

After N-methylaniline(0.45ml, 4.05mmol) was added to a mixture solution of 6-methyl-4-(7-methyl-4,5,6,7-tetrahydro thieno[2,3-c]pyridin-6-yl)-2-chloropyrimidine(0.75g, 2.7mmol) and dimethylformamide(10ml), 0.52g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 49.7%

M.P.: 175-178°C

^{1}H-NMR($CDCl_3$): δ 1.38(dd, 3H), 2.50(bs, 1H), 2.68-3.05(m, 4H), 3.45(m, 1H), 3.90(s, 3H), 4.27(dd, 1H), 5.30(qq, 1H), 6.02(d, 1H), 6.78(d, 1H), 7.10-7.35(m, 4H), 7.38-7.55(m, 2H), 13.50 (bs, 1H).

Example 55: Synthesis of 5,6-dimethyl-2-(2-methyl-4-fluoro-phenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]-pyridin-6-yl)pyrimidine hydrochloride

Step 1: 5,6-Dimethyl-4-(7-methyl-4,5,6,7-tetrahydrothieno [2,3-c]pyridin-6-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that N,N-dimethylformamide(20ml), 5,6-dimethyl-2,4-dichloropyrimidine(2.8g, 16mmol) prepared in Step 1 of Example 12 and 7-methyl-4,5,6,7-tetrahydro

thieno[2,3-c]pyridine (2.7g, 17.6mmol) in Preparation 2 were used as starting materials, 1.85g of the titled compound was prepared. (Yield: 39.4%)

Step 2: 5,6-Dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl) pyrimidine hydrochloride

After 4-fluoro-2-methylaniline(0.5ml, 4.6mmol) was added to a mixture solution of 5,6-dimethyl-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-2-chloropyrimidine(0.68g, 2.3mmol) and dimethylformamide(5ml), 0.12g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 12.4%

M.P.: >240°C

^{1}H-NMR($CDCl_3$): δ 1.60(d, 3H), 2.22(s, 3H), 2.43(s, 3H), 2.55(s, 1H), 2.72(bd, 1H), 2.80(m, 1H), 3.48(m, 1H), 4.30(m, 1H), 5.58(q, 1H), 6.76(d, 1H), 6.90(m, 2H), 7.18(d, 1H), 7.44(m, 1H), 9.55(s, 1H), 14.36(s, 1H).

Example 56: Synthesis of 5-methyl-2-(2-methyl-4-fluorophenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]-pyridin-6-yl)-6-ethylpyrimidine hydrochloride

Step 1: 5-Methyl-4-(7-methyl-4,5,6,7-tetrahydrothieno [2,3-c]-pyridin-6-yl)-6-ethyl-2-chloropyrimidine

In accordance with the same procedure as in Step 1 of Example 1, except that triethylamine(2.2ml), N,N-dimethyl formamide(20ml), 2,4-dichloro-5-methyl-6-ethylpyrimidine (2.7g, 14.1mmol) prepared in Preparation 4 and 7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine(2.4g, 15.7mmol) prepared in Preparation 1 were used as starting materials, 2.23g of the titled compound was prepared. (Yield: 51.3%)

Step 2: 5-Methyl-2-(2-methyl-4-fluorophenylamino)-4-

(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-6-ethylpyrimidine hydrochloride

After 4-fluoro-2-methylaniline(0.51ml, 4.59mmol) was added to a mixture solution of 5-methyl-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-6-ethyl-2-chloropyrimidine(0.74g, 2.4mmol) and dimethylformamide(5ml), 0.15g of the titled compound was obtained in accordance with the same procedure as in Step 2 of Example 1.

Yield: 14.4%

M.P.: 178-180°C

^{1}H-NMR(DMSO-d_6 + TFA): δ 1.07(t, 3H), 1.75(d, 3H), 1.95(s, 3H), 2.21(s, 3H), 2.35(m, 2H), 2.61(q, 2H), 3.34(m, 2H), 5.05(m, 1H), 6.81(d, 1H), 6.83-7.20(m, 3H), 7.50(d, 1H).

Example 57: Synthesis of 6-methyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

Step 1: 6-Methyl-2-chloro-4-hydroxypyrimidine

To a mixture solution of 6-methyl-2,4-dichloro pyrimidine (25g, 0.153mol) in tetrahydrofurane(170ml) was added 1N-NaOH solution(420ml) and stirred for 48 hours at room temperature. The reaction mixture was washed with ethyl ether to remove impurities, acidified with hydrochloric acid, and then extracted with ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulfate, concentrated under reduced pressure to give 13.5g of titled compound as yellow solid form. (Yield: 66.7%)

Step 2: 6-Methyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxypyrimidine

A mixture solution of 6-methyl-2-chloro-4-hydroxy-pyrimidine(6g, 37.5mmol) prepared in the above Step 1, 1-methyl-1,2,3,4-tetrahydroisoquinoline(11.6g, 78.8mmol) and

N,N-dimethylformamide(30ml) was stirred at 120 for 2 hours and cooled to give a solid. The resulting solid was dissolved in a mixture solution of dichloromethane and methanol and the undissolved materials were filtered off. The filtrate residue was washed many times with water, dried over anhydrous sodium sulfate, concentrated under reduced pressure to give 7.1g of titled compound. (Yield: 74.1%)

Step 3: 6-Methyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine

A mixture solution of 6-methyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxypyrimidine(7.0g, 27.4mmol) prepared in the above Step 2, phosphorous oxychloride(30ml) and N,N-dimethylaniline(2.3ml) was stirred at 90°C for 2 hours and cooled. The reaction mixture was added to ice water and basified with sodium bicarbonate and then was extracted with ethyl ether. The ethyl ether layer was dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give 4.5g of titled compound. (Yield: 60%)

Step 4: 6-Methyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

2-Methyl-4-fluoroaniline(1.1ml, 10.2mmol) was added to a mixture solution of 6-methyl-2-(1-methyl-1,2,3,4-tetra hydroisoquinolin-2-yl)-4-chloropyrimidine(1.5g, 5.5mmol) and dimethylformamide(10ml). The reaction solution was stirred for 3 hours and cooled to room temperature. The reaction mixture was diluted with dichloromethane and washed with water. Dichloromethane layer was separated, basified with aqueous sodium hydroxide solution, washed with water, and dried and concentrated in vacuo. The resulting residue was purified with silica gel column chromatography to give a free base form of titled compound as an oily form. The free

base form of titled compound was dissolved in ethyl ether, then hydrochloric acid was added thereto. The resulting solid was filtered, dried under reduced pressure to give 0.9g of titled compound.

Yield: 41%

M.P.: 157-160°C

^{1}H-NMR(DMSO-d_6): δ 1.42(bs, 3H), 2.25(s, 3H), 2.40(s, 3H), 2.90(bs, 2H), 3.55(bs, 1H), 4.40(bs, 1H), 5.60(bs, 1H), 6.40(s, 1H), 7.00-7.30(m, 6H), 7.40(bs, 1H), 10.60(bs, 1H), 12.35(bs, 1H).

Example 58: Synthesis of 6-methyl-4-(4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 4-fluoroaniline(0.8ml, 8.4mmol) was added to a mixture solution of 6-methyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(1.5g, 5.5mmol) and dimethylformamide(10ml), 1.1g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 52%

M.P.: 165-167°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.50(s, 3H), 3.00(bs, 2H), 3.60(bs, 1H), 4.50(bs, 1H), 5.75(bs, 1H), 6.38(bs, 1H), 7.00-7.50(m, 6H), 7.75(bs, 2H), 11.20(bs, 1H), 12.38(bs, 1H).

Example 59: Synthesis of 6-methyl-4-(2-methyl-4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]-pyridin-6-yl)pyrimidine hydrochloride

Step 1: 6-Methyl-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-4-hydroxypyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 6-methyl-2-chloro-4-hydroxy-

pyrimidine(6g, 37.5mmol) prepared in Step 1 of Example 57, and 7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine (12.07g, 78.75mmol) prepared in preparation 1 were used as starting materials, 6.9g of the titled compound was prepared.
(Yield: 70%)

Step 2: 6-Methyl-2-(7-methyl-4,5,6,7-tetrahydrothieno-[2,3-c]pyridine-6-yl)-4-chloropyrimidine

In accordance with the same procedure as in Step 3 of Example 57, except that 6-methyl-2-(7-methyl-4,5,6,7-tetra hydrothieno[2,3-c]pyridin-6-yl)-4-hydroxypyrimidine(6.5g, 24.9mmol) prepared in the above Step 1 was used as a starting material, 4.5g of the titled compound was prepared.
(Yield: 70%)

Step 3: 6-Methyl-4-(2-methyl-4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.38ml, 3.42mmol) was added to a mixture solution of 6-methyl-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-4-chloropyrimidine (0.5g, 1.8mmol) and dimethylformamide(10ml), 0.35g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.
Yield: 48%
M.P.: 135-137°C
^{1}H-NMR($CDCl_3$): δ 1.43(bs, 3H), 2.22(s, 3H), 2.42(s, 3H), 2.70(bs, 2H), 3.36(bs, 1H), 4.65(m, 1H), 5.70(m, 1H), 6.38(bs, 1H), 6.85(d, 1H), 7.04-7.30(m, 2H), 7.34-7.50(m, 2H), 10.58(bs, 1H), 12.42(bs, 1H).

Example 60: Synthesis of 6-methyl-4-(4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-pyrimidine hydrochloride

After 4-fluoroaniline(0.26ml, 2.74mmol) was added to a mixture solution of 6-methyl-2-(7-methyl-4,5,6,7-tetrahydro thieno[2,3-c]pyridin-6-yl)-4-chloropyrimidine(0.5g, 1.8mmol) and dimethylformamide(10ml), 0.30g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 42.6%

M.P.: 245-247°C

^{1}H-NMR(DMSO-d_6): δ 1.58(d, 3H), 2.42(s, 3H), 2.81(m, 2H), 3.48(m, 1H), 4.64(m, 1H), 5.75(m, 1H), 6.25(s, 1H), 6.90(d, 1H), 7.30(t, 3H), 7.42(d, 2H), 7.70(m, 2H).

Example 61: Synthesis of 6-ethyl-2-(2-methyl-4-fluorophenyl-amino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

Step 1: 6-ethyl-2-chloro-4-hydroxypyrimidine

In accordance with the same procedure as in Step 1 of Example 57, except that 6-ethyl-2,4-dichloropyrimidine (27.08g, 0.153mol) prepared in Preparation 2 was used as a starting material, 14.6g of the titled compound was prepared. (Yield: 66.7%)

Step 2: 6-Ethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxypyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 6-ethyl-2-chloro-4-hydroxy-pyrimidine (7.0g, 37.5mmol) prepared in the above Step 1 and 1-methyl-1,2,3,4-tetrahydroisoquinoline(11.04g, 75mmol) were used as starting materials, 8.1g of the titled compound was prepared. (Yield: 80%)

Step 3: 6-Ethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine

In accordance with the same procedure as in Step 3 of Example 57, except that 6-ethyl-2-(1-methyl-1,2,3,4-tetra hydroisoquinolin-2-yl)-4-hydroxypyrimidine(8.0g, 29.7mmol) prepared in the above Step 2 was used as a starting material, 4.9g of the titled compound was prepared. (Yield: 57.3%)

Step 4: 6-Ethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(1.1ml, 10.2mmol) was added to a mixture solution of 6-ethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(2.0g, 7.0mmol) and dimethylformamide(10ml), 1.1g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 38%

M.P.: 123-125°C

^{1}H-NMR(DMSO-d_6): δ 1.16-1.57(m, 6H), 2.27(s, 3H), 2.77-2.94 (m, 4H), 3.50(bs, 1H), 4.40(bs, 1H), 5.63(bs, 1H), 6.45(s, 1H), 7.08-7.52(m, 7H), 10.61(s, 1H), 12.27(s, 1H).

Example 62: Synthesis of 6-ethyl-4-(2-methyl-4-fluorophenyl-amino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

Step 1: 6-Ethyl-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxypyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 6-ethyl-2-chloro-4-hydroxy pyrimidine(7.0g, 37.5mmol) prepared in Step 1 of Example 59 and 1,2,3,4-tetrahydroisoquinoline(9.4ml, 75mmol) were used as starting materials, 8.1g of the titled compound was prepared. (Yield: 84.6%)

Step 2: 6-Ethyl-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 6-ethyl-2-(1,2,3,4-tetrahydroisoquinoline-2-yl)-4-hydroxypyrimidine(8.0g, 31.3mmol) prepared in the above Step 1 was used as a starting material, 4.7g of the titled compound was prepared. (Yield: 55%)

Step 3: 6-Ethyl-4-(2-methyl-4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.35ml, 3.15mmol) was added to a mixture solution of 6-ethyl-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(0.40g, 1.46mmol) and dimethylformamide(10ml), 0.51g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 88%

M.P.: 122-124°C

^{1}H-NMR(DMSO-d_6): δ 1.30(q, 3H), 2.24(s, 3H), 2.74-2.95(m, 4H), 3.88(t, 2H), 4.83(s, 2H), 6.44(s, 1H), 7.05-7.55(m, 7H), 10.62(s, 1H), 12.30(s, 1H).

Example 63: Synthesis of 6-ethyl-4-(4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 4-fluoroaniline(0.30ml, 3.17mmol) was added to a mixture solution of 6-ethyl-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(0.40g, 1.46mmol) and dimethylformamide(10ml), 0.44g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 78%

M.P.: 124-126°C

^{1}H-NMR($CDCl_3$): δ 1.41(q, 3H), 2.70-2.95(m, 4H), 4.05(bs, 2H), 4.95(s, 2H), 6.16(s, 1H), 6.35-6.80(t, 2H), 7.04-7.14(m,

4H), 7.66-7.75(dd, 2H), 11.05(s, 1H), 12.06(s, 1H).

Example 64: Synthesis of 6-ethyl-4-(N-methylphenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After N-methylaniline(0.10ml, 9.22mmol) was added to a mixture solution of 6-ethyl-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(1.20g, 4.38mmol) and dimethylformamide(10ml), 0.22g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 13%

M.P.: 130-132°C

^{1}H-NMR($CDCl_3$): δ 1.15(t, 3H), 2.97-3.15(m, 4H), 3.55(s, 3H), 4.38(bs, 2H), 5.10(bs, 2H), 5.50(s, 1H), 7.10-7.40(m, 6H), 7.50-7.60(m, 3H), 13.40(s, 1H).

Example 65: Synthesis of 5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

Step 1: 5,6-Dimethyl-2,4-dichloropyrimidine

A mixture solution of 5,6-dimethyl-2,4-dihydroxy pyrimidine(72g, 0.51mol), phosphorous oxychloride(250ml) and N,N-dimethylaniline(41ml) was heated to reflux for 3 hours and cooled to room temperature. The reaction mixture was added to ice water and the resulting solid was filtered and recrystallized from dichloromethane to give 58.5g of the titled compound. (Yield: 64.7%)

Step 2: 5,6-Dimethyl-2-chloro-4-hydroxypyrimidine

In accordance with the same procedure as in Step 1 of Example 57, except that 5,6-dimethyl-2,4-dichloropyrimidine (50.0g, 0.28mol) prepared in the above Step 1 was used as a starting material, 24.4g of the titled compound was

prepared. (Yield: 55%)

Step 3: 5,6-Dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxypyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 5,6-dimethyl-2-chloro-4-hydroxy pyrimidine(6.0g, 37.8mmol) prepared in the above Step 2 was used as a starting material, 7.6g of the titled compound was prepared. (Yield: 75%)

Step 4: 5,6-Dimethyl-2-(1-methyl-1,2,3,4-tetrahydro-isoquinolin-2-yl)-4-chloropyrimidine

In accordance with the same procedure as in Step 3 of Example 57, except that 5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxypyrimidine(7.0g, 26mmol) prepared in the above Step 3 was used as starting material, 3.9g of the titled compound was prepared. (Yield: 52%)

Step 5: 5,6-Dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.7ml, 6.3mmol) was added to a mixture solution of 5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(0.85g, 3.0mmol) and dimethylformamide(10ml), 0.9g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 72.6%

M.P.: 208-211°C

^{1}H-NMR(DMSO-d_6): δ 1.28(d, 3H), 2.16(s, 3H), 2.18(s, 3H), 2.55(s, 3H), 2.80(bd, 2H), 3.42(bd, 1H), 4.34(bd, 1H), 5.44(bd, 1H), 7.02(bd, 1H), 7.24(m, 6H), 9.65(s, 1H), 12.30(bd, 1H).

Example 66: Synthesis of (R)-5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydro-isoquinolin-2-yl)pyrimidine hydrochloride

Step 1: (R)-5,6-Dimethyl-2-(1-methyl-1,2,3,4-tetrahydro-isoquinoline-2-yl)-4-hydroxypyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 5,6-dimethyl-2-chloro-4-hydroxy pyrimidine(6.0g, 37.8mmol) prepared in Example 60 and (R)-1-methyl-1,2,3,4-tetrahydroisoquinoline(11.7g, 79.5mmol) were used as starting materials, 7.0g of the titled compound was prepared. (Yield: 68.8%)

Step 2: (R)-5,6-Dimethyl-2-(1-methyl-1,2,3,4-tetrahydro-isoquinolin-2-yl)-4-chloropyrimidine

In accordance with the same procedure as in Step 3 of Example 57, except that (R)-5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxypyrimidine (7.0g, 26mmol) prepared in the above Step 1 was used as a starting material, 3.2g of the titled compound was prepared. (Yield: 42.8%)

Step 3: (R)-5,6-Dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.82ml, 7.35mmol) was added to a mixture solution of (R)-5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(1.0g, 3.5mmol) prepared in the above Step 2 and dimethylformamide (10ml), 1.2g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 83%

M.P.: 207-209°C

^{1}H-NMR(DMSO-d_6): δ 1.28(d, 3H), 2.16(s, 3H), 2.18(s, 3H), 2.55(s, 3H), 2.80(bd, 2H), 3.42(bd, 1H), 4.34(bd, 1H), 5.44(bd, 1H), 7.02(bd, 1H), 7.24(m, 6H), 9.65(s, 1H), 12.30(bd, 1H).

Example 67: Synthesis of (S)-5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydro-isoquinolin-2-yl)pyrimidine hydrochloride

Step 1: (S)-5,6-Dimethyl-2-(1-methyl-1,2,3,4-tetrahydro-isoquinolin-2-yl)-4-hydroxypyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 5,6-dimethyl-2-chloro-4-hydroxy-pyrimidine(6.0g, 37.8mmol) prepared in Example 60 and (S)-1-methyl-1,2,3,4-tetrahydroisoquinoline(11.7g, 79.5mmol) were used as starting materials, 6.6g of the titled compound was prepared. (Yield: 64.8%)

Step 2: (S)-5,6-Dimethyl-2-(1-methyl-1,2,3,4-tetrahydro-isoquinolin-2-yl)-4-chloropyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that (S)-5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxypyrimidine (7.0g, 26mmol) prepared in the above Step 1 was used as a starting material, 3.5g of the titled compound was prepared. (Yield: 46.8%)

Step 3: (S)-5,6-Dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrohydroisoquinolin-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.82ml, 7.35mmol) was added to a mixture solution of (S)-5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(1.0g, 3.5mmol) obtained in the above Step 2 and dimethylformamide

(10ml), 1.0g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 69.2%

M.P.: 208-210°C

^{1}H-NMR(DMSO-d_6): δ 1.28(d, 3H), 2.16(s, 3H), 2.18(s, 3H), 2.55(s, 3H), 2.80(bd, 2H), 3.42(bd, 1H), 4.34(bd, 1H), 5.44(bd, 1H), 7.02(bd, 1H), 7.24(m, 6H), 9.65(s, 1H), 12.30(bd, 1H).

Example 68: Synthesis of 5,6-dimethyl-4-(4-fluorophenyl-amino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After 4-fluoroaniline(0.6ml, 6.3mmol) was added to a mixture solution of 5,6-dimethyl-2-(1-methyl-1,2,3,4-tetra hydroisoquinolin-2-yl)-4-chloropyrimidine(0.85g, 3.0mmol) and dimethylformamide(10ml), 0.62g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 52%

M.P.: 246-250°C

^{1}H-NMR(DMSO-d_6): δ 1.40(d, 3H), 2.18(s, 3H), 2.50(s, 3H), 2.88(bd, 2H), 3.42(bd, 1H), 4.42(bd, 1H), 5.62(bd, 1H), 7.18(m, 4H), 7.30(t, 2H), 7.63(q, 2H), 9.70(s, 1H), 12.30(bd, 1H).

Example 69: Synthesis of (R)-5,6-dimethyl-4-(4-fluorophenyl-amino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After 4-fluoroaniline(0.6ml, 6.3mmol) was added to a mixture solution of (R)-5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(0.85g, 3.0mmol) prepared in Step 2 of Example 61 and dimethylformamide(10ml), 0.50g of the titled compound was obtained in accordance with the same procedure as in Step 4

of Example 57.
Yield: 41.8%
M.P.: 245-248°C
^{1}H-NMR(DMSO-d_6): δ 1.40(d, 3H), 2.18(s, 3H), 2.50(s, 3H), 2.88(bd, 2H), 3.42(bd, 1H), 4.42(bd, 1H), 5.62(bd, 1H), 7.18(m, 4H), 7.30(t, 2H), 7.63(q, 2H), 9.70(s, 1H), 12.30(bd, 1H).

Example 70: Synthesis of (S)-5,6-dimethyl-4-(4-fluorophenyl-amino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After 4-fluoroaniline(0.6ml, 6.3mmol) was added to a mixture solution of (S)-5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinoline-2-yl)-4-chloropyrimidine(0.85g, 3.0mmol) prepared in Step 2 of Example 62 and dimethylformamide (10ml), 0.55g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.
Yield: 46%
M.P.: 245-247°C
^{1}H-NMR(DMSO-d_6): δ 1.40(d, 3H), 2.18(s, 3H), 2.50(s, 3H), 2.88(bd, 2H), 3.42(bd, 1H), 4.42(bd, 1H), 5.62(bd, 1H), 7.18(m, 4H), 7.30(t, 2H), 7.63(q, 2H), 9.70(s, 1H), 12.30(bd, 1H).

Example 71: Synthesis of 5,6-dimethyl-4-(N-methylphenyl-amino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After N-methylaniline(0.6ml, 5mmol) was added to a mixture solution of 5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(0.7g, 2.4mmol) and dimethylformamide(10ml), 0.45g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.
Yield: 47%

M.P.: 91-95°C

^{1}H-NMR($CDCl_3$): δ 1.32(s, 3H), 1.64(d, 3H), 1.90(bd, 1H), 2.72(s, 3H), 3.02(bd, 1H), 3.25(bd, 1H), 3.56(s, 3H), 3.70(bd, 1H), 5.05(bs, 1H), 5.78(bs, 1H), 7.20(m, 6H), 7.42(m, 3H), 13.44(s, 1H).

Example 72: Synthesis of (R)-5,6-dimethyl-4-(N-methylphenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After N-methylaniline(0.6ml, 5mmol) was added to a mixture solution of (R)-5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-chloropyrimidine(0.7g, 2.4mmol) prepared in the Step 2 of Example 61 and dimethylformamide(10ml), 0.50g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 52.7%

M.P.: 90-93°C

^{1}H-NMR($CDCl_3$): δ 1.32(s, 3H), 1.64(d, 3H), 1.90(bd, 1H), 2.72(s, 3H), 3.02(bd, 1H), 3.25(bd, 1H), 3.56(s, 3H), 3.70(bd, 1H), 5.05(bs, 1H), 5.78(bs, 1H), 7.20(m, 6H), 7.42(m, 3H), 13.44(s, 1H).

Example 73: Synthesis of (S)-5,6-dimethyl-4-(N-methylphenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After N-methylaniline(0.6ml, 5mmol) was added to a mixture solution of (S)-5,6-dimethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(0.7g, 2.4mmol) prepared in the Step 2 of Example 62 and dimethylformamide(10ml), 0.42g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 44.4%

M.P.: 91-94°C

^{1}H-NMR($CDCl_3$): δ 1.32(s, 3H), 1.64(d, 3H), 1.90(bd, 1H), 2.72(s, 3H), 3.02(bd, 1H), 3.25(bd, 1H), 3.56(s, 3H), 3.70(bd, 1H), 5.05(bs, 1H), 5.78(bs, 1H), 7.20(m, 6H), 7.42(m, 3H), 13.44(s, 1H).

Example 74: Synthesis of 5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl) pyrimidine hydrochloride

Step 1: 5,6-Dimethyl-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxypyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 5,6-dimethyl-2-chloro-4-hydroxy pyrimidine (6.0g, 37.8mmol) prepared in the Step 2 of Example 65 and 1,2,3,4-tetrahydroisoquinoline(10ml, 79.9mmol) were used as starting materials, 7.8g of the titled compound was prepared. (Yield: 81%)

Step 2: 5,6-Dimethyl-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine

In accordance with the same procedure as in Step 3 of Example 57, except that 5,6-dimethyl-2-(1,2,3,4-tetrahydro isoquinolin-2-yl)-4-hydroxypyrimidine(7.0g, 26mmol) prepared in the above Step 1 was used as starting materials, 4.1g of the titled compound was prepared. (Yield: 57.6%)

Step 3: 5,6-Dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinoline-2-yl)pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.3ml, 2.7mmol) was added to a mixture solution of (5,6-dimethyl-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(0.30g, 1.0mmol) and dimethylformamide(10ml), 0.12g of the titled compound was obtained in accordance with the same procedure

as in Step 4 of Example 57.
Yield: 30%
M.P.: 117-120°C
^{1}H-NMR(DMSO-d_6): δ 2.13(s, 3H), 2.16(s, 3H), 2.52(s, 3H), 2.81(t, 2H), 3.79(t, 2H), 4.74(s, 2H), 7.00(bd, 1H), 7.09-7.34(m, 6H), 9.16(s, 1H), 12.35(s, 1H).

Example 75: Synthesis of 5,6-dimethyl-4-(4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After 4-fluoroaniline(0.24ml, 2.5mmol) was added to a mixture solution of 5,6-dimethyl-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(0.33g, 1.2mmol) and dimethylformamide(10ml), 0.31g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.
Yield: 67%
M.P.: 128-130°C
^{1}H-NMR(DMSO-d_6): δ 2.13(s, 3H), 2.53(s, 3H), 2.90(t, 2H), 3.93(t, 2H), 4.86(s, 2H), 7.18-7.34(m, 6H), 7.63(m, 2H), 9.71(s, 1H), 12.20(bd, 1H).

Example 76: Synthesis of 5,6-dimethyl-4-(N-methylphenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride

After N-methylaniline(0.5ml, 4.2mmol) was added to a mixture solution of 5,6-dimethyl-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(0.6g, 2.0mmol) and dimethylformamide(10ml), 0.28g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.
Yield: 37%
M.P.: 209-211°C
^{1}H-NMR(DMSO-d_6): δ 1.24(s, 3H), 2.41(s, 3H), 2.98(t, 2H), 3.52(s, 3H), 4.07(t, 2H), 5.02(s, 2H), 7.24-7.45(m, 9H),

12.65(bd, 1H).

Example 77: Synthesis of 5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride

Step 1: 5,6-Dimethyl-4-(7-methyl-4,5,6,7-tetrahydrothieno-[2,3-c]pyridin-6-yl)-2-hydroxypyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 5,6-dimethyl-2-chloro-4-hydroxypyrimidine(6.0g, 37.8mmol) prepared in the Step 2 of Example 65 and 7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine (12.2g, 79.6mmol) prepared in Preparation 1 were used as starting materials, 6.5g of the titled compound was obtained. (Yield: 62.4%)

Step 2: 5,6-Dimethyl-4-(7-methyl-4,5,6,7-tetrahydrothieno-[2,3-c]pyridin-6-yl)-2-chloropyrimidine

In accordance with the same procedure as in Step 3 of Example 57, except that 5,6-dimethyl-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-4-hydroxypyrimidine (6.0g, 21.8mmol) prepared in the above Step 1 was used as a starting material, 3.5g of the titled compound was prepared. (Yield: 54.6%)

Step 3: 5,6-Dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl) pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.3ml, 3mmol) was added to a mixture solution of 5,6-dimethyl-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-4-chloropyrimidine (0.4g, 1.4mmol) and dimethylformamide(10ml), 0.14g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 24%
M.P.: 134-137°C
^{1}H-NMR(DMSO-d_6): δ 1.35(d, 3H), 2.14(s, 3H), 2.18(s, 3H), 2.42(s, 3H), 2.65(bd, 2H), 3.56(bd, 1H), 4.54(m, 1H), 5.56(bd, 1H), 6.84(d, 1H), 7.15-7.38(m, 3H), 7.41(d, 1H), 9.72(s, 1H), 12.44(bd, 1H).

Example 78: Synthesis of 5,6-dimethyl-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-4-(4-fluorophenyl-amino)pyrimidine hydrochloride

After 4-fluoroaniline(0.3ml, 3mmol) was added to a mixture solution of 5,6-dimethyl-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-4-chloropyrimidine (0.4g, 1.4mmol) and dimethylformamide(10ml), 0.15g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.
Yield: 26.5%
M.P.: 141-145°C
^{1}H-NMR(DMSO-d_6): δ 1.42(d, 3H), 2.16(s, 3H), 2.52(s, 3H), 2.70(bd, 2H), 3.38(m, 1H), 4.65(bd, 1H), 5.75(bd, 1H), 6.84(d, 1H), 7.30(m, 2H), 7.42(d, 1H), 7.61(m, 2H), 9.80(s, 1H), 12.62(bd, 1H).

Example 79: Synthesis of 5,6-dimethyl-4-(N-methylphenyl-amino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]-pyridin-6-yl)pyrimidine hydrochloride

After N-methylaniline(0.5ml, 4mmol) was added to a mixture solution of 5,6-dimethyl-2-(7-methyl-4,5,6,7-tetra-hydrothieno[2,3-c]pyridin-6-yl)-4-chloropyrimidine(0.64g, 2mmol) and dimethylformamide(10ml), 0.16g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.
Yield: 20%
M.P.: 117-120°C
^{1}H-NMR($CDCl_3$): δ 1.32(s, 3H), 1.65(d, 3H), 2.72(s, 3H),

2.78(bd, 1H), 3.20(bd, 1H), 3.51(bd, 1H), 3.56(s, 3H), 5.36(bd, 1H), 6.03(bd, 1H), 6.82(d, 1H), 6.88(m, 3H), 7.37-7.48(m, 3H), 14.52(s, 1H).

Example 80: Synthesis of 5-methyl-6-ethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydro-isoquinolin-2-yl)pyrimidine hydrochloride

Step 1: 5-Methyl-6-ethyl-2-chloro-4-hydroxypyrimidine

In accordance with the same procedure as in Step 1 of Example 57, except that 2,4-dichloro-5-methyl-6-ethyl pyrimidine(2.7g, 14.1mmol) prepared in Preparation 4 was used as a starting material, 1.8g of the titled compound was prepared. (Yield: 72%)

Step 2: 5-Methyl-6-ethyl-2-(1-methyl-1,2,3,4-tetrahydro-isoquinolin-2-yl)-4-hydroxypyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 5-methyl-6-ethyl-2-chloro-4-hydroxy pyrimidine(1.8g, 10.1mmol) prepared in the above Step 1 was used as a starting material, 2.4g of the titled compound was prepared. (Yield: 84%)

Step 3: 5-Methyl-6-ethyl-2-(1-methyl-1,2,3,4-tetrahydro-isoquinolin-2-yl)-4-chloropyrimidine

In accordance with the same procedure as in Step 3 of Example 57, except that 5-methyl-6-ethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxypyrimidine(2.4g, 8.5mmol) prepared in the above Step 2 was used as a starting material, 1.6g of the titled compound was prepared. (Yield: 62.4%)

Step 4: 5-Methyl-6-ethyl-4-(2-methyl-4-fluorophenyl-amino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.42ml, 3.8mmol) was added to a mixture solution of 5-methyl-6-ethyl-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloropyrimidine(0.6g, 2.0mmol) and dimethylformamide(10ml), 0.35g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 41%

M.P.: 270-272°C

^{1}H-NMR(DMSO-d_6): δ 1.22(t, 3H), 1.35(d, 3H), 2.16(s, 3H), 2.20(s, 3H), 2.75-3.00(m, 4H), 3.48(m, 1H), 4.20(m, 1H), 5.38(bs, 1H), 7.00-7.40(m, 7H).

Example 81: Synthesis of 4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta-[d]pyrimidine hydrochloride

Step 1: 2-chloro-4-hydroxycyclopenta[d]pyrimidine

In accordance with the same procedure as in Step 1 of Example 57, except that 2,4-dichlorocyclopenta[d]pyrimidine (2.7g, 14.3mmol) prepared in Step 3 of Example 29 was used as a starting material, 1.7g of the title compound was prepared.(Yield: 69.7%)

Step 2: 2-(1-Methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-hydroxycyclopenta[d]pyrimidine

In accordance with the same procedure as in Step 2 of Example 57, except that 2-chloro-4-hydroxycyclopenta[d]-pyrimidine(1.7g, 10.0mmol) prepared in the above Step 1 was used as a starting material, 2.2g of the titled compound was prepared. (Yield: 78.2%)

Step 3: 2-(1-Methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chlorocyclopenta[d]pyrimidine

In accordance with the same procedure as in Step 3 of

Example 57, except that 2-(1-methyl-1,2,3,4-tetrahydro-isoquinoline-2-yl)-2-hydroxycyclopenta[d]pyrimidine(2.2g, 7.8mmol) prepared in the above Step 2 was used as a starting material, 1.5g of the titled compound was prepared. (Yield: 64%)

Step 4: 4-(2-Methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride

After 2-methyl-4-fluoroaniline(0.46ml, 4.2mmol) was added to a mixture solution of 2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chlorocyclopenta[d]pyrimidine(0.6g, 2.0mmol) and dimethylformamide(10ml), 0.10g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 12%

M.P.: 165-168°C

^{1}H-NMR($CDCl_3$): δ 1.40(m, 2H), 1.62(d, 3H), 2.22(m, 2H), 2.30(s, 3H), 2.62-2.98(bd, 3H), 3.30(m, 2H), 3.70(bd, 1H), 4.73(bs, 1H), 5.32(bs, 1H), 6.98(m, 2H), 7.20(m, 5H), 14.02(bd, 1H).

Example 82: Synthesis of 2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

Step 1: 2-chloro-4-hydroxy-5,6,7,8-tetrahydroquinazoline

In accordance with the same procedure as in Step 1 of Example 57, except that 2,4-dichloro-5,6,7,8-tetrahydro quinazoline(6.4g, 31.6mmol) prepared in the Step 2 of Example 32 was used as a starting material, 4.2g of the titled compound was prepared. (Yield: 72%)

Step 2: 2-(1-Methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline

In accordance with the same procedure as in Step 2 of Example 57, except that 2-chloro-4-hydroxy-5,6,7,8-tetrahydroquinazoline (2.0g, 10.8mmol) prepared in the above Step 1 and 1-methyl-1,2,3,4-tetrahydroisoquinoline(3.3g, 22.4mmol) were used as starting materials, 1.1g of the titled compound was prepared. (Yield: 34.5%)

Step 3: 2-(1-Methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloro-5,6,7,8-tetrahydroquinazoline

In accordance with the same procedure as in Step 3 of Example 57, except that 2-(1-methyl-1,2,3,4-tetrahydroisoquinoline-2-yl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline (1.1g, 3.7mmol) prepared in the above Step 2 was used as a starting material, 0.7g of the titled compound was prepared. (Yield: 60.3%)

Step 4: 4-(2-Methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

After 4-fluoro-2-methylaniline(0.3ml, 2.7mmol) was added to a mixture solution of 2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloro-5,6,7,8-tetrahydroquinazoline(0.35g, 1.1mmol) and dimethylformamide(5ml), 0.15g of the titled compound was obtained in accordance with the same procedure as in Step 4 of Example 57.

Yield: 31%

M.P.: 181-184°C

^{1}H-NMR(DMSO-d_6): δ 1.32(d, 3H), 1.80(bd, 4H), 2.18(s, 3H), 2.85(bd, 4H), 3.40(bd, 1H), 3.65(bd, 2H), 4.25(m, 1H), 5.40(bd, 1H), 7.05-7.38(m, 7H), 9.62(s, 1H), 12.20(s, 1H).

Example 83: Synthesis of 4-(2-methyl-4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

Step 1: 2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline

In accordance with the same procedure as in Step 2 of Example 57, except that 2-chloro-4-hydroxy-5,6,7,8-tetra hydroquinazoline (2.0g, 10.8mmol) prepared in Step 1 of Example 82 and 1,2,3,4-tetrahydroisoquinoline (2.8g, 22.4mmol) were used as starting materials, 0.8g of the titled compound was prepared. (Yield: 26.3%)

Step 2: 2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-chloro-5,6,7,8-tetrahydroquinazoline

In accordance with the same procedure as in Step 3 of Example 57, except that 2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline(0.8g, 2.8mmol) prepared in the above Step 1 was used as a starting material, 0.6g of the titled compound was prepared. (Yield: 71.5%)

Step 3: 4-(2-Methyl-4-fluorophenylamino)-2-(1,2,3,4-tetra hydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride

After 4-fluoro-2-methylaniline(0.3ml, 2.7mmol) was added to a mixture solution of 2-(1,2,3,4-tetrahydroiso quinolin-2-yl)-4-chloro-5,6,7,8-tetrahydroquinazoline(0.3g, 1.0mmol) and dimethylformamide(5ml), 0.2g of the titled compound was prepared in accordance with the same procedure as in Step 4 of Example 57.

Yield: 47.1%

M.P.: 150-152°C

^{1}H-NMR(DMSO-d_6): δ 1.76(bd, 4H), 2.15(s, 3H), 2.81(bd, 4H), 3.46(bd, 2H), 3.77(bd, 2H), 4.74(s, 1H), 7.02-7.33(m, 7H), 9.59(s, 1H), 12.40(bd, 1H).

Test 1: Inhibition of proton pump(H^+/K^+ ATPase) activity

A proton pump enzyme source was prepared by the same method as in Experiment 1-1 of WO 94/14795. Further, the inhibitory effect of proton pump activity was measured by the same method as in Experiment 1-2 of WO 94/14795.

Namely, the proton pump activity stimulated by Mg^{++} was used as a negative comparative group, and the activity stimulated by Mg^{++} and K^+ was used as a positive comparative group. The comparative compound was omeprazole.

Test tubes were divided into 4 groups: Group 1 as negative comparative group(n=3), Group 2 as positive comparative group(n=3), Group 3(n=5X2) to be administered with the compound of the present invention and Group 4(n=5X2) to be administered with the comparative compound.

The inhibitory effects of Groups 3 and 4 on proton pump activity were measured by employing the compound prepared in Example and omeperazole, respectively, each of which was dissolved in dimethylsulfoxide at 5 different concentrations.

To each of Groups 1, 2, 3 and 4 were added 100μl of magnesium chloride(40mM) dissolved in 40mM Tris-HCl buffer(pH 6.0) and 100μ of the enzyme source. Then, 50μ of potassium chloride(50mM) and 50μl of ammonium chloride(6mM) dissolved in 40mM Tris-HCl buffer(pH 6.0) were added to all groups except for Group 1.

10μl of dimethylsulfoxide was added to each of Groups 1 and 2; and to Group 3 was added 10μl of the solution in which the compound prepared in Example was dissolved in dimethylsulfoxide at 5 different concentrations(n=5X2). To Group 4, 10μl of the solution prepared by dissolving omeprazole in dimethylsulfoxide at 5 different concentrations(37.6, 21.4, 12.2, 7.0 and 4.0μM) was added(n=5X2). 40mM Tris-HCl buffer(pH=6.0) was added thereto so as to make the total volume 400μl.

Thereafter, the test tubes of each Group were placed at 37°C for 30 minutes for the preincubation. 100μl of ATP

solution (6.6mM) was added until the reaction volume became 500μl. After the reaction was carried out at 37°C for 30 minutes, 25% cold trichloroacetic acid was added to terminate the enzyme reaction. The released inorganic phosphate was measured by an automatic analyzer(Express 550, Corning).

The difference between Group 1 and Group 2 represents the proton pump activity activated by K^+ only. The inhibition percentages of Groups 3 and 4 were calculated from Litchfield-wilcoxon equation[see, e.g., J. pharmacol. Exp. Ther., 96, 99(1949)]. The concentrations of the test compounds which inhibit 50% of the proton pump activity are represented as IC50 in Table 1.

Table 1

Test compound	IC50(μM) Test compound	IC50(μM) Omeprazole	Effect ratio
Example 1	5.4	5.8	1.08
Example 2	0.9	7.3	7.82
Example 3	3.5	7.3	2.11
Example 5	1.3	6.4	4.91
Example 6	4.3	6.4	4.91
Example 8	~12.5	7.7	~0.60
Example 9	~10.0	11.2	~1.12
Example 10	10.6	7.3	0.69
Example 12	0.6	5.8	9.83
Example 13	0.5	5.8	10.70
Example 14	0.7	5.8	8.70
Example 15	1.6	5.8	3.69
Example 16	1.5	5.8	3.80
Example 17	1.8	5.8	3.20
Example 18	4.2	11.4	2.69

Table 1 (continued)

Test compound	IC50(μM) Test compound	IC50(μM) Omeprazole	Effect ratio
Example 19	3.9	11.4	2.92
Example 20	4.4	11.4	2.59
Example 21	1.5	10.9	7.33
Example 22	1.4	10.9	7.26
Example 23	2.0	10.9	5.45
Example 24	0.6	10.9	19.33
Example 25	1.4	11.1	8.10
Example 26	0.8	12.6	15.62
Example 27	2.1	12.9	6.26
Example 28	>15.0	14.2	<0.95
Example 29	0.4	6.4	17.49
Example 30	~8.4	14.2	~1.69
Example 31	~15.0	7.1	~0.51
Example 32	1.2	10.1	8.40
Example 34	1.0	10.1	4.02
Example 35	2.5	10.1	4.02
Example 37	0.7	7.1	9.85
Example 38	2.2	7.1	3.24
Example 39	>15.0	14.2	<0.95
Example 40	0.7	6.4	9.17
Example 41	0.6	10.1	18.10
Example 42	1.5	7.3	4.95
Example 43	0.5	7.1	14.44
Example 44	~11.3	12.2	~1.08
Example 45	3.1	12.90	4.12
Example 46	~19.2	12.20	~0.60
Example 47	~5.0	7.70	~1.54
Example 48	~5.5	10.80	~1.97
Example 49	~10.8	12.20	~1.13
Example 50	~16.9	12.20	~0.70
Example 51	1.1	8.00	7.05
Example 52	0.5	11.40	21.11
Example 53	2.1	11.40	5.38
Example 54	20.6	10.10	0.49
Example 55	2.1	10.10	4.80

Table 1 (continued)

Test compound	IC50(μM) Test compound	IC50(μM) Omeprazole	Effect ratio
Example 57	3.3	11.5	3.50
Example 58	>25.0	11.3	<0.45
Example 59	~12.5	7.7	~0.60
Example 60	~15.0	7.7	~0.51
Example 61	~12.5	7.7	~0.60
Example 62	>20.0	14.2	<0.71
Example 63	~12.0	14.2	~1.18
Example 64	~10.0	14.2	~1.42
Example 65	1.0	5.8	6.04
Example 66	0.9	5.8	6.40
Example 67	1.3	5.8	4.50
Example 68	3.1	5.8	1.86
Example 69	2.9	5.8	2.00
Example 70	3.5	5.8	1.70
Example 74	0.5	10.1	22.00
Example 75	1.2	7.1	5.89
Example 77	3.6	11.4	3.16
Example 78	8.5	11.4	1.35
Example 81	0.4	6.4	15.82
Example 82	1.1	10.1	9.40
Example 83	1.4	7.3	5.34

Test 2: Inhibition of gastric secretion

In accordance with the method disclosed in journal [Shay, H., et al., Gastroenterology, 5, 43-61(1945), inhibitory effect on gastric secretion was carried out.

Male Sprague-Dawley rats having a body weight of 200±10g were divided into 3 groups(n=5) and fasted for 24 hours before the experiment with free access to water. Under ether anesthesia, the abdomen was incised, and the pylorus was ligated. As a comparative group, Group 1 was administered intraduodenally in a volume of 0.5mg/200g of 30% aqueous polyethylene glycol 400 solution. Groups 2 and 3 were administered intraduodenally with the compound of Example and omeprazole, respectively, each of which was suspended in 30% aqueous polyethylene glycol 400 solution at a concentration of 20mg/kg. After closing the abdominal cavity, the rats were placed for 5 hours and then killed by cervical dislocation. The stomach was extracted to obtain gastric juice.

The gastric juice was centrifuged at 1,000g to remove precipitates. The amount and acidity of the gastric juice were measured. Relative volumes, relative acid concentrations and relative acid outputs of the test compounds were calculated from equations(I), (II) and (III) and the results are shown in Table 2.

Relative volume ..... (I)
= (the average amount of gastric juice of Group 1 - the average amount of gastric juice of Group 2)
/ (the average amount of gastric juice of group 1- the average amount of gastric juice of Group 3)

Relative acid concentration..... (II)
= (the average acidity of Group 1 - the average acidity of Group 2)
/ (the average acidity of Group 1- the average acidity of Group 3)

Relative acid output ..... (III)
= (the total amount of acid output of Group 1- the total amount of acid output of Group 2)
/ (the total amount of acid output of Group 1- the total amount of acid output of Group 3).

Table 2

Compound	Rel. Vol. (%)	Rel. Conc. (%)	Relative Acid Output
Example 1	0.12	0.00	0.11
Example 2	0.92	0.8	0.89
Example 3	0.76	0.81	0.87
Example 4	0.99	0.56	0.87
Example 5	0.59	0.27	0.61
Example 6	0.64	0.28	0.64
Example 7	0.51	0.09	0.48
Example 8	0.43	0.12	0.42
Example 9	0.4	-0.03	0.3
Example 10	0.58	0.47	0.55
Example 11	0.99	0.41	0.82
Example 12	1.64	0.29	0.75
Example 13	1.72	0.46	0.81
Example 14	0.53	0.3	0.72
Example 15	0.8	1.06	0.99
Example 16	0.96	1.24	1.13
Example 17	0.82	0.97	0.89
Example 18	1.72	1.82	1.39
Example 19	1.8	1.86	1.43
Example 20	1.66	1.75	1.28
Example 21	1.06	0.88	0.97
Example 22	0.99	0.80	0.90
Example 23	0.92	0.78	0.88
Example 24	1.00	1.03	1.01
Example 25	1.06	0.80	0.92
Example 26	0.6	0.53	0.73
Example 27	0.7	0.61	0.81
Example 28	0.71	0.44	0.78
Example 29	0.56	0.31	0.6
Example 30	0.33	0.2	0.39

Table 2 (continued)

Compound	Rel. Vol. (%)	Rel. Conc. (%)	Relative Acid Output
Example 31	0.83	0.21	0.74
Example 32	1.03	0.97	0.91
Example 33	0.93	1.13	0.94
Example 34	0.99	1	0.99
Example 35	1.05	0.84	0.94
Example 36	0.65	0.05	0.41
Example 37	0.82	0.42	0.82
Example 38	0.74	0.37	0.74
Example 39	0.58	0.18	0.56
Example 40	0.71	0.36	0.74
Example 41	0.94	1.87	1.12
Example 42	1.15	1.4	1.15
Example 43	0.7	0.59	0.82
Example 44	0.27	0.33	0.41
Example 45	0.84	0.75	0.89
Example 46	0.73	0.44	0.74
Example 47	0.38	0.14	0.38
Example 48	0.17	0.04	0.16
Example 49	0.2	0.02	0.16
Example 50	0.72	0.29	0.66
Example 51	0.59	0.12	0.59
Example 52	1.34	0.94	1.12
Example 53	0.47	1.14	0.55
Example 54	0.86	0.23	0.75
Example 55	0.56	0.11	0.51
Example 56	0.01	0.08	0.08
Example 57	1.20	0.27	0.61
Example 58	0.58	0.16	0.35
Example 59	0.51	0.25	0.56
Example 60	0.65	0.28	0.65
Example 61	0.32	0.22	0.40
Example 62	0.61	0.31	0.67
Example 63	0.69	0.33	0.72
Example 64	0.34	0.26	0.40
Example 65	1.43	0.35	0.71
Example 66	1.49	0.47	0.85
Example 67	1.36	0.31	0.62
Example 68	1.52	0.23	0.67
Example 69	1.61	0.39	0.76
Example 70	1.30	0.22	0.61

Table 2 (continued)

Compound	Rel. Vol. (%)	Rel. Conc. (%)	Relative Acid Output
Example 71	0.25	-0.07	0.16
Example 72	0.34	0.16	0.25
Example 73	0.18	-0.05	0.13
Example 74	1.22	1.49	1.16
Example 75	1.30	1.62	1.20
Example 76	0.28	0.17	0.33
Example 77	1.48	1.63	1.30
Example 78	1.06	1.75	1.09
Example 79	0.54	1.28	0.49
Example 80	0.32	0.29	0.44
Example 81	0.56	0.47	0.68
Example 82	0.69	0.59	0.79
Example 83	1.19	1.29	1.13

Test 3. Reversibility Test

Gastric vesicles were prepared by the same method as in Experiment 4-1 of WO 94/14795. The inhibition mechanism of proton pump activity by the present invention compound was tested in accordance with the so-called Dilution and Washout method [see e.g., D. J. Keeling, et al., Biochemical Pharmacology, 42(1), 123-130(1991)].

Namely, test tubes were divided into two group, Groups 1 and 2. Each group was divided into four subgroups. 90μl of 5 mM Pipes/Tris buffer(pH 7.4) and 10μl of DMSO were added to subgroups 1 and 2 of each group. 90μl of 5 mM Pipes/Tris buffer(pH 7.4) and 10μl of the compound prepared in Example 43(50μM) were added to subgroups 3 and 4 of each group. To all two groups, was added 100μl of lyophilized vesicles at the concentration of 100μg protein/ml and then preincubated at 37°C for 15 minutes.

2mM $MgCl_2$ was added to subgroups 1 and 3 of Group 1. 2mM $MgCl_2$ and 10mM KCl were added to subgroups 2 and 4 of

Group 1. 3mM ATP was added to all subgroups of Group 1 until the final volume became 500μl. After incubation for 30 minutes, the inhibition of H^+/K^+-ATPase activity by the test compound was measured.

After preincubation as described above, each subgroup of Group 2 was diluted with 50-fold volume of 5 mM Pipes/Tris buffer(pH7.4) and then centrifuged for 60 minutes by means of Beckman ultracentrifuge(Model L8-80). The supernatant was discarded and washed out by 10 ml of 5 mM Pipes/Tris buffer(pH 7.4). The resulting pellet was suspended with 5mM Pipes/Tris buffer (pH7.4) until the volume became the same as the preincubation volume.

Thereafter, in accordance with the treatment to Group 1, each subgroup of Group 2 was treated with 2mM $MgCl_2$, 10mM KCl and 3mM ATP. And the final volume of each subgroup of Group 2 was made to be 500μl. After incubation at 37°C for 30 minutes, the inhibition of H^+/K^+-ATPase activity was measured.

And it was further measured in accordance with the same procedures as above, except the compound prepared in Example 75 was used as a test compound. The inhibition of H^+/K^+ ATPase activity before and after the Dilution and Washout procedures is shown in Table 3.

Table 3

Compound	Dilution & Washout	
	Before	After
Example 43	62	6
Example 75	66.6	15

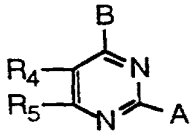
As shown in Table 3, the compounds of Examples 43 and 75 inhibit the enzyme activity by 62% and 66.6% before the Dilution and Washout procedure, whereas they show 6 or 15%

of inhibition of the enzyme activity after the Dilution and Washout procedure. This indicates that the inhibition of the enzyme activity of the present invention compounds is reversible.

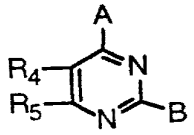
While the invention has been described with respect to the specific embodiments, it should be recognized that various modifications and changes may be made by those skilled in the art to the invention which also fall within the scope of the invention as defined as the appended claims.

What is claimed is :

1. Pyrimidine derivative compounds of formulae (I-1) and (I-2) inclusive of pharmaceutically acceptable salts thereof:



(I-1)

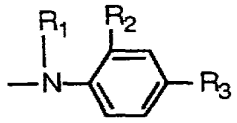


(I-2)

wherein:

R_4 and R_5, which may be the same or different, are independently hydrogen or a C_1-C_3 alkyl group, or jointly form a cyclopentyl or cyclohexyl ring;

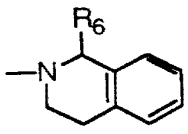
A is a group of formula(II):



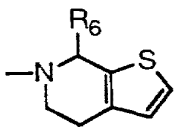
(II)

wherein R_1 and R_2 are, independently of each other, hydrogen or a C_1-C_3 alkyl group, and R_3 is hydrogen, a C_1-C_3 alkyl group or a halogen; and

B is 1-(substituted)-1,2,3,4-tetrahydroisoquinolin-2-yl of formula (III-1) or 7-(substituted)-4,5,6,7-tetrahydro-thieno[2,3-c]pyridin-6-yl of formula (III-2)



(III-1)



(III-2)

wherein R_6 is hydrogen or a C_1-C_3 alkyl group.

2. The compound of claim 1, which is selected from the group consisting of:
2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-methyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-methyl-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-2-(4-fluorophenylamino)pyrimidine hydrochloride;
6-methyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(2-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propylpyrimidine hydrochloride;
4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propyl-2-(4-fluorophenylamino)pyrimidine hydrochloride;
2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-6-propylpyrimidine hydrochloride;
5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,

3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl) pyrimidine hydrochloride;
5,6-dimethyl-2-(phenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-2-(4-phenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(2-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(4-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride;
2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride;
2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride;
2-(N-methylphenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinoline-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride;
6-methyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

6-methyl-2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinoline-2-yl)pyrimidine hydrochloride;
6-methyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
6-ethyl-2-(2-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazolinehydrochloride;
2-(4-fluorophenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride;
2-(N-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride;
2-(2-methylphenylamino)-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride;
6-methyl-2-(2-methyl-4-fluorophenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;

6-methyl-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]-pyridin-6-yl)-2-(4-fluorophenylamino)pyrimidine hydrochloride;

6-methyl-2-(N-methylphenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;

5,6-dimethyl-2-(2-methyl-4-fluorophenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;

5-methyl-2-(2-methyl-4-fluorophenylamino)-4-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)-6-ethylpyrimidine hydrochloride;

6-methyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

6-methyl-4-(4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

6-methyl-4-(2-methyl-4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;

6-methyl-4-(4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;

6-ethyl-2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

6-ethyl-4-(2-methyl-4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

6-ethyl-4-(4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

6-ethyl-4-(N-methylphenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

(R)-5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

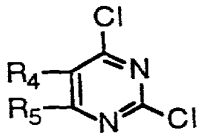
(S)-5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;

5,6-dimethyl-4-(4-fluorophenylamino)-2-(1-methyl-1,2,3,4-

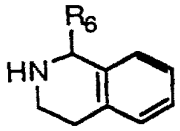
tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-4-(4-fluorophenylamino)-2-(1-methyl-1,2,-3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-4-(4-fluorophenylamino)-2-(1-methyl-1,2,-3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(N-methylphenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(R)-5,6-dimethyl-4-(N-methylphenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
(S)-5,6-dimethyl-4-(N-methylphenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(N-methylphenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
5,6-dimethyl-4-(2-methyl-4-fluorophenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;
5,6-dimethyl-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]-pyridin-6-yl)-4-(4-fluorophenylamino)pyrimidine hydrochloride;
5,6-dimethyl-4-(N-methylphenylamino)-2-(7-methyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-6-yl)pyrimidine hydrochloride;
5-methyl-6-ethyl-4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)pyrimidine hydrochloride;
4-(2-methyl-4-fluorophenylamino)-2-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)cyclopenta[d]pyrimidine hydrochloride;
2-(2-methyl-4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride; and
4-(2-methyl-4-fluorophenylamino)-2-(1,2,3,4-tetrahydroisoquinolin-2-yl)-5,6,7,8-tetrahydroquinazoline hydrochloride.

3. The compound of claim 1, wherein the pharmaceutically acceptable salts are hydrochlorides, sulfates, phosphates, nitrates, tartrates, fumarates, citrates, mesylates or acetates of the pyrimidine derivative compounds of formulae (I-1) and (I-2).

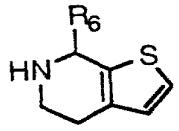
4. A process for preparing a pyrimidine derivative compound of formula (I-1), which comprises reacting a compound of formula(IV) with a compound of formula (V-1) or (V-2) to give a compound of formula(VI-1); and reacting the compound of formula(VI-1) with a compound of formula(VII):



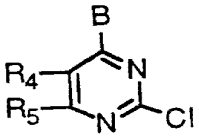
(IV)



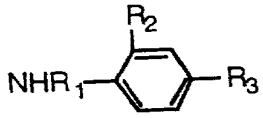
(V-1)



(V-2)



(VI-1)

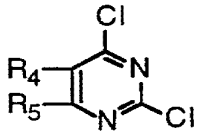


(VII)

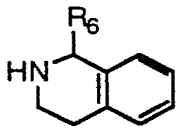
wherein A, B, R_1, R_2, R_3, R_4, R_5 and R_6 are the same as defined in claim 1.

5. The process of claim 4, wherein the reaction of the compound of formula (IV) with the compound of formula (V-1) or (V-2) is carried out in the presence of a solvent selected from the group consisting of dichloromethane, acetone, acetonitrile and dimethylformamide, and a base selected from the group consisting of triethylamine, N,N-dimethylaniline and pyridine.

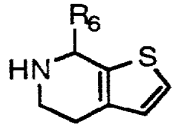
6. A process for preparing a pyrimidine derivative compound of formula (I-2), which comprises: hydrolyzing a compound of formula(IV) at its 4-position to give a compound of formula(VIII); reacting the compound of formula(VIII) with a compound of formula (V-1) or (V-2) to give a compound of formula(IX); chlorinating the compound of formula(IX) at its 4-position to give a compound of formula (VI-2); and then reacting the compound of formula (VI-2) with a compound of formula (VII):



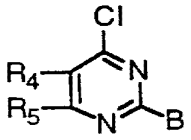
(IV)



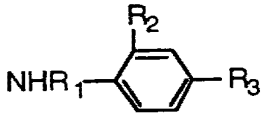
(V-1)



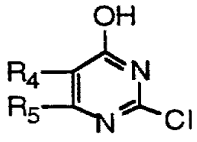
(V-2)



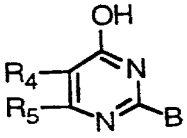
(VI-2)



(VII)



(VIII)

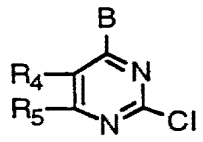


(IX)

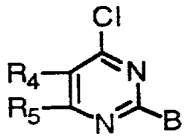
wherein A, B, R_1, R_2, R_3, R_4, R_5 and R_6 are the same as defined in claim 1.

7. The process of claim 6, where the reaction of the compound of formula (VIII) with the compound of formula (V-1) or (V-2) is carried out in the presence of a solvent selected from the group consisting of dichloromethane, acetone, acetonitrile and dimethylformamide, and a base selected from the group consisting of triethylamine, N,N-dimethylaniline and pyridine.

8. Pyrimidine derivative compounds of formulae (VI-1) and (VI-2):



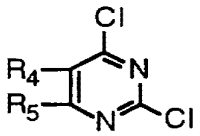
(VI-1)



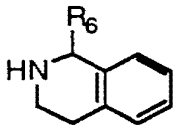
(VI-2)

wherein B, R_4 and R_5 are the same as defined in claim 1.

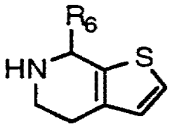
9. A process for preparing a compound of formula (VI-1), which comprises reacting a compound of formula(IV) with a compound of formula (V-1) or (V-2):



(IV)



(V-1)



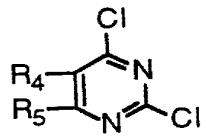
(V-2)

wherein R_4, R_5 and R_6 are the same as defined in claim 1.

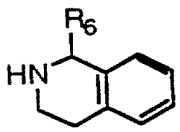
10. The process of claim 9, wherein the reaction of the compound of formula (IV) with the compound of formula (V-1) or (V-2) is carried out in the presence of a solvent selected from the group consisting of dichloromethane, acetone, acetonitrile and dimethylformamide, and a base selected from the group consisting of triethylamine, N,N-dimethylaniline and pyridine.

11. A process for preparing a compound of formula (VI-2), which comprises: hydrolyzing a compound of formula(IV) at its 4-position to give a compound of formula(VIII); reacting the compound of formula(VIII) with a compound of formula (V-1) or (V-2) to give a compound of formula(IX); and then chlorinating the compound of

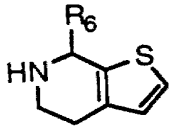
formula(IX) at its 4-position:



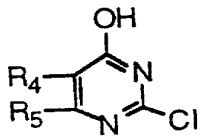
(IV)



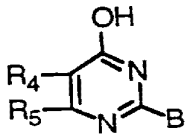
(V-1)



(V-2)



(VIII)



(IX)

wherein R_4, R_5 and R_6 are the same as defined in claim 1.

12. The process of claim 11, wherein the reaction of the compound of formula (VIII) with the compound of formula (V-1) or (V-2) is carried out in the presence of a solvent selected from the group consisting of dichloromethane, acetone, acetonitrile and dimethylformamide, and a base selected from the group consisting of triethylamine, N,N-dimethylaniline and pyridine.

13. A pharmaceutical composition comprising a therapeutically effective amount of any of the pyrimidine derivative compounds of claim 1 and a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 95/00105

A. CLASSIFICATION OF SUBJECT MATTER
IPC[6]: C 07 D 239/42,401/04,409/14; A 61 K 31/505,31/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC[6]: C 07 D 239/42,401/04,409/14; A 61 K 31/505,31/38

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AT

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DARC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	Chemical Abstracts, Vol.122, No.23, 05 June 1995 (Columbus, Ohio, USA), page 1041, column 1, abstract No.290883s, SHIBATA, Masahiro et al. "Preparation of substituted pyrimidine derivatives as analgesics and antiinflammatory agents", & Jpn. Kokai Tokkyo Koho JP 07 53,527 [95 53,527].	1-13
A	Chemical Abstracts, Vol.120, No.8, 21 February 1994 (Columbus, Ohio, USA), page 654, column 2, abstract No.86474g, KIMURA, Isami et al. "Pyrimidine derivative for treatment of ulcerative colitis", & Jpn. Kokai Tokkyo Koho JP 05,262,747 [93,262,747].	1-13
A	Chemical Abstracts, Vol.118, No.10, 08 March 1993 (Columbus, Ohio, USA), page 457, column 1, abstract No.87674z, TAKEDA, Dennai et al. "Oral preparations containing antiallergy pyrimidine derivative", & Jpn. Kokai Tokkyo Koho JP 04,305,526 [92,305,526].	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 October 1995 (20.10.95)	07 November 1995 (07.11.95)
Name and mailing address of the ISA/ AT AUSTRIAN PATENT OFFICE Kohlmarkt 8-10 A-1014 Vienna Facsimile No. 1/53424/535	Authorized officer Brus e.h. Telephone No. 1/5337058/32

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WO 9605199A1

(51) Internationale Patentklassifikation 6 :
C07D 471/04, A61K 31/435, C07C 59/245 // (C07D 471/04, 235:00, 221:00)

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(81) Bestimmungsstaaten: AU, BG, BR, BY, CA, CN, CZ, EE, FI, HU, JP, KR, LT, LV, MX, NO, NZ, PL, RO, RU, SG, SI, SK, UA, US, europäisches Patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Veröffentlicht
Mit internationalem Recherchenbericht.

(54) Title: IMIDAZOPYRIDINE SALT

(54) Bezeichnung: IMIDAZOPYRIDINSALZ

(57) Abstract

The invention concerns the compound 8-(2-methoxycarbonylamino-6-methyl-benzylamino)-2,3-dimethylimidazo-[1,2-a]pyridine-D,L-hemimalate and the therapeutic use of the compound.

(57) Zusammenfassung

Die Erfindung betrifft die Verbindung 8-(2-Methoxycarbonylamino-6-methylbenzylamino)-2,3-dimethylimidazo-[1,2-a]pyridin-D,L-hemimalat und ihre therapeutische Anwendung.

LEDIGLICH ZUR INFORMATION

Codes zur Identifizierung von PCT-Vertragsstaaten auf den Kopfbögen der Schriften, die internationale Anmeldungen gemäss dem PCT veröffentlichen.

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Imidazopyridinsalz

Anwendungsgebiet der Erfindung

Die Erfindung betrifft ein neues Imidazopyridinsalz, das in der pharmazeutischen Industrie als Wirkstoff für die Herstellung von Arzneimitteln verwendet werden soll.

Bekannter technischer Hintergrund

In der europäischen Patentanmeldung EP-A-0 033 094 werden Imidazo[1,2-a]pyridine beschrieben, die in 8-Position einen Arylsubstituenten tragen, der bevorzugt ein Phenyl-, Thienyl-, Pyridyl- oder ein durch Chlor, Fluor, Methyl, tert.-Butyl, Trifluormethyl, Methoxy oder Cyan substituierter Phenylrest ist. Als besonders interessante Arylreste sind in der EP-A-0 033 094 die Reste Phenyl, o- oder p-Fluorphenyl, p-Chlorphenyl und 2,4,6-Trimethylphenyl genannt, wovon die Reste Phenyl, o- oder p-Fluorphenyl und 2,4,6-Trimethylphenyl besonders bevorzugt sind. - In den europäischen Patentanmeldungen EP-A-0 204 285, EP-A-0 228 006, EP-A-0 268 989 und EP-A-0 308 917 werden Imidazo[1,2-a]pyridine beschrieben, die in 3-Position einen ungesättigten aliphatischen Rest, insbesondere einen (substituierten) Alkinylrest tragen. - In der europäischen Patentanmeldung EP-A-0 266 890 werden Imidazo[1,2-a]pyridine beschrieben, die in 8-Position durch einen Alkenyl-, Alkyl- oder Cycloalkylalkylrest substituiert sind.

Beschreibung der Erfindung

Es wurde nun gefunden, daß das Äpfelsäuresalz der nachfolgend näher beschriebenen Verbindung überraschende und besonders vorteilhafte Eigenschaften besitzt.

Gegenstand der Erfindung ist das Salz der Verbindung 8-(2-Methoxycarbonylamino-6-methylbenzylamino)-2,3-dimethylimidazo[1,2-a]pyridin mit D,L-Äpfelsäure, also das 8-(2-Methoxycarbonylamino-6-methylbenzylamino)-2,3-dimethylimidazo[1,2-a]pyridin-D,L-hemimalat und seine Enantiomeren.

Das 8-(2-Methoxycarbonylamino-6-methylbenzylamino)-2,3-dimethylimidazo-[1,2-a]pyridin kann so wie in den nachfolgenden Beispielen beschrieben hergestellt werden. Die Herstellung des Malats ist ebenfalls in den Beispielen beschrieben.

Die Abkürzung RT steht für Raumtemperatur, h steht für Stunde(n), min für Minute(n), Schmp. für Schmelzpunkt, Zers. für Zersetzung.

Beispiele

Endprodukt

1. 8-(2-Methoxycarbonylamino-6-methylbenzylamino)-2,3-dimethylimidazo[1,2-a]pyridin-D,L-hemimalat

a) Man trägt 5,0 g 8-(2-Methoxycarbonylamino-6-methylbenzylamino)-2,3-dimethylimidazo[1,2-a]pyridin in kleinen Portionen bei 40°C unter Rühren in eine Lösung von 1,04 g D,L-Äpfelsäure in 35 ml Wasser ein, rührt noch 2 h bei 40°C und über Nacht bei RT. Man stellt mit Natronlauge auf pH 4,7 und läßt unter Rühren im Eisbad kristallisieren. Man erhält 5,85 g (97,7 %) der Titelverbindung vom Schmp. 174-175°C (Zers.).

b) Man verfährt analog, wie nachfolgend unter A. beschrieben, und versetzt anstelle von Fumarsäure mit einer konzentrierten Lösung von 2,0 g D,L-Äpfelsäure in Wasser. Das schwer lösliche Salz wird abgesaugt und aus Isopropanol/Wasser umkristallisiert. Man erhält die Titelverbindung vom Schmp. 174-175°C (Zers.).

2. 8-(2-Methoxycarbonylamino-6-methylbenzylamino)-2,3-dimethylimidazo-[1,2-a]pyridin-L(-)-hemimalat

Verfährt man analog Beispiel 1.a) und verwendet L(-)-Äpfelsäure, erhält man die Titelverbindung vom Schmp. 174-175°C (Zers.).

Ausgangsverbindungen

A. 8-(2-Methoxycarbonylamino-6-methylbenzylamino)-2,3-dimethylimidazo-[1,2-a]pyridin

Eine Lösung von 4,1 g 8-Amino-2,3-dimethylimidazo[1,2-a]pyridin und 6,0 g (2-Chlormethyl-3-methylphenyl)carbamidsäuremethylester in 100 ml Aceton wird mit 1,1 g Natriumjodid und 6,7 g pulverisiertem, wasserfreiem Natriumcarbonat versetzt und 24 h bei RT gerührt. Man filtriert die anorganischen Salze ab, wäscht gut mit Aceton aus, engt das Filtrat im Rotationsverdampfer ein, versetzt mit Wasser und schüttelt mit Ethylacetat aus. Man engt im Rotationsverdampfer ein, löst den Rückstand in 50 ml Aceton und setzt eine Lösung von 1,8 g Fumarsäure in 180 ml Aceton zu. Aus der auf die Hälfte konzentrierten Lösung filtriert man 8,5 g Hemifumarat der Titelverbindung ab. Das Salz wird in einer Mischung von 40 ml Aceton und 100 ml Wasser bei 40°C mit verdünnter Natronlauge bis pH 9,5 neutralisiert und die ausgefallene freie Base aus Ethylacetat/Petrolether umkristallisiert. Man erhält 5,6 g (65 %) der Titelverbindung vom Schmp. 136-138°C.

B. 8-Amino-2,3-dimethylimidazo[1,2-a]pyridin

Die Verbindung ist aus EP-A-0 299 470 bekannt bzw. sie kann analog dazu wie dort beschrieben hergestellt werden.

C. 2-Methoxycarbonylamino-6-methylbenzylchlorid

Die Verbindung ist aus EP-A-0 308 917 bekannt bzw. sie kann analog dazu wie dort beschrieben hergestellt werden.

Gewerbliche Anwendbarkeit

Das 8-(2-Methoxycarbonylamino-6-methylbenzylamino)-2,3-dimethylimidazo-[1,2-a]pyridin-D,L-hemimalat besitzt wertvolle pharmakologische Eigenschaften, die es gewerblich verwertbar machen. Es weist insbesondere eine ausgeprägte Magensäuresekretionshemmung und eine ausgezeichnete Magen- und Darmschutzwirkung bei Warmblütern auf. Hierbei zeichnet sich die erfindungsgemäße Verbindung durch eine hohe Wirkungsselektivität, eine vergleichsweise lange Wirkungsdauer, eine gute enterale Wirksamkeit, das Fehlen wesentlicher Nebenwirkungen, eine große therapeutische Breite und insbesondere eine ausgezeichnete Bioverfügbarkeit aus.

Unter "Magen- und Darmschutz" wird in diesem Zusammenhang die Verhütung und Behandlung gastrointestinaler Krankheiten, insbesondere gastrointestinaler entzündlicher Krankheiten und Läsionen (wie z.B. Ulcus ventriculi, Ulcus duodeni, Gastritis, hyperazider oder medikamentös bedingter Reizmagen) verstanden, die beispielsweise durch Mikroorganismen (z.B. Helicobacter pylori), Bakterientoxine, Medikamente (z.B. bestimmte Antiphlogistika und Antirheumatika), Chemikalien (z.B. Ethanol), Magensäure oder Streßsituationen verursacht werden können. Die erfindungsgemäße Verbindung besitzt hierbei auch eine Eigenwirkung gegen den Keim Helicobacter pylori.

In ihren ausgezeichneten Eigenschaften erweist sich die erfindungsgemäße Verbindung an verschiedenen Modellen, in denen die antiulcerogenen und die antisekretorischen Eigenschaften bestimmt werden, überraschenderweise den aus dem Stand der Technik bekannten Verbindungen deutlich überlegen. Aufgrund dieser Eigenschaften ist die erfindungsgemäße Verbindung für den Einsatz in der Human- und Veterinärmedizin hervorragend geeignet, wobei sie insbesondere zur Behandlung und/oder Prophylaxe von Erkrankungen des Magens und/oder Darms verwendet wird.

Ein weiterer Gegenstand der Erfindung ist daher die erfindungsgemäße Verbindung zur Anwendung bei der Behandlung und/oder Prophylaxe der vorstehend genannten Krankheiten.

Ebenso umfaßt die Erfindung die Verwendung der erfindungsgemäßen Verbindung zur Herstellung von Arzneimitteln, die zur Behandlung und/oder Prophylaxe der vorstehend genannten Krankheiten eingesetzt werden.

Weiterhin umfaßt die Erfindung die Verwendung der erfindungsgemäßen Verbindung zur Behandlung und/oder Prophylaxe der vorstehend genannten Krankheiten.

Ein weiterer Gegenstand der Erfindung sind Arzneimittel, die die erfindungsgemäße Verbindung enthalten.

Die Arzneimittel werden nach an sich bekannten, dem Fachmann geläufigen Verfahren hergestellt. Als Arzneimittel wird die erfindungsgemäße Verbindung (= der Wirkstoff) entweder als solche, oder vorzugsweise in Kombination mit geeigneten pharmazeutischen Hilfs- oder Trägerstoffen in Form von Tabletten, Dragees, Kapseln, Suppositorien, Pflastern (z.B. als TTS), Emulsionen, Suspensionen oder Lösungen eingesetzt, wobei der Wirkstoffgehalt vorteilhafterweise zwischen 0,1 und 95 % beträgt und wobei durch die entsprechende Wahl der Hilfs- und Trägerstoffe eine auf den Wirkstoff und/oder auf den gewünschten Wirkungseintritt genau angepaßte galenische Darreichungsform (z.B. eine Retardform oder eine magensaftresistente Form) erzielt werden kann.

Welche Hilfs- bzw. Trägerstoffe für die gewünschten Arzneimittelformulierungen geeignet sind, ist dem Fachmann aufgrund seines Fachwissens geläufig. Neben Lösemitteln, Gelbildnern, Suppositoriengrundlagen, Tablettenhilfsstoffen und anderen Wirkstoffträgern können beispielsweise Antioxidantien, Dispergiermittel, Emulgatoren, Entschäumer, Geschmackskorrigentien, Konservierungsmittel, Lösungsvermittler, Farbstoffe oder insbesondere Permeationspromotoren und Komplexbildner (z.B. Cyclodextrine) verwendet werden.

Die Wirkstoffe können oral, parenteral oder percutan appliziert werden.

Im allgemeinen hat es sich in der Humanmedizin als vorteilhaft erwiesen, den Wirkstoff bei oraler Gabe in einer Tagesdosis von etwa 0,01 bis etwa

20, vorzugsweise 0,05 bis 5, insbesondere 0,1 bis 1,5 mg/kg Körpergewicht, gegebenenfalls in Form mehrerer, vorzugsweise 1 bis 4 Einzelgaben zur Erzielung des gewünschten Ergebnisses zu verabreichen. Bei einer parenteralen Behandlung können ähnliche bzw. (insbesondere bei der intravenösen Verabreichung des Wirkstoffs) in der Regel niedrigere Dosierungen zur Anwendung kommen. Die Festlegung der jeweils erforderlichen optimalen Dosierung und Applikationsart des Wirkstoffs kann durch jeden Fachmann aufgrund seines Fachwissens leicht erfolgen.

Soll die erfindungsgemäße Verbindung zur Behandlung der oben genannten Krankheiten eingesetzt werden, so können die pharmazeutischen Zubereitungen auch einen oder mehrere pharmakologisch aktive Bestandteile anderer Arzneimittelgruppen, wie Antacida, beispielsweise Aluminiumhydroxyd, Magnesiumaluminat; Tranquilizer, wie Benzodiazepine, beispielsweise Diazepam; Spasmolytika, wie z.B. Bietamiverin, Camylofin, Anticholinergica, wie z.B. Oxyphencyclimin, Phencarbamid; Lokalanaesthetika, wie z.B. Tetracain, Procain; gegebenenfalls auch Fermente, Vitamine oder Aminosäuren enthalten.

Hervorzuheben ist in diesem Zusammenhang insbesondere die Kombination der erfindungsgemäßen Verbindung mit Pharmaka, die die Säuresekretion hemmen, wie beispielsweise H_2-Blockern (z.B. Cimetidin, Ranitidin), H^+/K^+-ATPase-Hemmstoffen (z.B. Omeprazol, Pantoprazol), oder ferner mit sogenannten peripheren Anticholinergika (z.B. Pirenzepin, Telenzepin) sowie mit Gastrin-Antagonisten mit dem Ziel, die Hauptwirkung in additivem oder überadditivem Sinn zu verstärken und/oder die Nebenwirkungen zu eliminieren oder zu verringern, oder ferner die Kombination mit antibakteriell wirksamen Substanzen (wie z.B. Cephalosporinen, Tetracyclinen, Nalidixinsäure, Penicillinen oder auch Wismutsalzen) zur Bekämpfung von Helicobacter pylori.

Pharmakologie

Die ausgezeichnete Magenschutzwirkung und die magensäuresekretionshemmende Wirkung der erfindungsgemäßen Verbindung kann in Untersuchungen an tierexperimentellen Modellen nachgewiesen werden.

Prüfung der sekretionshemmenden Wirkung am perfundierten Rattenmagen

In der folgenden Tabelle 1 ist der Einfluß der erfindungsgemäßen Verbindung nach intraduodenaler Gabe auf die durch Pentagastrin stimulierte Säuresekretion des perfundierten Rattenmagens in vivo dargestellt.

Tabelle 1

Dosis (µmol/kg) i.d.	Hemmung der Säureausscheidung (%)
3	95

Methodik

Narkotisierten Ratten (CD-Ratte, weiblich, 200-250 g; 1,5 g/kg i.m. Urethan) wurde nach Tracheotomie das Abdomen durch einen medianen Oberbauchschnitt eröffnet und ein PVC-Katheter transoral im Ösophagus sowie ein weiterer via Pylorus derart fixiert, daß die Schlauchenden eben noch in das Magenlumen hineinragten. Der aus dem Pylorus führende Katheter führte über eine seitliche Öffnung in der rechten Bauchwand nach außen.

Nach gründlicher Spülung (ca. 50-100 ml) wurde der Magen mit 37°C warmer physiologischer NaCl-Lösung kontinuierlich durchströmt (0,5 ml/min, pH 6,8-6,9; Braun-Unita I). In dem jeweils im 15 Minuten-Abstand aufgefangenen Effluat wurde der pH-Wert (pH-Meter 632, Glaselektrode EA 147; ϕ = 5 mm,

Metrohm) sowie durch Titration mit einer frisch zubereiteten 0,01 N NaOH bis pH 7 (Dosimat 665 Metrohm) die sezernierte HCl bestimmt.

Die Stimulation der Magensekretion erfolgte durch Dauerinfusion von 1 µg/kg (= 1,65 ml/h) i.v. Pentagastrin (V. fem. sin.) ca. 30 min nach Operationsende (d.h. nach Bestimmung von 2 Vorfraktionen). Die zu prüfende Substanz wurden intraduodenal in 1 ml/kg Flüssigkeitsvolumen 60 min nach Beginn der Pentagastrin-Dauerinfusion verabreicht.

Die Körpertemperatur der Tiere wurde durch Infrarot-Bestrahlung und Heizkissen (automatische, stufenlose Regelung über rektalen Temperaturfühler) auf konstant 37,8 - 38°C gehalten.

Patentansprüche

1. 8-(2-Methoxycarbonylamino-6-methylbenzylamino)-2,3-dimethylimidazo-[1,2-a]pyridin-D,L-hemimalat und seine Enantiomeren.

2. Arzneimittel enthaltend die Verbindung nach Anspruch 1 zusammen mit üblichen pharmazeutischen Hilfs- und/oder Trägerstoffen.

3. Verbindung nach Anspruch 1 zur Anwendung bei der Verhütung und Behandlung gastrointestinaler Krankheiten.

4. Verwendung der Verbindung nach Anspruch 1 zur Herstellung von Arzneimitteln für die Verhütung und Behandlung gastrointestinaler Krankheiten.

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/EP 95/03139

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D471/04 A61K31/435 C07C59/245 //(C07D471/04,235:00, 221:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 033 094 (SCHERING) 5 August 1981 cited in the application see page 1, paragraph 1; claim 1	1,2
P,X	WO,A,94 18199 (BYK GULDEN LOMBERG) 18 August 1994 see page 2, line 26; claims 1,8; example 5	1-4

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
26 October 1995	- 7. 11. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Alfaro Faus, I

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interm al Application No
PCT/EP 95/03139

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-33094	05-08-81	AU-B-	540840	06-12-84
		AU-B-	6633781	30-07-81
		CA-A-	1167845	22-05-84
		JP-A-	56113782	07-09-81
		OA-A-	6727	30-06-82
WO-A-9418199	18-08-94	AU-B-	6039194	29-08-94
		NO-A-	953187	14-08-95

INTERNATIONALER RECHERCHENBERICHT

Intern: ales Aktenzeichen
PCT/EP 95/03139

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
IPK 6 C07D471/04 A61K31/435 C07C59/245 //(C07D471/04,235:00, 221:00)

Nach der Internationalen Patentklassifikation (IPK) oder nach der nationalen Klassifikation und der IPK

B. RECHERCHIERTE GEBIETE

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IPK 6 C07D A61K

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Während der internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)

C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie°	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
A	EP,A,0 033 094 (SCHERING) 5.August 1981 in der Anmeldung erwähnt siehe Seite 1, Absatz 1; Anspruch 1	1,2
P,X	WO,A,94 18199 (BYK GULDEN LOMBERG) 18.August 1994 siehe Seite 2, Zeile 26; Ansprüche 1,8; Beispiel 5	1-4

☐ Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

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Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
26.Oktober 1995	- 7. 11. 95

Name und Postanschrift der Internationale Recherchenbehörde	Bevollmächtigter Bediensteter
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INTERNATIONALER RECHERCHENBERICHT

Angaben zu Veröffentlichungen, die zur selben Patentfamilie gehören

Intern ales Aktenzeichen
PCT/EP 95/03139

Im Recherchenbericht angeführtes Patentdokument	Datum der Veröffentlichung	Mitglied(er) der Patentfamilie		Datum der Veröffentlichung
EP-A-33094	05-08-81	AU-B-	540840	06-12-84
		AU-B-	6633781	30-07-81
		CA-A-	1167845	22-05-84
		JP-A-	56113782	07-09-81
		OA-A-	6727	30-06-82
WO-A-9418199	18-08-94	AU-B-	6039194	29-08-94
		NO-A-	953187	14-08-95

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(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

(57) Abstract

A pharmaceutical composition for oral administration for the treatment of acute pain and inflammation comprises an inclusion complex of a non-steroidal anti-inflammatory drug or a pharmaceutically acceptable salt thereof and a cyclodextrin, and a physiologically acceptable alkali agent selected from the group consisting of alkali and alkaline earth metal carbonates, bicarbonates, phosphates and hydroxides, and water-soluble amines, in an amount equivalent to between 2 and 30 molar equivalents inclusive of the non-steroidal anti-inflammatory drug, the alkali agent being capable of forming an alkaline diffusion layer around the composition in the gastrointestinal tract.

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PHARMACEUTICAL COMPOSITION COMPRISING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

BACKGROUND OF THE INVENTION

This invention relates to a pharmaceutical composition for oral administration comprising an inclusion complex of a non-steroidal anti-inflammatory drug (NSAID) and a cyclodextrin, and an alkali agent.

Oral treatment with non-steroidal anti-inflammatory drugs has the disadvantage of gastrointestinal side effects, particularly local gastric irritation. Contact between the NSAID and the mucosa is believed to constitute an important factor in the pathogenesis of gastric irritation [Bianchi, P.G et al. Why are non-steroidal anti-inflammatory drugs important

in peptic ulceration? Alimentary Pharmacology and Therapeutics 1987, 1, 5405-5475]. Commercial preparations of NSAID's for oral administration include enteric coated tablets which release the drug in the duodenum so as to avoid local gastric irritation. This however has the disadvantage that peak plasma levels of the drug are reached between one to four hours after administration of the enteric coated tablets.

An example of an NSAID is diclofenac which is a phenylacetic acid NSAID with potent anti-inflammatory and analgesic actions highly utilized in the treatment of acute and chronic pain especially when associated with inflammation e.g. post operative pain, rheumatism, arthritis, gout, musculo-skeletal injury and trauma. In order to reduce the lag-time associated with enteric coated diclofenac tablets, a non-enteric coated tablet and dispersible dosage form have recently been commercialized with indications for short-term treatment of acute inflammatory conditions, although gastro-intestinal side effects are frequent, particularly local gastric irritation [Martindale Extra Pharmacopoeia Edition 30].

There is therefore a need for oral pharmaceutical compositions of NSAIDs e.g. diclofenac, which provide rapid absorption with minimized gastrointestinal irritation.

The properties of cyclodextrins and numerous inclusion complexes are well known and have been reviewed in detail [Szejtli, J. Cyclodextrin Technology (1988) Kluwer Academic Publishers, Dordrecht].

Depending on solvent conditions, a dissolved inclusion complex exists in equilibrium between uncomplexed host and guest and complexed host/guest. Orally administered cyclodextrin-drug inclusion complexes generally result

in rapid absorption of the drug, facilitated by the cyclodextrin, whereas the cyclodextrin is not absorbed to any significant extent. Additionally cyclodextrin inclusion complexes of certain drugs have been shown to reduce gastrointestinal side effects [Frömming, K-H & Szejtli, J. Cyclodextrins in Pharmacy (1994), Kluwer Academic Publishers]. Cyclodextrins therefore possess ideal properties as drug carriers. Cyclodextrins and their inclusion complexes possess favourable flow, binding and compaction properties facilitating tablet compression.

The diffusability of a diclofenac (acid) complex with beta cyclodextrin has been reported [Availability of NSAIDH β-Cyclodextrin Inclusion Complexes. Orienti, I., Cavallari, C. and Zecchi, V. Arch. Pharm (Weinheim) 1989, 322, 207-211]. The complex was found to be poorly soluble at pH 2.

The effect of addition of buffering agents to tablet and capsule formulations of sparingly soluble acidic drugs e.g. furosemide, is known to enhance the dissolution rate of the drug in gastric media [Marais, A.F. & van der Watt J.G. Relationship between the pH of the diffusion layer and the dissolution rate of furosemide; Drug Development and Industrial Pharmacy 1991, 17, 1715-1720].

South African Patent No 94/5930 in the name of Smithkline Beecham PLC discloses a pharmaceutical composition for oral consumption in aqueous solution comprising a drug/beta-cyclodextrin complex, wherein the composition further comprises a pharmaceutically acceptable acid-base couple, in a quantity sufficient to cause the drug/beta-cyclodextrin complex to dissolve when the composition is mixed with cold water and provide a solution with acid or neutral pH. It is essential to this pharmaceutical composition that it contain the combination of an acid and a base couple,

which may or may not be effervescent.

SUMMARY OF THE INVENTION

According to the invention there is provided a pharmaceutical composition for oral administration comprising an inclusion complex of a non-steroidal anti-inflammatory drug or a pharmaceutically acceptable salt thereof and a cyclodextrin, and a physiologically acceptable alkali agent selected from the group consisting of alkali and alkaline earth metal carbonates, bicarbonates, phosphates and hydroxides, and water soluble amines, in an amount equivalent to between 2 and 30 molar equivalents inclusive of the non-steroidal anti-inflammatory drug, the alkali agent being capable of forming an alkaline diffusion layer around the composition in the gastrointestinal tract.

The NSAID may be any suitable NSAID such as for example diclofenac, indomethacin, naproxen, ibuprofen, mefenamic acid, piroxicam, tenoxicam, lornoxicam, or a pharmaceutically acceptable salt thereof.

The cyclodextrin may be any suitable cyclodextrin but is preferably a beta-cyclodextrin which may be substituted for example with methyl or hydroxypropyl groups, or preferably an unsubstituted beta-cyclodextrin. However, for tenoxicam and lornoxicam, the cyclodextrin is preferably alpha-cyclodextrin.

The alkali agent, when it is an amine, may be selected from ammonium hydroxide, tris(hydroxymethyl)aminomethane, ethanolamine and diethanolamine.

The alkali agent is preferably sodium hydrogen carbonate, also known as sodium bicarbonate, or tris(hydroxymethyl)aminomethane, also known as tromethamine.

The pharmaceutical composition is preferably formulated as a tablet or a capsule.

The pharmaceutical composition may also contain conventional excipients including binders such as starch and microcrystalline cellulose, diluents such as lactose, disintegrating agents such as sodium carboxymethylcellulose, and lubricants.

The NAISD:cyclodextrin inclusion complex preferably has a mass ratio of NSAID:cyclodextrin of between 1:0,85 and 1:5 inclusive, more preferably between 1:1 and 1:2,5 inclusive.

The active component is preferably an inclusion complex of diclofenac optionally in the form of a pharmaceutically acceptable salt such as diclofenac sodium or diclofenac potassium, and an unsubstituted beta-cyclodextrin. The mass ratio of diclofenac to beta-cyclodextrin is preferably between 1:1,8 and 1:9 inclusive, more preferably between 1:3 and 1:5 inclusive. The unit dose of the complex may be equivalent to between 10 and 100 mg inclusive diclofenac, but preferably between 20 and 60 mg inclusive diclofenac.

The active component may also be an inclusion complex of lornoxicam and alpha-cyclodextrin. The mass ratio of lornoxicam to alpha-cyclodextrin is preferably between 1:2,6 and 1:13 inclusive. The unit dose of the complex may be equivalent to between 2 and 10 mg inclusive lornoxicam, but

preferably between 4 and 8 mg lornoxicam.

The pharmaceutical composition may also contain at least one further active ingredient such as for example morphine, codeine, propoxyphene or paracetamol, or meprobamate.

The pharmaceutical composition of the invention may be used in the treatment of acute pain and inflammation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of plasma concentration versus time from Example 3; and

Figure 2 is a graph of the mean dissolution rate of the tablets of Example 5.

DESCRIPTION OF EMBODIMENTS

The invention relates to a pharmaceutical composition for oral administration comprising an inclusion complex of an NSAID or a pharmaceutically acceptable salt thereof and a cyclodextrin, and a physiologically acceptable alkali agent selected from the group consisting of alkali and alkaline earth metal carbonates, bicarbonates, phosphates and hydroxides, and water soluble amines, in an amount equivalent to between 2 and 30 molar equivalents inclusive of the NSAID, the alkali agent being capable of forming an alkaline diffusion layer around the composition in the gastrointestinal tract.

It is to be noted that the pharmaceutical composition of the invention must

not contain any additional acid agent which can form an acid-base couple with the alkali agent, or the alkali agent will not be capable of forming an alkaline diffusion layer around the composition in the gastrointestinal tract.

The incorporation of an alkali agent in the formulation of an NSAID-cyclodextrin complex improves release of the NSAID from the complex in gastric media.

The alkali further functions to neutralize the microenvironment surrounding the disintegrating tablet in the gastrointestinal tract, particularly the stomach. creating an alkaline diffusion layer. When administered to healthy human volunteers, non-enteric coated diclofenac sodium-beta cyclodextrin tablets according to the invention containing diclofenac shows a superior extent of absorption as measured by the area under the data curve (AUDC) parameter when compared with commercial non-enteric coated diclofenac tablets used as reference product. The variable C_{max} extends over the upper bound of the bioequivalence range relative to the reference product and the T_{max} variable is shorter by about 50 %. Tablets according to the invention containing diclofenac therefore appear to exhibit an increased rate of absorption relative to the reference product. An increased rate of absorption is likely to reduce contact time between diclofenac and the intestinal mucosa thereby reducing the potential for gastric irritation. In addition, presentation of the drug to the gastric mucosa as an inclusion complex may further reduce gastric irritation. The same effect is applicable to other NSAID's.

An inclusion complex for use in the pharmaceutical composition of the invention may be prepared as follows:

(1) pre-mixture of the required ratios of pre-screened NSAID and cyclodextrin;

(2) transfer to a suitable mixing vessel;

(3) gradual addition of deionised purified water with vigorous mixing until a paste-like consistency is reached;

(4) kneading of the paste for a suitable period of time, for example from 0,25 to 1 hour, with further occasional addition of deionised purified water if necessary to maintain the paste-like consistency until the inclusion complex is formed;

(5) drying the product of step (4); and

(6) screening the product of step (5).

To form the pharmaceutical composition of the invention, the inclusion complex is blended with the alkali agent and with suitable excipients and then formed into a suitable oral dosage form such as tablets or capsules.

The preferred active components of the pharmaceutical composition of the invention are an inclusion complex of diclofenac or a pharmaceutically acceptable salt thereof and an unsubstituted beta-cyclodextrin or lornoxicam or a pharmaceutically acceptable salt thereof and alpha-cyclodextrin. Various examples utilising such inclusion complexes will now be given.

Example 1

Diclofenac sodium (6,6 g) and beta-cyclodextrin (23,4g) are screened (30 mesh) and tumble mixed. The mixture is transferred to a mortar. Deionised water (10-15 ml) is gradually added with vigourous mixing to produce a uniform paste. Vigorous mixing is continued for 0,5 hours ensuring a uniform paste-like consistency throughout the operation. The mixture is oven dried at 40°C. The dried mass is crushed and passed through 30 mesh screen. The powder is homogenised in a powder mixer for 10 minutes. The product contains 21 % m/m diclofenac sodium as determined by HPLC. The

water content of the product is between 9 and 11 % m/m as determined by Karl Fisher titration. The molecular composition of the product thus corresponds to 1 molecule diclofenac sodium, 1 molecule beta cyclodextrin and between 7 and 10 water molecules. The particle size of the product corresponds to 90% less than 30 microns as measured under a light microscope. The morphology of the complex resembles very fine fractured crystalline particles.

Example 2

The inclusion complex of beta-cyclodextrin-diclofenac sodium obtained in Example 1 was formulated into tablets with the following unit composition:

Diclofenac sodium-beta cyclodextrin complex (equivalent to 50 mg diclofenac sodium)	220mg
Sodium hydrogen carbonate	200mg
Microcrystalline cellulose	130mg
Starch	44mg
Magnesium stearate	6mg
	600 mg

The sodium hydrogen carbonate, microcrystalline cellulose and starch are premixed in a blender. The diclofenac sodium-beta cyclodextrin complex is added to the mixture and blended. The magnesium stearate is screened in and blended. The mixture is compressed into tablets with a compression force of about 100 N. The tablets are optionally film-coated.

Example 3

Tablets prepared according to Example 2 were administered to six healthy

human volunteers in a double blind cross-over trial against a commercial non-enteric coated diclofenac potassium 50 mg tablet as reference product. Each candidate received 2 x 50 mg diclofenac products and plasma diclofenac concentration was measured at 20 minute intervals. The pharmacokinetic data is summarized in Table 1 and the plasma concentration versus time curves are shown in Figure 1 (data from Bioequivalence Study FARMOVS 20/94).

Table 1 Summary of Pharmacokinetic data for Diclofenac (n = 6)

		Reference Product Dose: 2 x [50mg diclofenac potassium tablet]			**Test Product** Dose: 2 x [diclofenac/beta-cyclodextrin tablet equivalent to 50 mg diclofenac sodium]		
Variable	**Unit**	**Geometric Mean**	**SD**	**Range**	**Geometric Mean**	**SD**	**Range**
C_{max}	(ng/ml)	3050	1,22	2253 - 3825	3992	1.46	2440 - 6260
T_{max}	(h)	0,67		0,33 - 0,67	0,33		0,33 - 0,67
AUDC	(ng.hr/ml)	2827	1,17	2423 - 3631	3047	1.24	2230 - 3898

C_{max} = maximum plasma concentration obtained
T_{max} = time to reach C_{max}
AUDC = area under the data curve

The test product was well tolerated with no reports of adverse effects. Peak plasma levels of diclofenac from the beta-cyclodextrin complex were obtained within about 20 minutes (mean T_{max} = 0,33 hr) or half the time required for commercial non-enteric coated diclofenac preparation. The T_{max} for conventional enteric coated diclofenac tablets is significantly longer (one

hour or more) owing to lag-time to reach the duodenum. The 90 % confidence interval for the mean of the variable C_{max} of the test product extends over the upper bound of the bioequivalence range relative to the reference product and the AUDC value falls within the conventional bioequivalence range of 80 to 125 %. The test product is bioequivalent to the reference product with respect to extent of absorption of diclofenac and seems to have a higher rate of absorption than the test product. The formulation according to the invention thus provides diclofenac in a form which is apparently more rapidly absorbed than conventional enteric and non-enteric coated formulations of diclofenac without affecting the extent of absorption. The invention thus has the advantage of providing therapeutic plasma levels of diclofenac in a relatively short time and thus enables a rapid onset of pharmacological action. Apart from the potential gastroprotectant effect created by the cyclodextrin inclusion complex, the apparent increased rate of absorption is likely to decrease the contact time of diclofenac with the gastrointestinal mucosa leading to a reduced extent of local gastric irritation associated with oral diclofenac treatment.

It is proposed that on dilution in the stomach of a tablet according to Example 1, an alkaline diffusion layer created by the alkali is established. The complex rapidly dissolves in the diffusion layer and then diffuses into the bulk fluid where dilution takes place, followed by dissociation and absorption of free diclofenac.

Example 4

Lornoxicam (3,71g) and alpha-cyclodextrin (24,30g) are screened (30 mesh) and tumble mixed. The mixture is transferred to a mortar. Deionised water (10-15ml) is gradually added with vigorous mixing to produce a uniform paste. Vigorous mixing is continued for 1 hour. The mixture is dried in

vacuo at 40°C. The dried mass is crushed and passed through 30 mesh screen. The powder is homogenized in a tumble mixer for 10 minutes. The product contains 13% by mass lornoxicam.

Example 5

The inclusion complex of alpha-cyclodextrin-lornoxicam obtained in Example 4 was formulated into tablets with the following unit composition:

Lornoxicam-alpha-cyclodextrin complex (equivalent to 4mg lornoxicam)	30,2 mg
Tromethamine	30,0 mg
Microcrystalline cellulose	26,2 mg
Starch	12,4 mg
Magnesium stearate	1,2 mg
TOTAL	100 mg

The tablets were prepared as described in Example 2.

Figure 2 is a graph of the mean dissolution rate of 6 lornoxicam-alpha cyclodextrin tablets prepared according to Example 5. The dissolution rate in purified water (pH ~6.5, temperature 37°C) with a paddle speed of 50 rpm shows that 80% is dissolved within 30 minutes. The release rate of lornoxicam from the system is consistent with pharmacopoeal specifications for highly soluble drugs (e.g. 80% dissolved within 45 minutes) indicating the solubilizing potential of tablets prepared according to the invention.

The pharmaceutical composition of the invention is preferably for use in the treatment of acute pain and inflammation.

CLAIMS

1 A pharmaceutical composition for oral administration comprising an inclusion complex of a non-steriodal anti-inflammatory drug or a pharmaceutically acceptable salt thereof and a cyclodextrin, and a physiologically acceptable alkali agent selected from the group consisting of alkali and alkaline earth metal carbonates, bicarbonates, phosphates and hydroxides, and water soluble amines, in an amount equivalent to between 2 and 30 molar equivalents inclusive of the non-steriodal anti-inflammatory drug, the alkali agent being capable of forming an alkaline diffusion layer around the composition in the gastrointestinal tract.

2 A pharmaceutical composition according to claim 1 wherein the non-steroidal anti-inflammatory drug or a pharmaceutically acceptable salt thereof is selected from the group consisting of diclofenac, indomethacin, naproxen, ibuprofen, mefenamic acid, piroxicam, tenoxicam, lornoxicam, and pharmaceutically acceptable salts thereof.

3 A pharmaceutical composition according to claim 1 or claim 2 wherein the cyclodextrin is selected from the group consisting of an alpha-cyclodextrin, an unsubstituted beta-cyclodextrin, and a substituted beta-cyclodextrin.

4 A pharmaceutical composition according to any one of claims 1 to 3 wherein the alkali agent is sodium bicarbonate.

5 A pharmaceutical composition according to any one of claims 1 to 3 wherein the alkali agent is tromethamine.

6 A pharmaceutical composition according to any one of claims 1 to 5 wherein the mass ratio of the non-steroidal anti-inflammatory drug to cyclodextrin in the inclusion complex is between 1:0,85 and 1:5 inclusive.

7 A pharmaceutical composition according to claim 6 wherein the mass ratio of the non-steroidal anti-inflammatory drug to cyclodextrin in the inclusion complex is between 1:1 and 1:2,5 inclusive.

8 A pharmaceutical composition according to claim 1 wherein the inclusion complex is an inclusion complex of diclofenac or a pharmaceutically acceptable salt thereof and an unsubstituted beta-cyclodextrin.

9 A pharmaceutical composition according to claim 8 wherein the mass ratio of diclofenac to beta-cyclodextrin is between 1:1,8 and 1:9 inclusive and the unit dose of the complex is equivalent to between 10 and 100 mg inclusive diclofenac.

10 A pharmaceutical composition according to claim 9 wherein the mass ratio of diclofenac to beta-cyclodextrin is between 1:3 and 1:5 inclusive and the unit dose of the complex is equivalent to between 20 and 60 mg inclusive diclofenac.

11 A pharmaceutical composition according to claim 1 wherein the inclusion complex is an inclusion complex of lornoxicam and alpha-cyclodextrin.

12 A pharmaceutical composition according to claim 11 wherein the

mass ratio of lornoxicam to alpha-cyclodextrin is between 1:2,6 and 1:13 inclusive and the unit dose of the complex is equivalent to between 2 and 10 mg inclusive lornoxicam.

13 A pharmaceutical composition according to any one of claims 1 to 12 which includes a further active ingredient selected from the group consisting of morphine, codeine, propoxyphene, paracetamol, and meprobamate.

14 A pharmaceutical composition according to any one of claims 1 to 13 which includes one or more conventional excipients.

15 A pharmaceutical composition according to any one of claims 1 to 14 in the form of a tablet or a capsule.

Fig 1

PLASMA DICLOFENAC CONCENTRATIONS

Geometric mean values

(n=6)

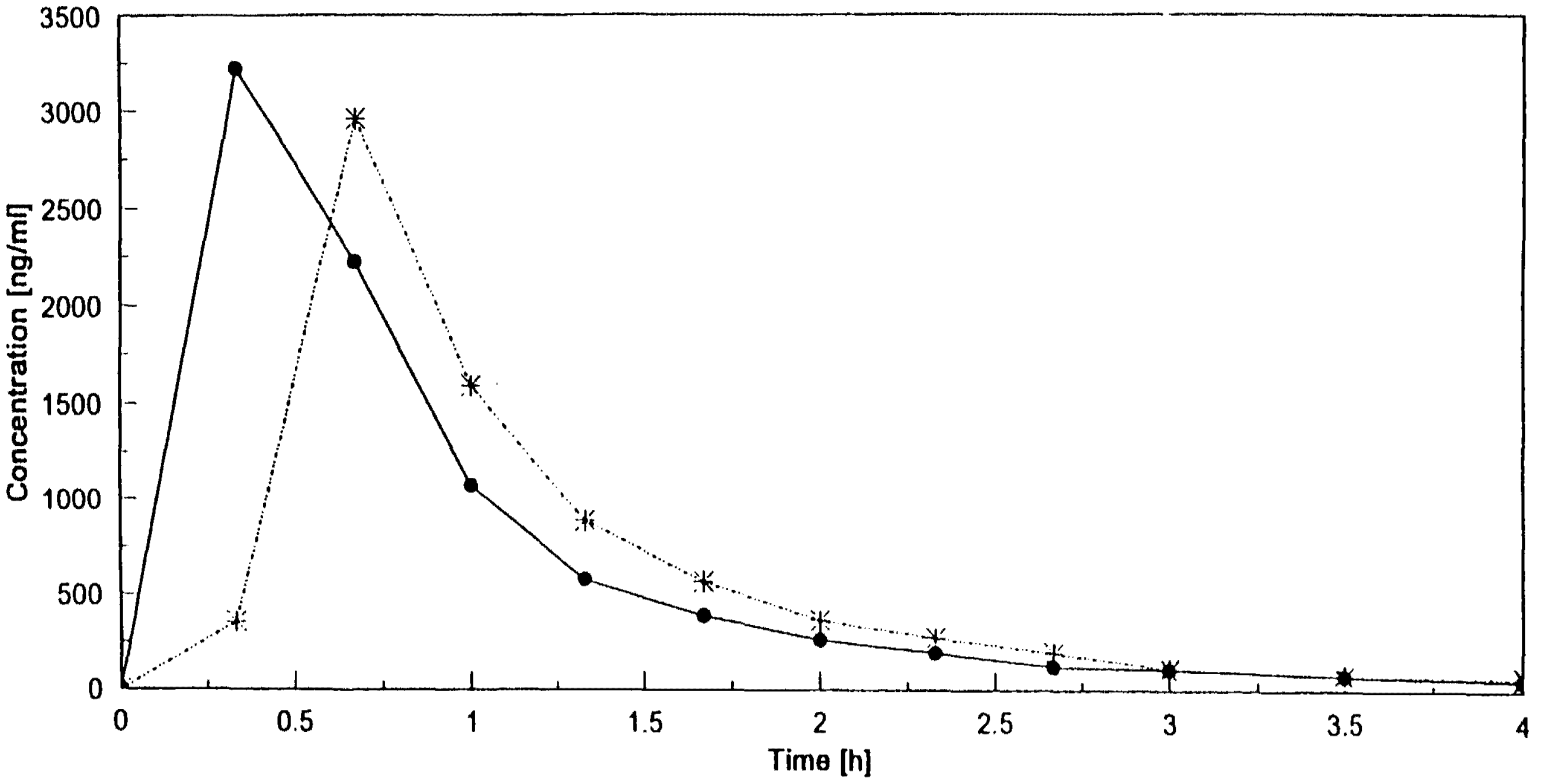
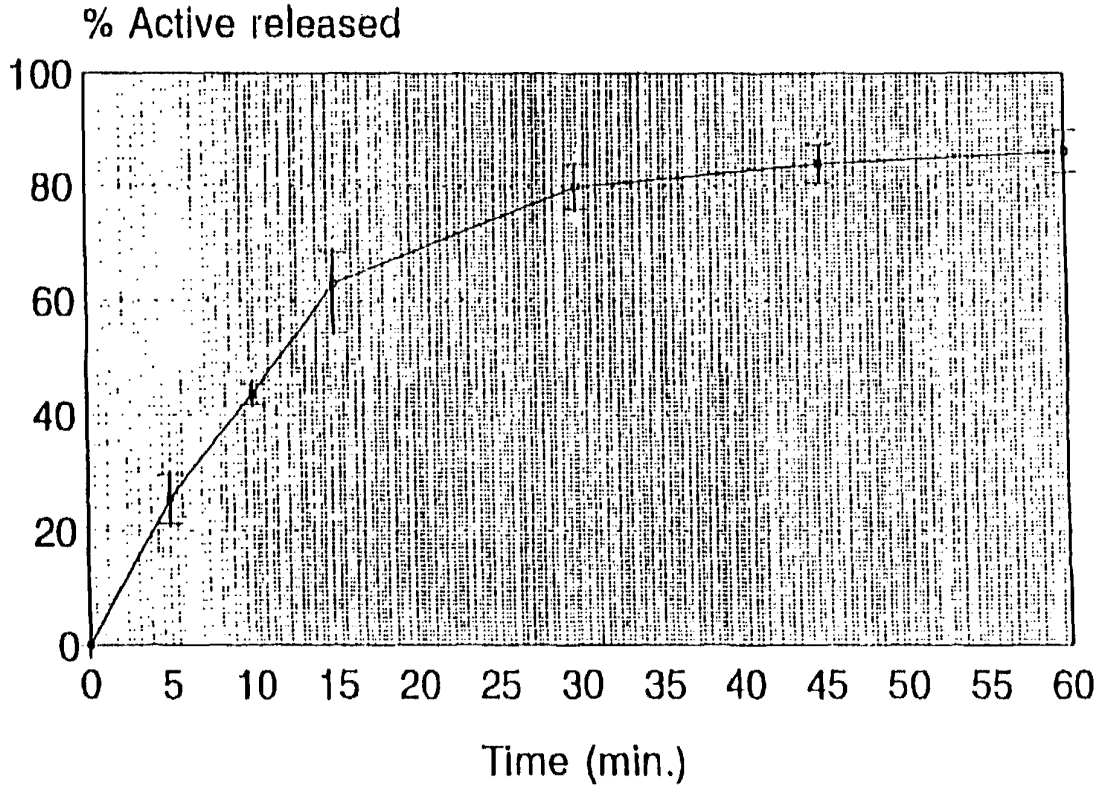


Fig 2

Lornoxicam 4mg

Media: H2O



Mean of 6 individual tablets

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 95/02679

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/19 A61K47/48 A61K47/02 A61K47/18 A61K31/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,5 362 758 (I.AHMED) 8 November 1994 see claims 1,10,12	1-4
X,P	WO,A,95 04528 (SMITHKLINE BEECHAM PLC,GB) 16 February 1995 cited in the application see claims see examples see page 3, line 17 - line 19 see page 3, line 27 - line 30 see page 4, line 30 - line 33	1-4,6,7, 14,15
E	WO,A,95 32737 (SOUTH AFRICAN DRUGGISTS LIMITED,ZA) 7 December 1995 see the whole document	1-3,6,7

[ ] Further documents are listed in the continuation of box C.

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14 February 1996	23.06.96

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Form PCT/ISA/210 (second sheet) (July 1992)

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...information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-5362758	08-11-94	NONE	
WO-A-9504528	16-02-95	AU-B- 7609994	28-02-95
WO-A-9532737	07-12-95	AU-B- 2531295	21-12-95

CORRECTED VERSION*

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(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

(57) Abstract

A pharmaceutical composition for oral administration for the treatment of acute pain and inflammation comprises an inclusion complex of a non-steroidal anti-inflammatory drug or a pharmaceutically acceptable salt thereof and a cyclodextrin, and a physiologically acceptable alkali agent selected from the group consisting of alkali and alkaline earth metal carbonates, bicarbonates, phosphates and hydroxides, and water-soluble amines, in an amount equivalent to between 2 and 30 molar equivalents inclusive of the non-steroidal anti-inflammatory drug, the alkali agent being capable of forming an alkaline diffusion layer around the composition in the gastrointestinal tract.

*(Referred to in PCT Gazette No. 34/1998, Section II)

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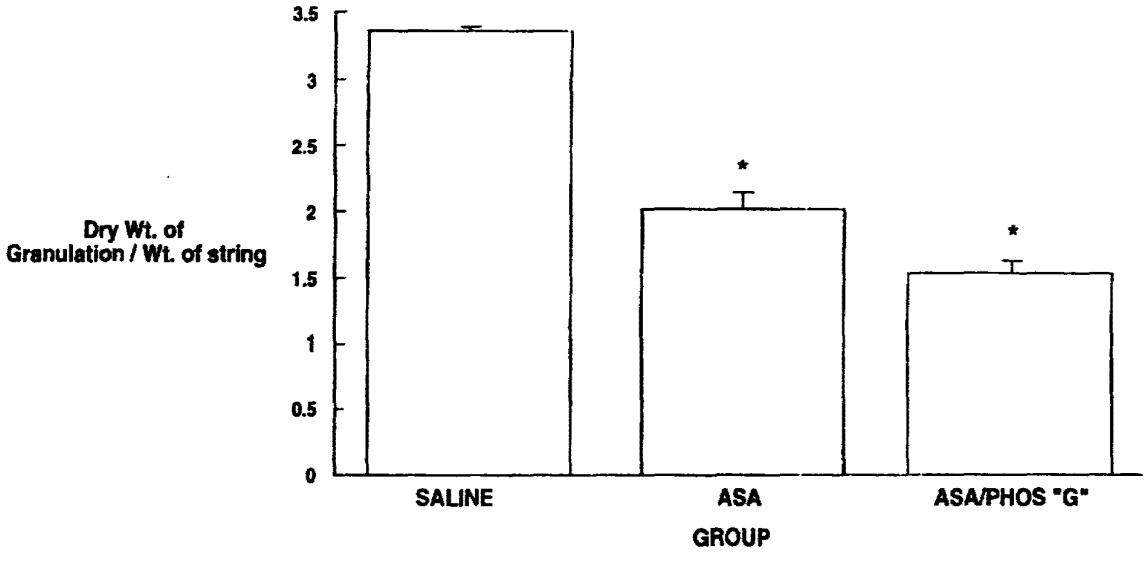
(74) Agent: MAYFIELD, Denise, L.; Akin, Gump, Strauss, Hauer & Feld, L.L.P., Suite 1900, 816 Congress Avenue, Austin, TX 78701 (US).

(54) Title: METHODS OF ENHANCING THE THERAPEUTIC ACTIVITY OF NSAIDS AND COMPOSITIONS OF ZWITTERIONIC PHOSPHOLIPIDS USEFUL THEREIN



(57) Abstract

Disclosed are compositions comprising non-steroid anti-inflammatory drugs (NSAID's) complexed with zwitterionic, neutral phospholipids, or both, having reduced gastrointestinal irritating effects and enhanced anti-pyretic, analgesic, and anti-inflammatory activity. Also disclosed are improved methods of using the complexes for treating fever, inflammation, and preventing platelet aggregation. In some embodiments, the anti-pyretic activity of sub-therapeutically used amounts of NSAID's are enhanced to elicit anti-pyretic activity *in vivo* when associated (noncovalently) with zwitterionic phospholipids, such as dipalmitoyl phosphatidyl choline.

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METHODS OF ENHANCING THE THERAPEUTIC ACTIVITY OF NSAIDS AND COMPOSITIONS OF ZWITTERIONIC PHOSPHOLIPIDS USEFUL THEREIN

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the fields of pharmacology and medicine and more particularly, it concerns the treatment and prevention of fever, pain and inflammation with non-steroidal anti-inflammatory drugs (NSAIDs) complexed with phospholipids, and in other embodiments in further combination with neutral lipids. The invention also provides methods for retarding platelet aggregation, and the application of these methods in treating cardiovascular and vascular diseases as it relates to platelet activity.

2. Description of the Related Art

The consumption of NSAIDs among the general populace is unparalleled by any other drug class due to their great efficacy in the treatment of pain, inflammation and fever (Rainsford, 1985). The widespread usage of these drugs is anticipated to increase even further due to their efficacy in the treatment of osteoarthritic and generalized aches and pain as the elderly increase as a percentage of the population (Alexander *et al.*, 1985; Jolobe and Montgomery, 1984), and as NSAIDs are employed in the treatment/prevention of stroke and cardiovascular disease.

The major concern with these developments relates to the tendency of NSAIDs to induce gastrointestinal (GI) mucosal lesions, perforations and bleeding resulting in significant morbidity and mortality, even in occasional NSAID users (Rainsford, 1989; Graham, 1989; Allison *et al.*, 1992). Strategies to reduce the gastroduodenal injurious effects of these drugs with enteric coatings, have had limited success due to the delayed therapeutic actions of these specially packaged NSAIDs (Alpsten *et al.*, 1982; Mojaverian *et al.*, 1987).

Although it is clear that the GI side-effects of NSAIDs are in part attributable to their ability to inhibit the biosynthesis of gastroprotective prostaglandins, a significant amount of evidence exists that NSAIDs act locally on the mucosa to induce GI ulcers and bleeding by a prostaglandin-independent mechanism (Rainsford, 1989; Whittle *et al.*, 1980; Whittle, 1981; Ligumsky *et al.*, 1982; McCormack and Brune, 1987).

The present inventor and others have obtained evidence that the mucosa of the stomach and other regions of the GI tract have hydrophobic, non-wettable properties, that protect the underlying epithelium from gastric acid and other luminal toxins (Hills *et al.*, 1983; Goddard *et al.*, 1987; Goddard *et al.*, 1990; Kao *et al.*, 1990). This biophysical characteristic, which can be quantified by contact angle analysis, appears to be attributable to the presence of an extracellular lining of surfactant-like phospholipid on the luminal aspects of the mucus gel layer (Goddard *et al.*, 1990; Kao *et al.*, 1990). Evidence has also come forth that these zwitterionic phospholipids are synthesized in surface mucus cells of the stomach, as well as those present in discrete submucosal glands of the GI tract, where they are stored in specific organelles and secreted by a prostaglandin-dependent pathway (Kao and Lichtenberger, 1991). It has also been reported that aspirin and other NSAIDs have the ability to rapidly transform the gastric mucosa from a non-wettable to a wettable state within minutes after luminal administration, thereby increasing the tissue's susceptibility to the corrosive actions of gastric acid (Hills *et al.*, 1983; Goddard *et al.*, 1987; Goddard *et al.*, 1990; Kao *et al.*, 1990).

One solution to this problem has been to formulate injectable solutions of NSAIDs and thus bypass the GI tract completely. The low water solubility of these drugs, however, has caused problems with this technique. Stable, injectable solutions of indoleacetic and indanacetic acid derivatives have been developed to address this problem, by complexing these NSAIDs with phosphatidylcholine and phosphatidylethanolamine derivatives (See US Patent 4,309,420).

US Patent 4,421,747 describes NSAIDs complexed with phospholipids for oral administration. These complexes were shown to retain their anti-inflammatory action and to have reduced ulcer formation in rats. However, no enhancement of therapeutic effects was reported with these preparations.

WO 91/16920 (Vical Inc.) relates to phospholipid prodrug derivatives of a salicylate or non-steroidal, anti-inflammatory drug. These preparations are made by combining salicylic acid or NSAID with a phospholipid in the presence of a coupling agent, thereby producing a covalently linked NSAID-phospholipid compound. These prodrugs are described as useful in reducing the toxicity of high dose, long term usage of NSAID preparations.

JP 3176425 (Nippon Shinyaku KK) relates to compositions including non-steroidal, anti-inflammatory drugs together with neutral lipids and phospholipids in a fat and oil emulsion. Although the method of preparation is not described in the abstract, these compositions appear to be encapsulated in lipid, such as in a micelle. The combination of the drug with the neutral lipids and the phospholipids is described as not affecting the drug's pharmacological actions.

JP 63048228 (Toa Eiyo KK) relates to topically applied compositions that include non-steroidal anti-inflammatory drug together with phospholipid and a "disintegrator". The disintegrator is described as providing for a preparation with improved dispersability and increased absorptivity. JP 63048226 (Ono Pharmaceutical KK) relates to compositions that include a phospholipid base (such as phosphatidylcholine) and an anti-inflammatory agent (such as acetylsalicylic acid and indomethacin). KK JP 58150508 (Ono Pharmaceutical) relates to topical compositions that include a phospholipid base (such as phosphatidylcholine) and an anti-inflammatory agent (such as acetylsalicylic acid and indomethacin).

US Patent 4,369,182 (Nattermann & CIE), relates to inflammation-preventing pharmaceutical compositions for oral administration. The compositions are prepared

and then lyophilized into powder form. The described compositions include natural and synthetic phospholipids (dipalmitoylphosphatidylcholine (DPPC)), in combination with nonsteroidal agents including salicylic acid, acetyl-salicylic acid, diflunisal, indomethacin, glucametacine, acemetacin, sulindac, ibuprofen, naproxen, tolmetin and other NSAID's. Also described are NSAID's in combination with phosphatidylcholine preparations, named phospholipons. US Patent 4,421,747 relates to methods of alleviating inflammation with compositions as described in the '182 patent.

Despite the extensive work in the area of NSAIDs, a need continues to exist in the art for preparations that include reduced amounts of this useful class of drug without loss of therapeutic efficacy. Methods and compositions that provide for similar or enhanced anti-pyretic, anti-inflammatory, anti-platelet and analgesic activity at lower doses than currently prescribed for pharmacological activity would also render this very valuable class of drugs available to those previously unable to tolerate standard and/or prolonged therapeutic regimens of NSAID.

SUMMARY OF THE INVENTION

The present invention seeks to overcome these and other drawbacks inherent in the prior art by providing compositions and methods capable of maintaining and/or improving the pharmacological activity of non-steroidal anti-inflammatory drugs by noncovalent association with zwitterionic phospholipids. In some embodiments, these preparations may further include neutral lipids, such as the triglycerides.

The present invention illustrates the ability of one or more zwitterionic phospholipids to enhance the fever-reducing potential of an NSAID. Pharmacological activity of low dose NSAID to reduce inflammation and pain may also be observed, and in some cases enhanced by chemically associating the NSAID with zwitterionic phospholipid, such as phosphatidyl choline (PC), dipalmitoylphosphatidylcholine (DPPC), and other disaturated phosphatidyl cholines, and the like. In some embodiments, the association of NSAID and zwitterionic phospholipid is of a non-

covalent nature. In still other embodiments, the NSAID and zwitterionic phospholipid compositions may be further described as including more or less equimolar amounts of these ingredients. The compositions may also, of course, comprise a pharmaceutically acceptible carrier in any form, such as solid powder, gel or liquid form.

The present invention focusses techniques that are demonstrated in some cases to enhance the therapeutic activities of NSAIDs. This is accomplished without the disadvantage of hindering the pharmacological activity or therapeutic bioavailability of the drug rendering the preparations effective even at low doses.

Accordingly, the present invention provides in one aspect a method for enhancing the antipyretic activity of a nonsteroidal anti-inflammatory drug (NSAID). This method comprises providing a non-covalently associated combination of a zwitterionic phospholipid with an amount of a nonsteroidal anti-inflammatory drug that provides reduced anti-pyretic activity in the absence of the zwitterionic phospholipid. Some embodiments of the present preparations are further described as being essentially free of anionic phospholipid, or as including an amount of anionic phospholipid that is biologically inert and/or not an active component, of the preparation.

The term "essentially free" as used in the description of the present invention, is understood to mean compositions that include less than about 0.10% of anionic phospholipid, and in even further defined embodiments, less than 0.01% anionic phospholipid. As used in the description of the present invention, the term zwitterionic phospholipid embraces a wide range of phospholipids, including but not limited to phosphatidylcholine, phosphatidylserine, phosphalidylethanolamine, sphingomyelin and other ceramides, as well as various other zwitterionic phospholipids. In some embodiments, these compositions are essentially free of anionic phospholipid.

In other embodiments of the described method, the amount of nonsteroidal anti-inflammatory drug is defined as an amount that provides reduced antipyretic activity in the absence of the zwitterionic phospholipid. Such amounts of the drug sub-therapeutically effective amounts thereof. This activity or lack of activity is observed in the absence of zwitterionic phospholipid, while the same or about the same amount of the NSAID does demonstrate pharmacological activity in the presence of zwitterionic phospholipid. In this regard, the phenomenon is observed that the combination of low amounts of nonsteroidal anti-inflammatory drugs with phospholipid have potent pharmacological activity, while doses of the drug alone (i.e., without zwitterionic phospholipid) do not.

The present method employs compositions that may include any variety of those drugs generally classified as nonsteroidal anti-inflammatory drugs. By way of example, these drugs include ibuprofen, piroxicam, salicylate, aspirin, naproxen, indomethacin, diclofenac, or any mixture thereof. In particular embodiments, the nonsteroidal anti-inflammatory drug is salicylate. By way of example and not limitation, NSAID's useful in the practice of the invention, include those noted in Table 1.

TABLE 1

Nonsteroidal Anti-Inflammatory Drugs To Be Used in Combination with Zwitterionic Phospholipids

Propionic acids	Fenoprofen calcium	Nalfon
	Flurbiprofen	Ansaid
	Suprofen	
	Benoxaprofen	
	Ibuprofen (prescription)	Motrin
	Ibuprofen (200 mg. over the counter)	Nuprin, Motrin 1B
	Ketoprofen	Orduis, Oruvall
	Naproxen	Naprosyn
	Naproxen sodium	Aleve, Anaprox, Aflaxen
	Oxaprozin	Daypro
Acetic acids	Diclofenac sodium	Voltaren
	Diclofenac potassium	Cataflam
	Etodolac	Lodine
	Indomethacin	Indocin
	Ketorolac tromethamine (intramuscular)	Acular, Toradol
	Ketorolac (oral)	Toradol
Ketones	Nabumetone	Relafen
	Sulindac	Clinoril
	Tolmetin sodium	Tolectin
Fenamates	Meclofenamate sodium	Meclomen
	Mefenamic acid	Ponstel
Oxicams	Piroxicam	Dolibid
Salicylic acid	Diflunisal	Feldene
	Aspirin	
Pyrazolin acid	Oxyphenbutazone	Tandearil
	Phenylbutazone	Butazolidin

NSAIDs such as benoxaprofen, ketoprofen, oxaprozin, etodolac, ketorolac tromethamine, ketorolac and nabumetone, together with zwitterionic phospholipid, comprise still other particular embodiments of the invention, again both with and without neutral lipid.

In particular embodiments, the zwitterionic phospholipid in the compositions is dipalmitoyl phosphatidylcholine, phosphatidyl choline, or a mixture thereof.

The pharmacological, particularly anti-pyretic, activity of NSAIDs, is shown to be enhanced several fold over preparations with similar doses without zwitterionic phospholipid. Surprisingly, the present inventors have found that combination of the aforedescribed nonsteroidal anti-inflammatory drugs with zwitterionic phospholipid dramatically enhances the anti-pyretic activity and potency of the drug, even at sub-therapeutically active doses, and in some cases from about 2-fold to about 6-fold relative to non-phospholipid containing preparations. Hence, the methods are expected to be particularly efficacious in reducing fever in a mammal having reduced tolerance for NSAID's.

Amounts ranging between one-tenth and one-half that typically necessary to illicit a fever-reducing response in a mammal may thus be realized employing the present inventive methods and compositions. In this regard, it is expected that amounts of between 2 mg/kg to about 300 mg/kg will provide fever-reducing therapeutic

activity. Of course, the amount/dose used will depend in specific cases on the particular pharmacological characteristics of the NSAID or combination of NSAIDs included. Further defined ranges of the drug expected to provide the anti-pyretic activity herein disclosed range from between about 10 to about 150 mg/kg or about 20 or 50 mg/kg to about 150 mg/kg.

The present inventors have also observed the claimed compositions are useful for enhancing the platelet retarding activity of a non-steroidal anti-inflammatory drug. This method for inhibiting platelet aggregation comprises providing a non-covalently associated combination of zwitterionic phospholipid and an amount of non-steroidal anti-inflammatory agent that provides reduced inhibition of platelet aggregation in the absence of zwitterionic phospholipid. In some embodiments, this composition is essentially free of anionic phospholipid and/or includes an amount of anionic phospholipid that is a biologically inert component of the preparation. The amounts of non-steroidal anti-inflammatory drug employed as part of the composition, again, is relatively low, and may be further described as an amount that generally provides reduced pharmacological activity in the absence of zwitterionic phospholipid. Again, the zwitterionic phospholipid of choice is, in some embodiments, DPPC, PC, or a combination thereof.

The compositions of the aforedescribed methods may further include a neutral lipid, such as a triglyceride. For a partial listing of representative neutral lipids, such as the triglycerides, reference is specifically made to U.S. Patent Number 4,950,656

and 5,043,329. Both saturated and unsaturated triglycerides may be employed in the present compositions, and include such triglycerides as tripalmitin (saturated), triolein and trilinolein (unsaturated). However, these particular triglycerides are listed here for convenience only, and are merely representative of a variety of useful triglycerides, and is further not intended to be inclusive.

Turning now to another aspect of the invention, methods for enhancing the analgesic activity of a non-steroidal anti-inflammatory drug are provided. These methods again comprise providing a non-covalently associated composition comprising zwitterionic phospholipid and an amount of a non-steroidal anti-inflammatory drug that provides reduced pharmacological activity in the absence of zwitterionic phospholipid. In some embodiments, these compositions are essentially free of anionic phospholipid, or include amounts of anionic phospholipid that are biologically inert. In particular embodiments, the non-steroidal anti-inflammatory drug is one or more of those listed in Table 1. In particular embodiments, the NSAID is aspirin, salicylate, a salt thereof, or a combination thereof. While any of a number of different zwitterionic phospholipids may be employed in the composition of the method, in some embodiments, the phospholipid is dipalmitoyl phosphatidyl choline, phosphatidyl choline, or a combination thereof. In these and other embodiments of the described method, an anionic phospholipid that may be excluded or included only in biologically inert amounts is phosphatidyl glycerol (PG).

In still another aspect, the invention provides methods of enhancing the anti-inflammatory activity of non-steroidal anti-inflammatory drugs. The method comprises proving a non-covalently associated combination of zwitterionic phospholipid with an amount of a non-steroidal anti-inflammatory drug. The amount of NSAID in the composition is again defined as an amount that provides reduced pharmacological activity in the absence of zwitterionic phospholipid. In some embodiments, the method employs compositions that are essentially free of anionic phospholipids, such as the DPPG or PG, or includes amounts of anionic phospholipid that are biologically and pharmacologically inert.

In particular embodiments, the composition is further defined as comprising an equimolar amount of NSAID and zwitterionic phospholipid. The present inventors' studies demonstrate the described combination of ingredients provides an enhancement of the anti-inflammatory activity and potency of these drugs compared to drug preparations that do not include zwitterionic phospholipid. Also demonstrated is the reduction in anti-inflammatory activity observed where pharmacologically active (i.e., non-biologically insert amounts) amounts of anionic phospholipid, such as PG (PI), are included in the preparation. Hence, preparations that include pharmacologically active amounts of DPPG or PI would not necessarily provide for the same enhanced anti-inflammatory activity, or the aforedescribed enhanced pharmacological activity (i.e., antipyretic or enhanced reduction in platelet aggregation activity), described in the present methods. Pharmacologically active amounts of other negatively-charged

phospholipids would also not be contemplated as particularly useful in view of these results.

Turning now to still a further aspect of the present invention, a method for enhancing the antipyretic potential of subtherapeutically effective amounts of nonsteroidal anti-inflammatory drug is disclosed. In some embodiments, the method comprises again combining zwitterionic phospholipid with an amount of non-steroidal anti-inflammatory drug to provide a noncovalently associated composition thereof. In some embodiments, the composition is further defined as essentially free of biologically active amounts of anionic (or negatively charged) phospholipid.

As used in the description of the above method, a sub-therapeutically effective amount of NSAID is defined as an amount that provides reduced antipyretic activity in the absence of a zwitterionic phospholipid. The enhancement in activity of low amounts of NSAID illustrated in the various *in vivo* studies disclosed herein demonstrate that while doses of aspirin of about 9 mg/kg in combination with the zwitterionic phospholipid, DPPC, did provide for a fever reducing (anti-pyretic) pharmacologic response, treatment with this same dose of NSAID without phospholipid did not.

The present invention also discloses particular pharmaceutical preparations. These pharmaceutical preparations are further described as suitable for enteral or oral administration, and comprise a non-covalently associated combination of zwitterionic phospholipid, non-steroidal anti-inflammatory drug, and a pharmaceutically acceptable

carrier. These compositions are formulated to provide a non-covalently linked composition that is further described as being essentially free of biologically active (pharmacologically active) amounts of anionic phospholipid, and in some embodiments, essentially free of DPPG.

In some applications, the non-steroidal anti-inflammatory drug is one or more of those listed in Table 1, such as naproxen, indomethacin, diclophenac, salicylate, aspirin, or any mixture thereof. In particular embodiments, the non-steroidal anti-inflammatory drug of choice is salicylate. While any variety of zwitterionic phospholipids may be employed alone or in combination with the described drugs, some of the representative phospholipids include phosphatidyl choline, dipalmitoyl phosphatidylcholine, phosphatidyl serine, other zwitterionic phospholipids, or mixtures thereof. The compositions may further include a neutral lipid, such as a triglyceride. Representative triglycerides are described in U.S. Patent 4,950,656, which reference is specifically incorporated herein by reference for this purpose. In particular embodiments, the pharmaceutical preparation is defined as comprising an equimolar amount of zwitterionic phospholipid and NSAID.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, anti-oxidant, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions and

methods described herein is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

As used in the description of the present invention, the term "sub-therapeutically effective amount", particularly as it is used to described the amount of NSAID employed, is defined as an amount of the NSAID that provides reduced pharmacological (i.e., anti-pyretic) activity in the absence of non-covalent association with a zwitterionic phospholipid.

It is understood that as used in the present disclosure and appended claims, the terms "a" and "an," as in "an element" or "a molecule" are intended to include one or more items or elements, and in no way limit the description or claimed element to one element or item.

The following abbreviations are employed in the description of the invention:

PI = phosphatidyl inositol

PC = phosphatidylcholine

PG = phosphatidylglycerol

L-NAME = N-nitro-L-arginine Methyl Ester

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1A. Photograph of test tubes containing 30 nM of the sodium salts of one of the five NSAIDs tested in chloroform in the absence and presence of 30 nM DPPC (naproxen, indomethacin, diclofenac, salicylate, aspirin).

FIG. 1B. The concentration of each NSAID in solution increases (quantified by UV absorption) in proportion to the molar equivalents of DPPC dissolved in chloroform (Naproxen = -▲-; Aspirin = -●-; Salicylate = -□-, Diclofenac = -△-; Indomethacin = -○-).

FIG. 2A. The DPPC-induced reduction in ASA's solubility in water was maximal when both reactants were present in equimolar concentration over a 60 min. incubation period at 25°C.

FIG. 2B. Time course at 25°C of the reduction in ASA's solubility in water induced by the presence of equimolar concentrations of DPPC. In contrast, the concentration of ASA in water was not significantly changed over time by the addition of an

equimolar concentration of the anionic phospholipid, DPPG as a lipidic suspension (ASA only = -●-; DPPG/ASA = -□-; DPPC/ASA = -o-).

FIG. 3. The passive diffusion of ASA from water into cyclohexane is markedly accelerated by the presence of zwitterionic (DPPC) but not anionic (DPPG) phospholipids in the aqueous solution (ASA/DPPC = -o-; ASA/DPPG = -□-; DPPC = -●-; ASA = -x-).

FIG. 4A. The injurious potential of salicylate and ASA to induce gastric lesions (in rats subsequently challenged with 0.6N HCl), is remarkably decreased when the NSAIDs are intragastrically administered in association with DPPC (without DPPC = clear bar; with DPPC = hatched bar).

FIG. 4B. The injurious potential of non-salicylate NSAIDs to induce GI bleeding (in rats pre- and post-treated with L-NAME to increase the animal's susceptibility to the NSAID) is remarkably decreased when the drugs are intragastrically administered in association with DPPC (without DPPC = clear bar; with DPPC = hatched bar).

FIG. 4C. The anti-pyretic activity of the NSAID (aspirin - dose 90 mg/kg) is not diminished, and is augmented if administered as a lipidic suspension. Asterisk (*) represents a statistically significant difference between the values of rats treated with the NSAID alone (-DPPC) and those treated with the NSAID/DPPC complex (+ DPPC) (Saline = -●-; ASA = -□-; ASA/DPPC = -o-).

FIG. 5. Anti-inflammatory action of ASA and Phospholipon G™ (Phospholipid "G") as determined by the implanted string assay as described. Dosage is 90 mg/kg ASA. Each bar represents data for n=5 rats.

FIG. 6. Antipyretic activity of ASA\DPPC complex at a subthreshold ASA dosage (9.0 mg/kg) (Saline = -□-; ASA (9.0 mg/kg) = -▲-; ASA/DPPC (9 mg/kg) = -o-).

FIG. 7. Antipyretic activity of aspirin (ASA) alone when administered at doses which range from 2.5-90.0 mg/kg. In this and all subsequently figures the test agents were intragastrically administered 18 hrs after the rats were subcutaneously injected with 2g/kg Brewer's Yeast to induce a 0.5-1.0°C increase in body temperature. It can be appreciated that doses of aspirin of < 10 mg/kg failed to reduce the fever during the 4 hour study period (Saline = -◇-; ASA, 2.5 mg/kg = -□-; ASA, 5 mg/kg = -o-; ASA, 10 mg/kg = -△-; ASA, 20 mg/kg = -■-; ASA, 45 mg/kg = -●-; ASA, 90 mg/kg = -▲-).

FIG. 8. In contrast to the above pattern aspirin (ASA) when complexed with an equimolar concentration of dipalmitoylphosphatidylcholine (DPPC) effectively reduced fever at a dose of 9.0 mg/kg, whereas the anionic phospholipid, DPPG, failed to augment aspirin anti-pyretic activity at this same sub-threshold dose. This figure also demonstrates that anionic phospholipid is essentially biologically inert in terms of enhancing the anti-pyretic action of aspirin (ASA, 9.0 mg/kg = -●-; ASA/DPPC, 9.0

mg/kg = -□-; ASA/DPPG, 9.0 mg/kg = -o-; n=5/grp; * = p <0.05 vs ASA; † = p <0.05 vs ASA/DPPG).

FIG. 9. ASA at a subthreshold dose of 4.5 mg/kg, which had no antipyretic activity alone, effectively reduced fever when complexed with an equimolar concentration of DPPC (H_2O = -□-; H_2O/DPPC (4.5 mg/kg) = -▲-; ASA (4.5 mg/kg) = -⊖-; ASA/DPPC (4.5 mg/kg) = --o--).

FIG. 10. Compilation of all the data at subthreshold doses (2.5-9.0 mg/kg) of ASA. ASA alone (open symbols) data demonstrates the enhancement of antipyretic activity when ASA was complexed to DPPC (closed symbols) (Saline = -*-; ASA, 9mM = -□-; ASA, 4.5 mM = -o-; ASA, 2.5 mM = --△--; ASA/DPPC, 9mM = -■-; ASA/DPPC, 4.5mM = -●-; ASA/DPPC, 2.25 mM = --▲--).

FIG. 11. Dose-response analysis of the antipyretic activity of ASA alone and the ASA/DPPC (equimolar ratio) complex 1 hr (FIG. 11) (ASA = -●-; ASA/DDPC = -□-) after intragastric administration. The potency of the ASA, as reflected by the ED_{50} is increased ~ 10 fold when it is administered with the zwitterionic phospholipid.

FIG. 12. Dose response analysis of the anti-pyretic activity of ASA alone and the ASA/DPPC (equimolar ratio) complex 2 hours after intragastric administration.

FIG. 13. Effect of varying the ASA:DPPC ratio from 1:1 on antipyretic activity at 1 hr post intragastric administration. It can be appreciated that the ability of the zwitterionic phospholipid to enhance the antipyretic activity of ASA was lost when the molar concentration of DPPC was increased (from unity) by a factor of 4 or decreased by a factor of 10 (ASA = 9.01 mg/kg).

FIG. 14. Effect of varying the ASA:DPPC ratio from 1:1 on the antipyretic activity of the complex 2 hours post intragastric administration (Legends same as in FIG. 13) (ASA = 9.01 mg/kg).

FIG. 15. At subthreshold doses the antipyretic efficacy of ASA could be clearly enhanced (0-120 min. post-administration), if the NSAID was administered as a microemulsion containing DPPC and tripalmitin (TP). In all cases the molar ratio of ASA:DPPC was maintained at 1:1, whereas the TP was administered in excess (weight ratio of DPPC:TP = 1:4) (n = 5/grp; * = $p>0.05$ vs ASA/DPPC; ASA/DPPC 1 mg/kg = -●-; ASA/DPPC/TP, 1 mg/kg = --o--; ASA/DPPC, 9 mg/kg = --▲--; ASA/DPPC/TP, 9 mg/kg = --△--).

FIG. 16. It can be appreciated that the addition of the neutral lipid provided a further enhancement of antipyretic activity, even over that of ASA/DPPC, as can best be seen with the 1 mg/kg subthreshold ASA dose (n = 10/grp; * = $p<0.05$ vs ASA/DPPC; ASA, 1 mg/kg = -◆-; ASA/DPPC = -o-; ASA/DPPC/TP = --●--).

FIG. 17. The ability of DPPC to promote the movement of aspirin from water into a lipidic cyclohexane phase (as a membrane model) is greatly accelerated by the presence of the neutral lipid, tripalmitin (TP) to form a microemulsion. This may provide an explanation why the presence of neutral lipids further promote the therapeutic potency of the NSAID/DPPC complex (DPPC + TP (1:4), 2.5mM + ASA, 10mM (sonicated) = -o-; DPPC, 2.5mM + ASA, 10mM (sonicated) = -□-; TP, 2.5 mM + ASA, 10 mM = -■-).

FIG. 18. The ability of DPPC to enhance the anti-pyretic activity of ASA was seen with indomethacin (Saline = -●-; indomethacin (10 mg/kg) = -□-; indomethacin (10 mg/kg)/DPPC = -o-).

FIG. 19. Demonstrates the ability of DPPC to enhance the anti-pyretic activity of naproxen (25 mg/kg dose) (Saline = -●-; naproxen (25 mg/kg) = -□-; naproxen (25 mg/kg)/DPPC = -o-).

FIG. 20. Demonstrates the ability of DPPC to enhance the anti-pyretic activity of diclofenac, 10 mg/kg (Saline = -□-; diclofenac (10 mg/kg) = -□-; diclofenac (10 mg/kg)/DPPC = -o-; n = 5/grp; * = $p<0.05$ vs. DICLO).

FIG. 21. Demonstrates the ability of DPPC to enhance the anti-pyretic activity of salicylic acid (SA) (70 mg/kg dose) (Saline = -◆-; SA (70 mg/kg) = -o-; SA (70 mg/kg)/DPPC = -●-; n = 5/grp, * = $p<0.05$ vs. SA/DPPC).

FIG. 22. Antipyretic activity of ASA (18 mg/kg) and ASA/DPPC complex with IP injection of omeprazole (150 mg/kg) two hours prior to NSAID dosage. ASA (saline) = - ● -; ASA/DPPC (Sal) = - o -; ASA (omeprazole) = --▲--; ASA/DPPC (omep) = --△--; n = 5/grp; * = $p<0.05$ vs. ASA (omep); † = $p<0.05$ vs. ASA (Sal).

FIG. 23. Antipyretic activity of ASA (18 mg/kg) and ASA/DPPC complex with IP injection of Ranitidine (2 mg/kg) 1 hour prior to NSAID dosage. ASA (saline) = - ●-; ASA (saline) = - o -; ASA (Ranitidine) = --▲--; ASA/DPPC (Ranitidine) = --△--; n = 5/grp; * = $p<0.05$ vs. ASA (rani); † = $p<0.05$ vs. ASA (Sal).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention arises from the discovery that aspirin and other NSAIDs chemically associate with zwitterionic phospholipids, such as dipalmitoyl phosphatidylcholine (DPPC). Important embodiments of the invention include methods of enhancing the various therapeutic activities of NSAID's, such as anti-pyretic, anti-inflammatory, and analgesic pharmacological activities. Surprisingly, these responses are observed without evidence of gastrointestinal side effects as demonstrated in acute and chronic animal models of NSAID injury in the present disclosure.

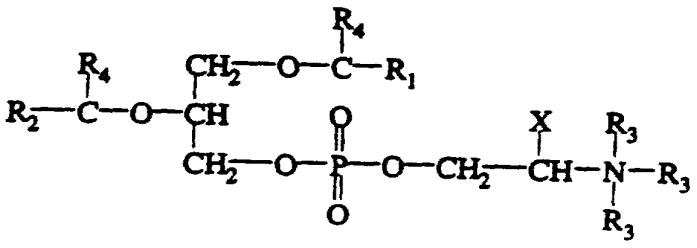
The data disclosed herein indicates that NSAIDs have the capacity to chemically associate with zwitterionic phospholipids in both organic and aqueous solvent systems, and in doing so, both classes of molecules undergo profound changes in their physical and chemical properties. Complex formation in aqueous solvent systems is shown to

occur more efficiently at pH values at or slightly below the pKa of the NSAID. Therefore, without being bound by any theory, it is contemplated that the intermolecular bonding is not covalent, but is instead both hydrophobic and electrostatic, with the latter association being between the negatively charged carboxyl group of the NSAID and the positively charged nitrogen of the phospholipid. This possible interaction has been supported by computer assisted molecular modelling programs (Quanta and CHARMm), which also indicate that the NSAID/phospholipid complex has a lower molecular free energy (greater thermodynamic stability) than either reactant alone.

According to the present invention, it would be expected that orally administered NSAIDs would chemically associate with the intrinsic zwitterionic phospholipids that coat the luminal aspects of the mucus gel layer of the upper GI tract. A description of luminal aspects of the mucus gel layer is described in Goddard *et al.*, (1990) and Kao *et al.* (1990). While not intending to be limited to any particular mechanism of action, this intermolecular association is thought to be the basis for the attenuation in surface activity and/or the loss of stability of the interfacial extracellular phospholipid layer, and to culminate in an NSAID induced decrease in mucosal hydrophobicity and barrier properties.

Description of the Lipid Compounds

The phospholipids of the present invention are characterized generally by the formula:



wherein R_1 and R_2 are saturated or unsaturated substitutions ranging from 8 to 32 carbon atoms; R_3 is H or CH_3, and X is H or COOH; and R_4 is = 0 or H_2.

As will be appreciated by those of skill in the art, the foregoing chemical structure defines a zwitterionic phospholipid structure and embraces a wide range of phospholipids, including but not limited to phosphatidyl cholines, phosphatidyl ethanolamines, phosphatidyl serines and various other zwitterionic phospholipids.

Other phospholipids that may be employed in the composition include: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin, and other ceramides, and mixtures thereof.

Description of the NSAID's

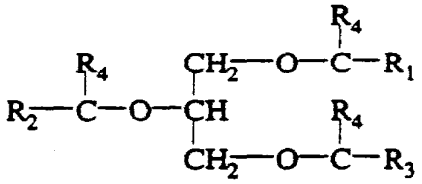
A few of the non-steroidal anti-inflammatory agents that may be employed in the methods and compositions disclosed herein include by way of example: pyrazolones, phenylkbutazonc (4-butyl-1,2-diphenylpyrazolidine-3,5-dion), and oxyphenbutazone (4-butyl-2-(4-hydroxyphenyl)-1-p-phenylpyrazolidine-3,5-dion), salicylic acid derivatives such as salicylic acid salicylic acid amide, acetyl-salicylic acid, benorilate (4-acetamidophenyl-o-acetylsalicylate), and diflunisal (5-(2,4-difluorophenyl)-salicylic acid); Indoles, especially indometacine and its analogs such as indometacine (1-(p-chlorobenzyl)-5-methoxy-2-methylindole acetic acid), glucametacine (1-(p-chlorobenzoyl-5-methoxy-2-methylindole-3-yl acetic acid glucose amide), acemetacine (1-(p-chlorobenzoyl)-5-methoxy-3-methylindole-3-acetic acid-glycolic acid-ester), and sulindac (5-fluor-2-methyl-1-p-(methylsulphenyl)-benzylidene-indene-3-acetic acid); Phenyl acetic acid or phenyl propionic acid derivatives such as ibuprofen (2-(4-isobutylphenyl)-propinic acid); naproxen (2-(6-methoxy-2-naphthyl)-propinic acid), alclofenac (4-allyloxy-3-chlorophenyl-acetic acid), ketoprofen (2-(3-benzylphenyl)benzoic acid), diclofenac (2-(2,6-dichlorophenylamino)-phenylacetic acid), fenoprofen (2-(3-phenyloxyphenyl)-acetic acid), tolmetin (1-methyl-5-(p-toluyl)-pyrrole-2-yl-acetic acid), flurbiprofen (2-2-fluorobiphenyl-4-ye-proprionic acid), and suprofen (p-2-thenoyl-hydratropic acid) phenyl-propionic acid); Anthranilic acids and their nitrogen analogs such as flufenamino acid (N-(m-trifluoromethylphenyl)-anthranilic acid), mefenamino acid (N-(2,3-dimethylphenyl)-anthranilic acid), and niflumin acid (2-(3-trifluoromethylaminolino)-nicotinic acid).

Phospholipid compounds found to be particularly useful in the practice of the present invention are dilinoleoyl phosphatidylcholine (DLL-PC), dipalmitoyl phosphatidylcholine (DPPC) and egg phosphatidylcholine (Egg-PC or PC_e). In DPPC, a saturated phospholipid, the saturated aliphatic substitution R_1 and R_2 are CH_3--$(CH_2)_{14}$, R_3 is CH_3 and X is H. In DLL-PC, an unsaturated phospholipid, R_1 and R_2 are CH_3--$(CH_2)_4$--CH=CH--CH_2--CH=CH--$(CH_2)_7$, R_3 is CH_3 and X is H. In Egg PC, which is a mixture of unsaturated phospholipids, R_1 primarily contains a saturated aliphatic substitution (e.g., palmitic or stearic acid), and R_2 is primarily an unsaturated aliphatic substitution (e.g., oleic or arachidonic acid).

Description of the Neutral Lipids

Neutral lipids form another component of some embodiments of the compositions described herein. This class of lipids include the triglycerides.

The triglycerides useful in the practice of the present invention are generally characterized by the formula:



wherein R_1, R_2 and R_3 are each saturated or unsaturated substitutions ranging from 4 to 32 carbon atoms; and R_4 is either $=O$ or H_2.

As will be appreciated, this structure embraces a wide range of triglycerides, both saturated and unsaturated, and include, for example, triglycerides such as tripalmitin (saturated), triolein and trilinolein (both unsaturated). A further listing of saturated and unsaturated fatty acids that can be esterified or ether-linked to the triglyceride in question is provided in U.S. 5,032,585, which is specifically incorporated herein by reference.

In a particular anticipated pharmaceutical preparation, the preparation will be provided in a pill form suitable for human ingestion, and contain about 2 to about 300 mg per kg aspirin or salicylate, together with an equimolar amount of PC, DPPC, or a combination thereof, or any other zwitterionic phospholipid. In the described methods, the compositions include the NSAID and the zwitterionic phospholipid in molar ratios ranging from about 1:0.1 to about 1:20, and preferably from about 1:0.5 to about 1:2. In a most preferred embodiments, the ingredients are included in a molar ratio of about 1:1.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to

constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1
GI LEGIONARY AND BLEEDING - EFFECT OF NSAID AND ZWITTERIONIC PHOSPHOLIPID COMPOSITIONS

The present example demonstrates the utility of the present invention for providing compositions that reduce the GI-related side-effects commonly associated with NSAIDS.

In order to retard the ability of NSAIDs to interact with the extracellular phospholipid lining of the mucus gel layer, several NSAIDs were preassociated with zwitterionic phospholipids and the effect was determined in various rat ulcer models. The present example shows that ability of the NSAIDs to induce acute and/or chronic GI lesions and bleeding was remarkably decreased when the drugs were administered as a complex with DPPC or related phospholipids. Surprisingly, the anti-pyretic and anti-inflammatory activity of aspirin appeared to be consistently enhanced when associated with zwitterionic phospholipids. This is in contrast to previous side effects of other formulations that reportedly suffer from reduced therapeutic efficacy or onset (Alpsten *et al.*, 1982; Mojaverian *et al.*, 1987).

Ulcer Models

Gastric lesions were acutely induced in rats in accordance with the following techniques. For the salicylate-based NSAIDs, fasted male Sprague Dawley rats (150-200g) were intragastrically injected with saline (control), ASA or salicylate, or the drugs preassociated with an equimolar concentration of DPPC (all solutions adjusted to a pH of 3.1). Ten minutes later, the rats were intragastrically challenged with 1 ml of 0.6 N HCl. Gastric lesions were macroscopically scored 60 minutes later in accordance with a previously outlined method (Lichtenberger *et al.*, 1983, incorporated herein by reference).

In order to investigate the effects of non-salicylate NSAIDs to induce GI bleeding, fasted rats were subcutaneously injected with N-nitro-L-arginine Methyl Ester (L-NAME), 1hr. before and 1 and 6 hrs. after intragastrically receiving 1 ml of the NSAIDs; indomethacin, diclofenac, and naproxen administered alone and in association with an equimolar concentration of DPPC. The Nitric Oxide synthesis inhibitor, L-NAME, is administered before and after the NSAID to increase the rat's sensitivity to the drug in accordance with the method of Chen et. al., *Gastroenterology* **104**: A53, 1993). Eighteen hours after receiving the NSAID, the distal half of the intestine was excised and flushed with 10 ml of saline. The hemoglobin (Hb) concentration of the intestinal perfusate was measured as an estimate of GI bleeding in accordance with a previously described method (Lichtenberger *et al.*, 1983).

Rats were treated with ASA over a two week period to investigate the chronic effects of NSAID exposure (in the presence and absence of phospholipids) on hematocrit, gastric mucosal hydrophobicity, and granuloma formation. In order to ensure that the rat had an empty stomach prior to receiving the daily intragastric dose of ASA, they were placed on a reverse lighting schedule (9AM/lights off; 5PM/lights on), and were only provided access to chow during the day (dark period). The test solutions (saline, ASA and ASA/Phospholipon 90G complex) were intragastrically administered between 8AM - 9AM daily during the two week study period. In these chronic exposure experiments, ASA was complexed with an equimolar concentration of Phospholipon 90G (purified soya lecithin, prepared and obtained from Nattermann GmbH of Cologne, Germany) instead of DPPC. At the completion of the study period, blood was collected into a capillary tube for the determination of hematocrit and the stomach was excised for contact angle analysis.

One of a number of NSAIDs was intragastrically administered to rats alone or preassociated with an equimolar concentration of DPPC or Phospholipon 90 G (purified soya lecithin, prepared by Nattermann GmbH of Cologne Germany) in several models of acute and chronic injury of the upper GI tract. Two contrasting animal models were employed to determine the ability of DPPC to protect against acute NSAID injury to the GI tract. For salicylate-based NSAIDs, which primarily induce stomach injury, gastric lesions were scored in fasted rats who were initially treated with aspirin or salicylate alone or complexed with DPPC and challenged 10 minutes later with a supra-physiological dose of HCl. For non-salicylate NSAIDs

(indomethacin, diclofenac and naproxen) which predominantly induce injury to the mid-distal regions of the small intestine, the quantity of intraluminal blood was assessed in the distal half of the small intestine of rats who were pre- and post-treated with the Nitric Oxide synthetase inhibitor, L-NAME (N-nitro-L-arginine methyl ester).

The results, shown in FIG. 4A and FIG. 4B, respectively, clearly demonstrate (in rats sensitized to the drugs) that the injurious potential of both salicylate-based and non-salicylate NSAIDs to induce acute GI lesions and bleeding is significantly reduced, by $>85\%$, if the NSAID is preassociated with the zwitterionic phospholipid prior to administration. Similarly, daily administration of aspirin to rats over a 2 week period resulted in a significant fall in both hematocrit and gastric mucosal hydrophobicity, which was prevented in rats that received the aspirin/Phospholipon G complex (Table 2).

Table 2. Effect of DPPC on Aspirin's Chronic GI Side Effects

Group	Gastric Mucosal Hydrophobicity (degrees, Contact Angle)[b]	Hematocrit
Saline (Control)	52.2 ± 2.7(6)	51.7 ± 0.4 (10)
Aspirin (90mg/kg)	15.8 ± 6.6[c](6)	47.8 ± 0.2[c](10)
Aspirin + Phospholipon G	55.3 ± 5.8[c,d](6)	53.1 ± 0.4[c,d](10)

[b] Gastric mucosal hydrophobicity was measured by contact angle analysis as described in Hills *et al.*, 1983; Goddard *et al.*, 1987; Goddard *et al.*, 1990.

[c] $p < 0.05$ in comparison to saline-treated control values.

[d] $p < 0.05$ in comparison to values of aspirin-treated rats.
(n) number of rats/group

EXAMPLE 2 - SOLUBILITY STUDIES; COMPLEX FORMATION BETWEEN NSAIDS and ZWITTERIONIC PHOSPHOLIPIDS

To demonstrate the effect of DPPC on the solubility of the sodium salts of the five NSAIDs (naproxen, indomethacin, diclofenac, salicylate and aspirin) in chloroform, each NSAID was added to chloroform at a 30 nM final concentration. DPPC was dissolved in the chloroform that was contained in half the tubes at a final concentration which ranged between 5 - 40 nM, prior to the addition of the NSAID salt. DPPC, as well as PC and other zwitterionic phospholipids useful in the practice of the invention, may also be dissolved in other organic solvents, such as ethanol, in the practice of the present invention. The tubes were gently mixed at 25°C for 16 hrs, after which they were photographed and/or centrifuged (2,000g for 15 min) and the supernatant collected to determine the concentration of NSAID

in solution. The latter was assessed by measuring the NSAID's UV absorbance at 290 nm, and comparing these readings to the appropriate standard curves for each NSAID. It should be noted that for each of the five NSAIDs a linear relationship existed between the drug's concentration (nM) in water and the UV absorbance reading. The equations for the regression lines for each of the NSAIDs were as follows: aspirin, $y = 2.603x - 0.004$, $r = 0.999$; salicylate, $y = 8.780x + 0.123$, $r = 0.999$; indomethacin, $y = 18.325x + 0.156$, $r = 0.999$; diclofenac, $y = 21.523x + 0.008$, $r = 0.999$; and naproxen, $y = 3.732x + 0.005$, $r = 0.999$. In all cases the readings for the unknowns fell within the linear portion of the standard curves. Further, the presence of DPPC in the solvent did not interfere with these analyses, as it failed to contribute to the UV absorbance reading in the absence of the NSAID.

To assess the effect of DPPC on the solubility of the sodium salt of ASA in water, ASA was dissolved in water at a final concentration of 30 nM (pH adjusted to 6.0), and its intrinsic fluorescence (290 nm/excitation; 406 nm/emission) was monitored. DPPC was present as a lipidic suspension in half the tubes at a final concentration which ranged between 15 - 60 nM. The tubes were gently mixed at 25 °C for the desired incubation period, after which they were centrifuged (2,000g for 15 min) and the supernatant collected to determine the concentration of ASA in solution. Once again a linear relationship ($y = 14.02 + 0.353$, $r = 0.999$) was found between the fluorescent reading and the concentration of aspirin in solution.

It can be appreciated from FIG. 1A and FIG. 1B that the sodium salts of these NSAIDs are insoluble in chloroform unless an equimolar or greater concentration of DPPC is added to the organic solvent, at which point complete solvation takes place. Conversely the sodium salts of the NSAIDs are readily soluble in either saline or water, and are removed from solution as a complex within minutes after an equimolar concentration of DPPC is added as a lipidic suspension. The solubility of aspirin in saline can be followed either by monitoring its intrinsic fluorescence or radioactivity (employing ^{14}C-labelled aspirin). FIG. 2A and FIG. 2B demonstrate that the injection of DPPC into an aqueous solution results in the precipitation of the NSAID, presumably as a complex with the phospholipid. The rapid change in solubility of the NSAID in both the organic and aqueous solvent systems does not occur if DPPC is substituted by the anionic phospholipid, dipalmitoylphosphatidylglycerol (DPPG).

EXAMPLE 3 - GRANULOMA FORMATION AND ENHANCED ANTI-INFLAMMATORY ACTIVITY

The present example demonstrates the utility of the present methods for enhancing the anti-inflammatory activity of ASA accomplished when ASA, or other non-steroidal anti-inflammatory drug complexed with Phospholipon 90G. A foreign-body granuloma model widely used by those of skill in the art to assess anti-inflammatory action was employed.

The inventors used the rat model of foreign-body granuloma formation. This model is recognized by those of skill in the art as a representative model for

granuloma formation and anti-inflammatory activity, as described in Ucelay *et al.*, (1988); and Castro *et al.*, (1980). These references are specifically incorporated herein for the purpose of providing details associated with the use of this model.

Methods

The above model has been successfully employed in both rats and invertebrates to quantify this most basic component of a tissue's response to injury (Ucelay *et al.*, 1988; Castro *et al.*, 1980; Clatworthy *et al.*, 1994, each incorporated herein by reference). Sterile tared cotton string was surgically implanted (bilaterally) under the abdominal skin of ether anesthetized rats on day 1 of the study period, and then randomly placing rats in a group to be daily treated with saline, aspirin or the NSAID complexed with Phospholipon 90G over a two-week period.

At the completion of the study period the string with the adherent granuloma tissue was surgically dissected from the euthanized rats and dried in a vacuum for several days at room temperature, until a baseline dry weight was obtained. The difference between this value and the initial dry weight of the string prior to implantation divided by the latter value provided an estimate of the weight of granuloma tissue. This technique proved to be very reproducible and accurate, as determined both by the close agreement between the changes in weight of the two pieces of string that were implanted contralaterally in each animal ($< 12.5\%$

difference in values between the right and left string); and the low variance (< 8%) in values of granuloma formation within a group of animals.

Improvement in the anti-inflammatory efficacy of ASA was observed over a 2 week study period. This effect is seen when the NSAID was administered as a complex with Phospholipon G at concentrations below the maximally effective dose for this particular drug (see Table 3, FIG. 5).

Table 3. Effect of Aspirin ± DPPC Formulations on Foreign-Body Granuloma Formation.

Group	± DPPC	n	Granuloma Formation[a]
Saline (Control)	-	5	3.20 ± 0.10
ASA (90 mg/kg)	-	5	1.90 ± 0.10[b]
	+	5	1.4 ± 0.10[b,c]
ASA (140 mg/kg)	-	6	1.07 ± 0.09[b]
	+	6	1.00 ± 0.15[b]

[a] The values represent the dry weight of the string with adherent granuloma tissue - dry weight of string/dry weight of the string.

[b] $p < 0.05$ in comparison to saline-treated control values.

[c] $p < 0.05$ in comparison to values of rats treated with NSAIDs alone.

EXAMPLE 4 - CONTACT ANGLE ANALYSIS

The present example is provided to describe the model which was used to examine the surface tension reducing action of the compositions of a combination of non-steroidal anti-inflammatory agent and zwitterionic phospholipid.

Contact angle analysis was performed with the use of a goniometer on excised gastric mucosal tissue, that was lightly blotted and dried, as previously outlined (Hills *et al.*, 1983; Goddard *et al.*, 1987; Goddard *et al.*, 1990, each incorporated herein by reference). Briefly, this was accomplished by applying a droplet of water ($\sim 5\mu l$) to the tissue surface, and employing the telescopic eyepiece of the goniometer to measure the maximal angle that is dissected at the triple point, where the solid/liquid/ and air interface meet.

EXAMPLE 5 - ANTIPYRETIC ACTIVITY

An established rat fever model was used in the present example to demonstrate the utility of the invention for enhancing the anti-pyretic activity of a NSAID by combining these class of agents with a phospholipid.

The rat fever model involves the injection of rats with Brewer's yeast (2g/kg, s.c.) to induce an increase in fever of 0.5 -1.5°C. These models are described in Adams *et al.* (1968) and Ucelay *et al.* (1988), which references are specifically incorporated herein for this purpose. The animals were intragastrically treated (instilled) with either saline, 90 mg/kg ASA, or 90 mg/kg ASA preassociated with an equimolar concentration of DPPC. Similar antipyretic analyses were performed with the sodium salts of the following NSAIDs: diclofenac (10 mg/kg), indomethacin (10 mg/kg) and naproxen (30 mg/kg), alone and complexed with an equimolar concentration of DPPC. All test solutions were titrated to a pH of 4.5 prior to intragastric administration. Rectal temperatures were

monitored in conscious, restrained rats at all indicated times. This technique provided a very reliable and reproducible estimate of the antipyretic activity of NSAID formulations as indicated by the fact the variance within a group was low, with the standard errors < 5% of the mean values, and the fact that the difference in mean temperature values for a given group varied < 2 % between separate experiments.

EXAMPLE 6 - PHARMACEUTICAL COMPOSITIONS

The complexes described in the present example are preferably for oral administration, for example, with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds (i.e., NSAID and Zwitterionic phospholipid and/or neutral lipid) may be incorporated with excipients or carriers and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain enough active NSAID compound to achieve an effective plasma level of the active drug. Aspirin, for example, would be provided in doses of from about 10 mgm, or about 20 mg, or 32.5 mg or even up to 60 mg, or 300 mgs/kg would be contained in each tablet or dose, where employed for administration to animals having a weight of about 60 kg - 70 kg. The dose will vary depending on the NSAID or combinations of NSAIDs used. The amount of the NSAID in particular preparations is more generally described as

an amount of NSAID or combination of NSAIDs effective to provide a pharmacologically active plasma concentration of the drug when used in combination with zwitterionic phospholipid. These amounts are determinable by one of ordinary skill in the pharmaceutical arts given the data disclosed herein, and general pharmaceutical references such as Remingtons Pharmaceutical Sciences.

The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose, aspartame or saccharin may be added, or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both.

A syrup of elixir may contain the active compounds sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor, again formulated for oral administration. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active

compounds may be incorporated into sustained-release preparation and formulations.

The present compositions may also be formulated as injectable formulations, or as formulations suitable for enteral administration, according to those techniques known to those of ordinary skill in the medicinal arts.

EXAMPLE 7
Lipid-Permeability of NSAID/Phospholipid Complex

The present example illustrates the utility of the invention for providing compositions and methods for enhancing the bio-absorption and bio-availability of NSAID's.

The diffusion of aspirin (alone and complexed with phospholipid) from water into cyclohexane was used as a model to estimate the membrane permeability of the drug.

Permeability Analysis

The sodium salt of ASA (also salicylate) was dissolved in 5 ml of water at a final concentration of 100mM (pH adjusted to 6.0) and gently stirred at 25°C. An equal volume of cyclohexane was layered over the aqueous solution and the entry of the NSAID into the organic phase was monitored fluorometrically over time. In order to determine the effect of phospholipid association on the lipid permeability of the NSAID, ASA (or salicylate) at the above concentration was sonicated in the

presence of 0.5 mM phospholipid (DPPC or DPPG) in water (adjusted to a pH of 7), and its rate of diffusion into the cyclohexane phase was measured fluorometrically. This was accomplished by removing 1 ml of the top phase chloroform solution by pipette, injecting it into the cuvette to obtain the fluorescence reading, and returning the sample to the incubation vessel to assure that the volume did not change. This entire process could be completed in < 30 seconds.

In these studies, the concentration of NSAID:phospholipid in water was adjusted to a molar ratio of 200:1, creating a large driving force to promote NSAID flux into the hydrocarbon phase, and minimizing the turbidity encountered with high phospholipid concentrations.

Results

Under neutral conditions, the passive diffusion of aspirin across an aqueous - hydrocarbon interface, as assessed fluorometrically, was negligible unless it was chemically associated with the zwitterionic phospholipid, DPPC (See Figure 3). Furthermore, this increase in the flux rate into the organic phase, was simply not a consequence of liposomal encapsulation since the NSAID failed to enter cyclohexane if the anionic phospholipid, DPPG was substituted for DPPC. These studies also revealed that DPPC promoted the flux of sodium-salicylate from the aqueous to the organic phase in a similar manner.

EXAMPLE 8
ANTI-INFLAMMATORY AND ANTI-PYRETIC ACTIVITY OF NSAID/PHOSPHOLIPID COMPLEX

This example demonstrates the utility of the present invention for enhancing the fever-reducing potential of the NSAID (20 mg/kg dose) when chemically associated with a zwitterionic phospholipid. The ability of NSAIDs (administered alone and complexed with DPPC) to reduce fever in rats was determined. Fever was induced 18 hrs prior to drug treatment by the subcutaneous administration of Brewer's yeast (Adams *et al.*, 1968; Ucelay *et al.*, 1988).

The results (See FIG. 4C) indicate that the anti-pyretic activity of the ASA/DPPC complex was significantly greater than that of the NSAID alone, at all time periods examined.

The enhancement in anti-pyretic activity of the NSAID/DPPC complex compared to the NSAID alone over the first three hours after intragastric administration, was observed to a lesser degree with sodium salts of the following drugs; diclofenac (-0.29°C), indomethacin (-0.28°C), and naproxen (-0.30°C).

EXAMPLE 9
ENHANCEMENT OF ANTIPYRETIC ACTIVITY OF ASA/DPPC COMPLEX AT SUBTHRESHOLD ASA DOSAGE

The antipyretic activity of the ASA/DPPC complex was determined in rats as in Example 8 (supra), this time at much lower doses, more than 2 times lower than in Example 8. In the present example, a dosage of 9.0 mg/kg ASA was

administered either alone or complexed with DPPC. The data from this study is summarized in FIG. 6.

As can be seen in FIG. 6, ASA alone does not have significant antipyretic activity at this dosage level, however ASA complexed with DPPC does have significant antipyretic activity over a five hour period. The dosage level in the present example is a 10-fold reduction of the standard dosage of 90 mg/kg, as used in Example 8, and reported in FIG. 4C.

Based on the recommended human dosages of 90 mg/kg for juvenile rheumatoid arthritis, or 325 to 650 mg for antipyretic or analgesic treatment in adults (See pages 1110-1111, Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Company, Easton, Pennsylvania, 1990, incorporated herein by reference), it is expected that ASA complexed with DPPC, or other zwitterionic phospholipid, at a 10-fold lower dosage, i.e. approximately 9 mg/kg for juvenile rheumatoid arthritis or 32.5 to 65 mg for antipyretic activity in adults, would be as effective as the normal dosage of ASA alone to provide fever-reducing activity.

EXAMPLE 10
ANALGESIC EFFECT OF COMPLEXED-NSAIDS

It is contemplated that, in light of the demonstrated enhancement of the effects of NSAIDs (anti-inflammatory and antipyretic) when complexed with a zwitterionic phospholipid, or even further administered in combination (i.e., in a mixture) with a neutral lipid (triglyceride) that the analgesic effects of these drugs

will also be enhanced when complexed with the phospholipids. Two established pain tests, using rats, may be employed to demonstrate enhancement of this effect. Tail retraction occurs in response to a noxious stimulus that generates pain. Tail retraction response may be observed and timed in rats given a placebo in order to determine a base line reaction time. If after receiving either DPPC-complexed or uncomplexed aspirin the time latency between pain induction (with a laser heat source) and tail retraction increases, this is a direct reflection of the analgesia. Retraction times will be compared between placebo and aspirin-treated rats. The test will be conducted with varying dosages and varying times after the administration of the placebo, aspirin, and/or other NSAIDs, or complex to determine if dosage or duration have some effect on the strength of the analgesia.

A model for analgesic effect in rats involves animals injected with formalin. The rats are injected under the dorsal surface of the right hind paw with a 0.05 ml volume of 15% formalin and saline solution (Helmstetter and Fanselow, 1987). The treated rats are then given the aspirin, and/or other NSAIDs, aspirin/DPPC or PC complex, or placebo at various times before and after injection with the irritating solution. The rats are then placed in a cage and their behavioral responses to the painful stimulus are be observed (employing a video camera system) and graded as follows: (1) freezing - an absence of all activity other than respiration; (2) paw lifting - the rat holds its treated paw close to its body; (3) paw licking - the rat either licks the treated paw or has some other type of mouth contact with it; and (4) general activity - this involves any other type of general movement. The

behavioral response of the rat is indicative of its sensitivity. The formalin test, along with the tail flick test, comprises two distinct and separate tests to evaluate pain sensitivity and analgesia effectiveness.

EXAMPLE 11
Effects of Low Dose Aspirin (9 mg/kg) Alone and as a Complex with Phospholipid/Neutral Lipids on Foreign-Body Granuloma Formation in Rats[a]

The present example is provided to demonstrate the utility of the present invention for reducing inflammation. The string granulona model described herein was again used to demonstrate the activity of the presently disclosed methods for treating this condition.

Sterile tared string was surgically implanted. Five days later rats were intragastrically administered twice a day with either saline, ASA alone, or ASA lipid mixtures. Rats were sacrificed after each rat received 5 doses of the test compounds and the string and granuloma excised and weighed.

*$p < 0.05$ vs. ASA alone. ASA = Aspirin;

DPPC = Dipalmitoylphosphatidylcholine,

TP = Tripalmitin; TO = Triolein

TABLE 3

Saline	ASA	ASA DPPC/TP	ASA PhospholiponG/TO
4.08(8)	3.89(8)	3.44(8)	3.49(8)*
±25	±0.06	±0.24	±0.17

Table 3. As demonstrated in the data provided in Table 3, a phospholipid (DPPC)/neutral lipid (TO) microemulsion appeared to modestly enhance the anti-inflammatory activity of ASA when the NSAID was administered at a subthreshold dose (9 mg/kg) for this activity.

EXAMPLE 12
Effect of DPPC on Aspirin's Inhibitory Effect on Platelet Aggregation

The present example demonstrates the ability of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) to inhibit cellular activation resulting in either platelet aggregation or the synthesis and release of inflammatory mediators. The activity is shown to be enhanced if the NSAIDs are administered as a complex with zwitterionic phospholipids alone or together with neutral lipids.

High Ristocetin (1.5 mg/ml) Test		
	% Platelet Aggregation	
ASA Conc	-DPPC	+DPPC
0.00	93%	100%
0.01mM	100%	100%
0.1 mM	83%	87%
1.0 mM	95%	35%
1.0 mM[a]	100%	84%
Low Ristocetin (0.75) mg/ml Test		
	% Platelet Aggregation	
ASA Cone	-DPPC	+DPPC
0.00	75%	94%
0.01mM	65%	12%
0.1mM	26%	20%
1.0mM	80%	6%
1.0mM[a]	39%	15%

Abbreviations: ASA = aspirin

DPPC = dipalmitoylphosphatidylcholine

DPPC was added at a conc. equimolar to the aspirin.

In the absence of aspirin, DPPC was added at a final concentration of 1 mM.

[a] Second study.

The advantage of this invention is it will allow the NSAIDs to be administered at a lower than normal dose, due to their enhanced efficacy and potency-thus increasing their effectiveness and minimizing their side-effects on the GI tract and other organ systems.

A number of embodiments of the invention would include the combination of NSAIDs with zwitterionic phospholipids alone and together with neutral lipids. These combinations would both increase the efficacy and potency of NSAIDs to inhibit the activation of: 1) platelets; 2) neutrophils; 3) monocytes/macrophages; 4) lymphocytes; 5) Pans and 7) other bone-marrow derived cell types.

Considering the interest in the pharmaceutical industry in the role of NSAIDs in the prevention of cardiovascular disease and tissue/joint inflammation, the presently described pharmaceutical preparations would provide alternative clinical management protocols with a improved bioavailability at lower doses of the sometimes irritative NSAID regimen.

This list would include at minimum the 20-40 pharmaceutical companies presently marketing an NSAID.

EXAMPLE 13

Anti-Secretory Agents together with NSAID and Lipids

The present example demonstrates the utility of the present invention for compositions that include an anti-secretory drug, such as Tagamet, or other histamine type 2 receptor antagonist, or Omeprazole (Prilosec™, or other proton-pump inhibitor or H^+/K^+ATPase inhibitors), either before or along with an NSAID complexed with phospholipid and/or neutral lipid (such as a triglyceride). These embodiments of the invention are further described in Example 14.

While not intending to be limited to any particular mechanism of action, by including a phospholipid and/or neutral lipid, the poor absorption of NSAID's that sometimes results with an anti-secretory agent administered therewith, may to some degree be prevented or lessened. Hence, the bioavailability and therapeutic action of the NSAID when administered together with an anti-secretory agent may be maintained, and in some cases enhanced.

The present example also demonstrates that the therapeutic (antipyretic) activity of aspirin, and other NSAIDs, is attenuated if animals are pre-treated with an agent that inhibits gastric acid secretion. Omeprazole (sold under the name Prilosec™) is in the class of "proton pump inhibitors", also called "H^+/K^+ ATPase inhibitors" that act by irreversibly binding to and inhibiting H^+/K^+ ATPase of the perietal cell, the rate limiting enzyme in gastric HCI secretion. Ranitidine (sole under the name Zantac™) is in the class of "H_2 receptor antagonists" that prevents

histamine from binding to its type-2 receptor on the parietal cell to inhibit gastric acid secretion. It can be appreciated from figures 22 and 23 that the blocking effect of these two classes of antisecretory drugs on the therapeutic actions of the NSAID, however, is overcome if the NSAID is complexed with a zwitterionic phospholipid. As demonstrated in Figures 23 and 23, aspirin at a dose of 20 mg/kg failed to reduce fever in rats if the NSAID was administered in conjunction with either the proton pump inhibitor (Prilosec™) or a histamine blocker, Ranitidine (Zantac™). This block in therapeutic activity due to inhibition of gastric acid secretin was overcome if the NSAID was administered as a complex with phospholipid, DPPC.

EXAMPLE 14 - Therapeutic Regimens

The present example is provided to demonstrate various therapeutic combination regimens for treating fever, inflammation and pain. These regimens include the administration of NSAIDs together with phospholipid and/or neutral lipid.

Agents that include phospholipid, such as lecithin - tablets and the like, may be used as part of the regimens disclosed herein for the enhancement of NSAID activity.

Therapeutic Regimen

For use as an improved regimen (e.g., anti-pyretic, platelet aggregation, analgesic), the present invention contemplates an initial administration of the

phospholipid, such as in a tablet or phospholipid-containing agent, such as lecithin tablets, that contain phospholipid (e.g., phosphatidyl choline).

Either at the same time or following the administration of a phospholipid or phospholipid containing agent, the patient would then be given an NSAID. The NSAID may also be administered in combination with the phospholipid as a single composition, or alternatively as a combination with both a phospholipid and a neutral lipid, such as a triglyceride (e.g., TO).

The present inventors also propose regimens that include the administration of an NSAID and phospholipid and/or neutral lipid, either before or at the same time as an anti-secretory drug, such as Tagamet® and Prilosec™. This is because antisecretory drugs are observed by the present inventor to reduce the anti-pyretic action of NSAID's. It is expected that the inclusion of phospholipid and/or neutral lipid will improve the observed reduced absorption of NSAID's observed when NSAIDs are administered with an antisecretory agent alone. Typically, this reduced absorption required that a higher dose of the NSAID be administered to the patient in order to provide the desired therapeutic effect.

Combinations of NSAIDs with zwitterionic phospholipids alone or in combination with neutral lipids will promote the ability of this family of drugs to influence certain target cells, such as neutrophils, platelets, eosinophils, macrophages, and others. In doing so, said phospholipids will increase the efficacy

and potency of the NSAIDs to inhibit the cellular cyclo-oxygenase and the formation of arachidonic acid-derived products and other agents involved in cellular aggregation, adhesion, and the synthesis and release of inflammatory mediators and/or cytokines.

* * *

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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WHAT IS CLAIMED IS:

1. A method for enhancing the anti-pyretic activity of a non-steroidal anti-inflammatory drug comprising:

providing a non-covalently associated composition of a zwitterionic phospholipid with an amount of non-steroidal anti-inflammatory agent that provides reduced anti-pyretic activity in the absence of the zwitterionic phospholipid.

2. The method claim 1 wherein the composition is further defined as comprising an equimolar amount of zwitterionic phospholipid and non-steroidal anti-inflammatory agent.

3. The method of claim 1 wherein the non-steroidal anti-inflammatory drug is salicylate, aspirin, naproxen, indomethacin, diclofenac, or a mixture thereof.

4. The method of claim 1 wherein the anti-pyretic activity of the non-steroidal anti-inflammatory drug is enhanced about 2-fold to about 6-fold in the presence of the zwitterionic phospholipid compared to non-steroidal anti-inflammatory drug without phospholipid.

5. The method of claim 1 wherein the non-steroidal anti-inflammatory drug is salicylate.

6. The method of claim 1 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidylcholine.

7. The method of claim 1 wherein the amount of non-steroidal anti-inflammatory drug is about 2 mg to about 300 mg.

8. A method of inhibiting platelet aggregation comprising:

providing a non-covalently associated combination of zwitterionic phospholipid and an amount of non-steroidal anti-inflammatory agent that provides reduced inhibition of platelet aggregation in the absence of zwitterionic phospholipid.

9. The method of claim 8 wherein the composition is further defined as comprising an equimolar amount of zwitterionic phospholipid and non-steroidal anti-inflammatory agent.

10. The method of claim 8 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidyl choline.

11. The method of claim 8 wherein the non-steroidal anti-inflammatory drug is salicylate, aspirin, naproxen, indomethacin, diclofenac, or a mixture thereof.

12. The method of claim 1 or 8 wherein the composition further comprises neutral lipid.

13. The method of claim 12 wherein the neutral lipid is triglyceride.

14. A method for enhancing the analgesic activity of a non-steroidal anti-inflammatory drug comprising:

providing a non-covalently associated composition of a zwitterionic phospholipid and an amount of a non-steroidal anti-inflammatory drug that provides reduced pharmacological activity in the absence of zwitterionic phospholipid.

15. A method for enhancing the anti-inflammatory activity of a non-steroidal anti-inflammatory drug comprising:

providing a non-covalently associated composition of a zwitterionic phospholipid with an amount of a non-steroidal anti-inflammatory drug that provides reduced pharmacological activity in the absence of zwitterionic phospholipid.

16. The method of claim 15 wherein the composition is further defined as comprising an equimolar amount of zwitterionic phospholipid and non-steroidal anti-inflammatory drug.

17. The method of claim 14 or 15 wherein the non-steroidal anti-inflammatory drug is salicylate, naproxen, indomethacin, diclofenac, aspirin, or a mixture thereof.

18. The method of claim 14 or 15 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidyl choline.

19. The method of claim 14 or 15 wherein the composition further comprises a neutral lipid.

20. The method of claim 20 wherein the neutral lipid is a triglyceride.

21. A pharmaceutical preparation comprising a non-covalently associated combination of nonsteroidal anti-inflammatory agent, zwitterionic phospholipid, and neutral lipid.

22. The pharmaceutical preparation of claim 24 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidyl choline and the neutral lipid is tripalmitin.

23. The pharmaceutical preparation of claim 21 further defined as essentially free of anionic phospholipid.

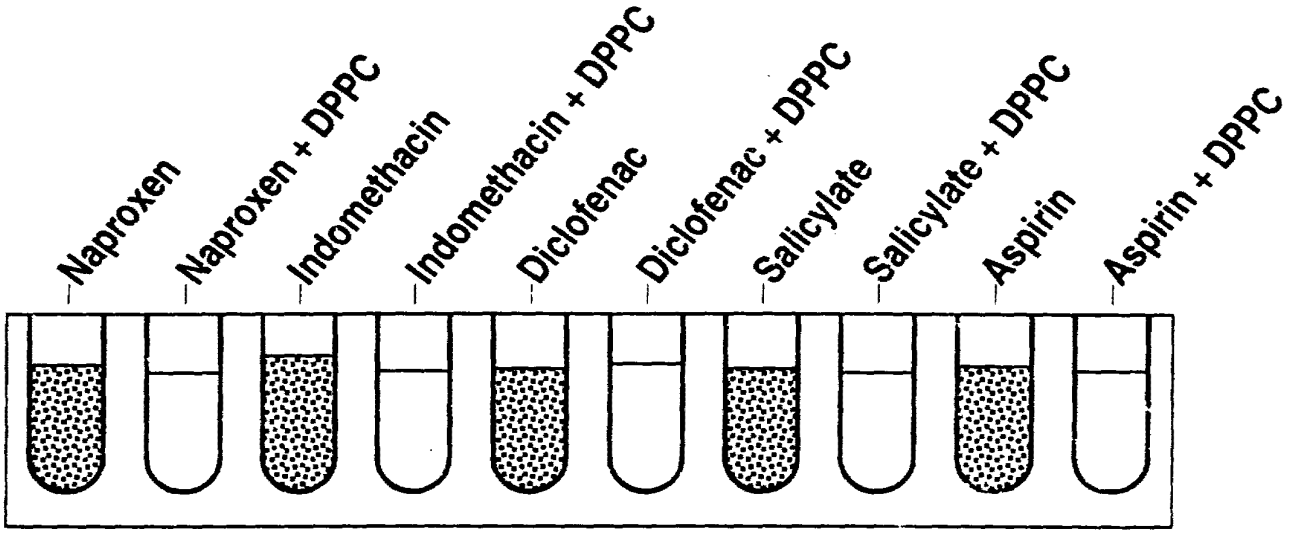
Naproxen
Naproxen + DPPC
Indomethacin
Indomethacin + DPPC
Diclofenac
Diclofenac + DPPC
Salicylate
Salicylate + DPPC
Aspirin
Aspirin + DPPC

Fig. 1A

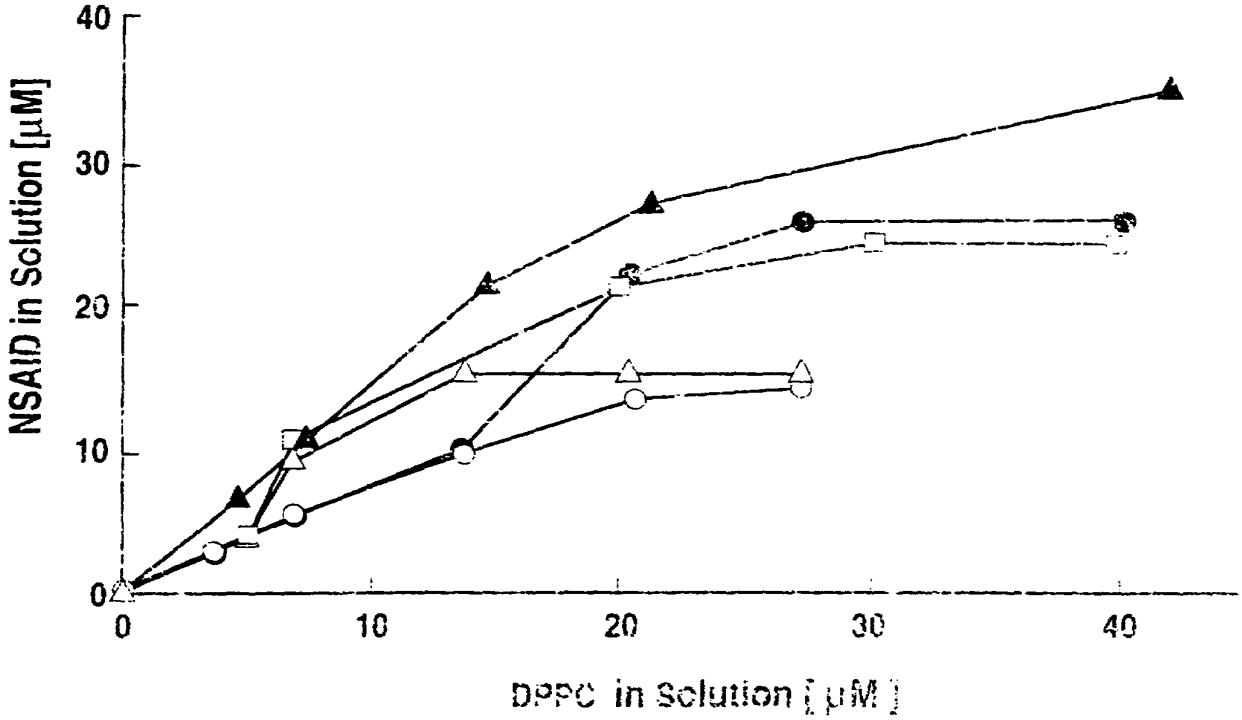
NSAID in Solution [µM]
40
30
20
10
0
0
10
20
30
40
DPPC in Solution [µM]

Fig. 1B

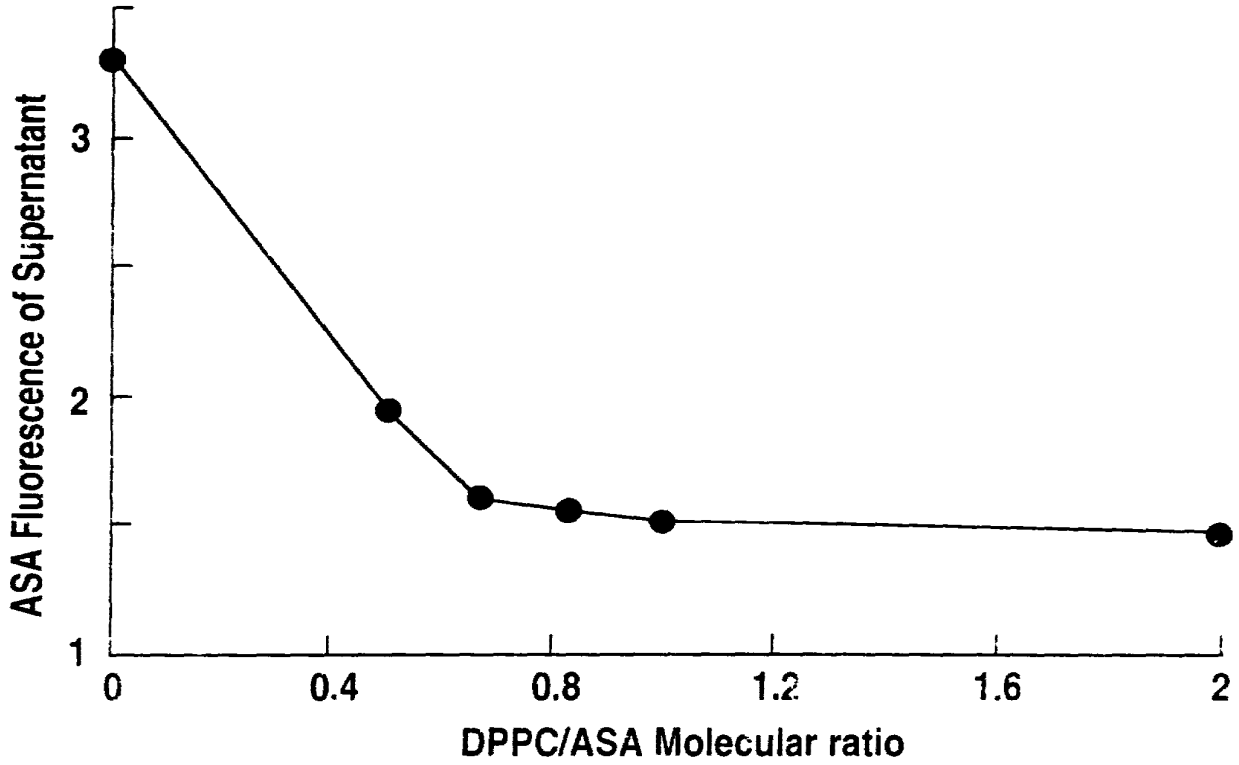
ASA Fluorescence of Supernatant
3
2
1
0
0.4
0.8
1.2
1.6
2
DPPC/ASA Molecular ratio

Fig. 2A

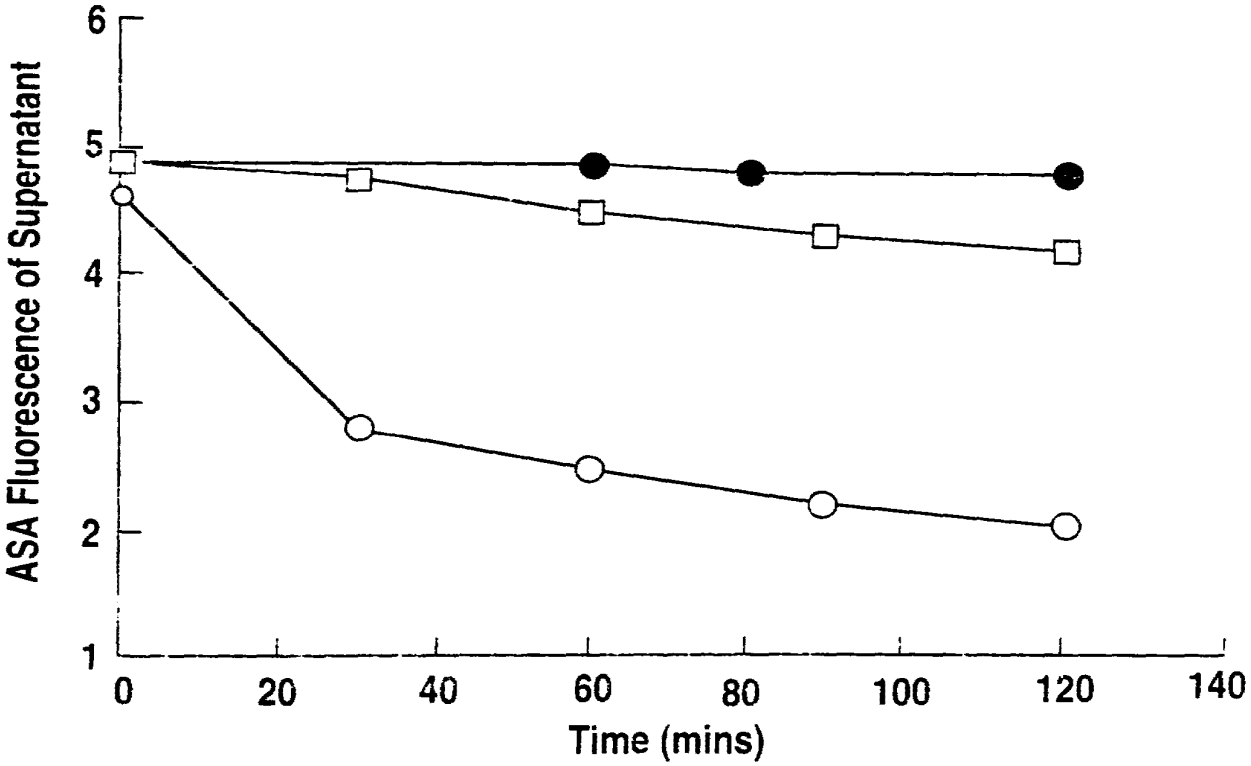
ASA Fluorescence of Supernatant
6
5
4
3
2
1
0
20
40
60
80
100
120
140
Time (mins)

Fig. 2B

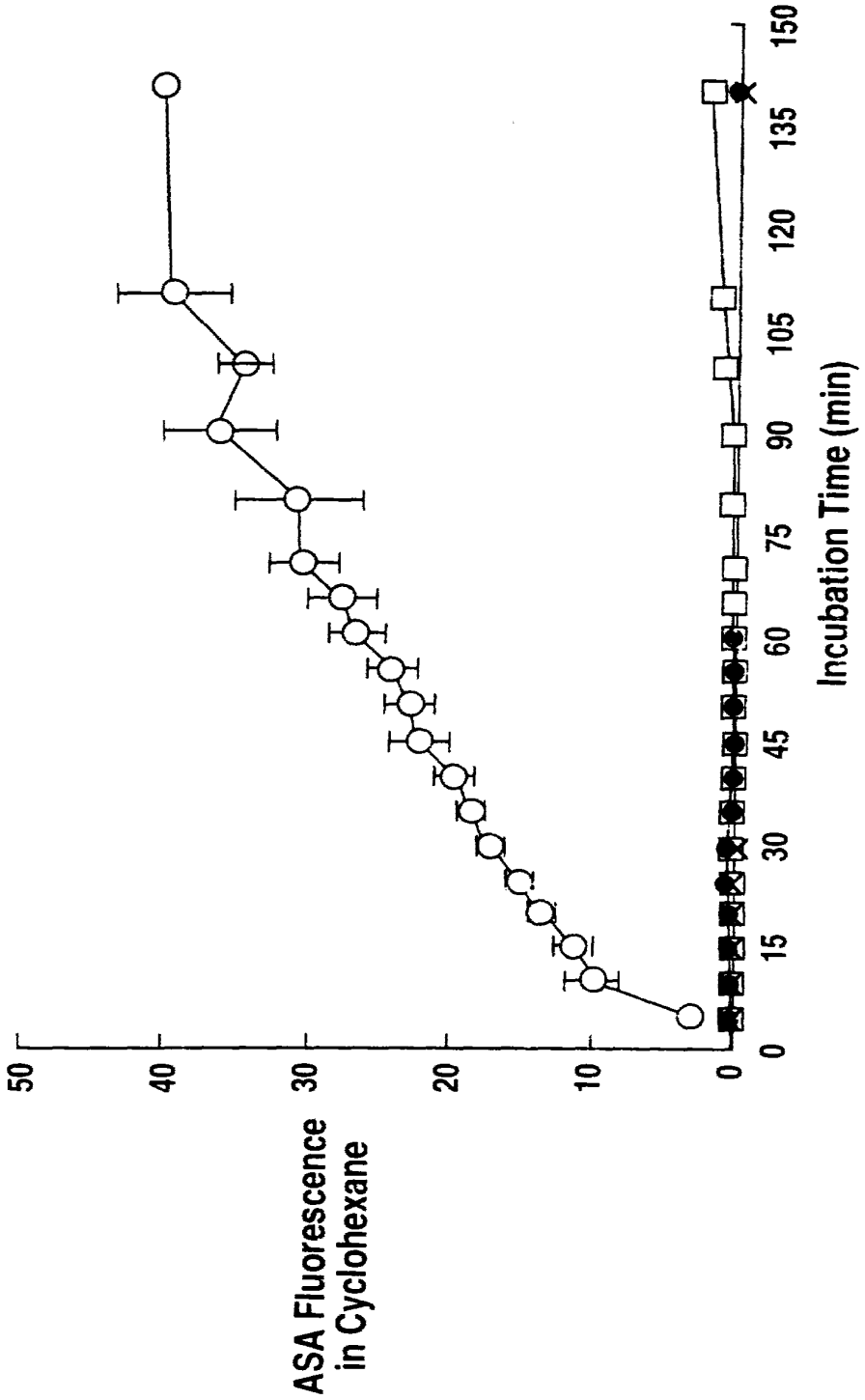
ASA Fluorescence in Cyclohexane
0
10
20
30
40
50
Incubation Time (min)
0
15
30
45
60
75
90
105
120
135
150

Fig. 3

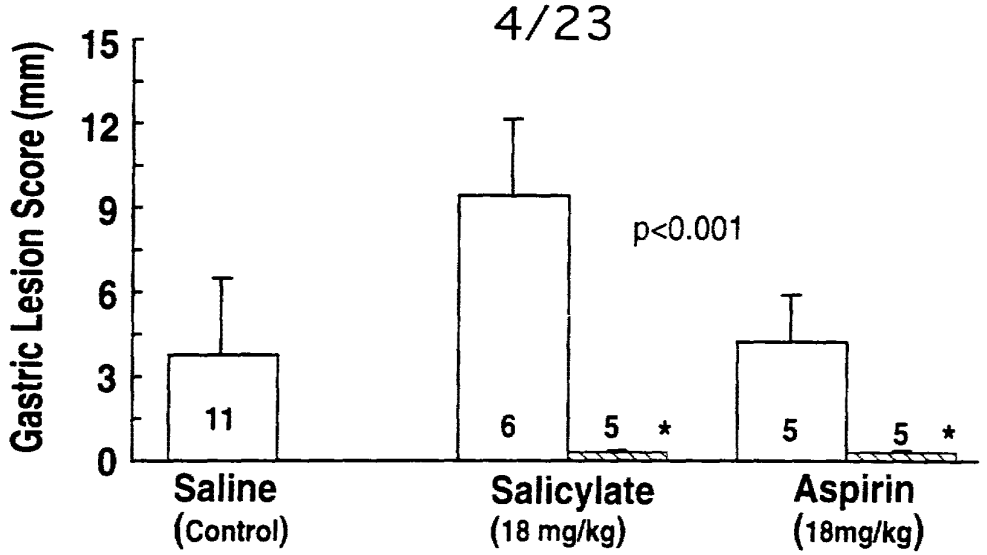

Fig. 4A

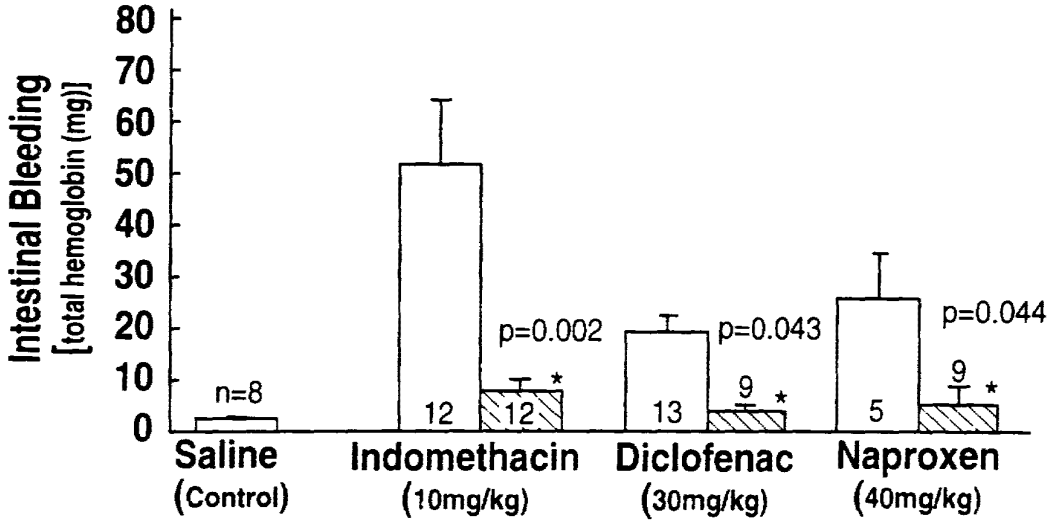

Fig. 4B

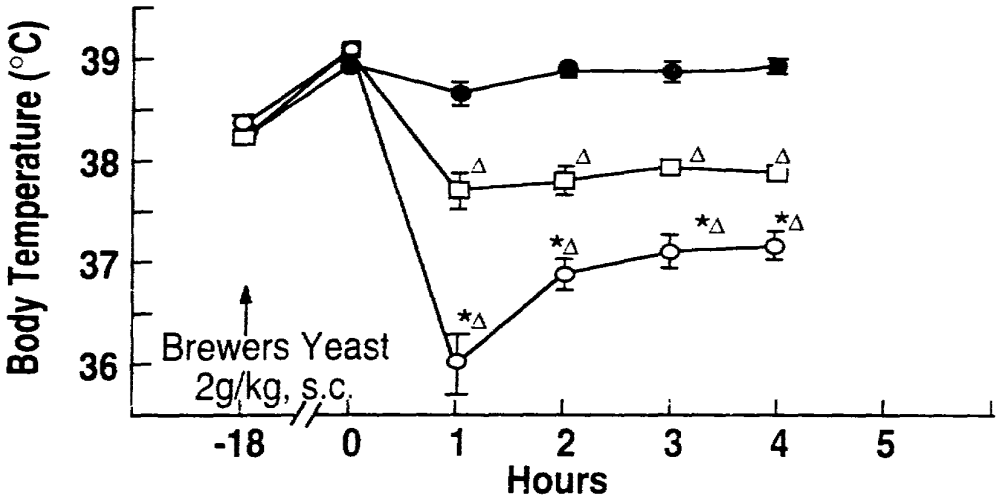

Fig. 4C

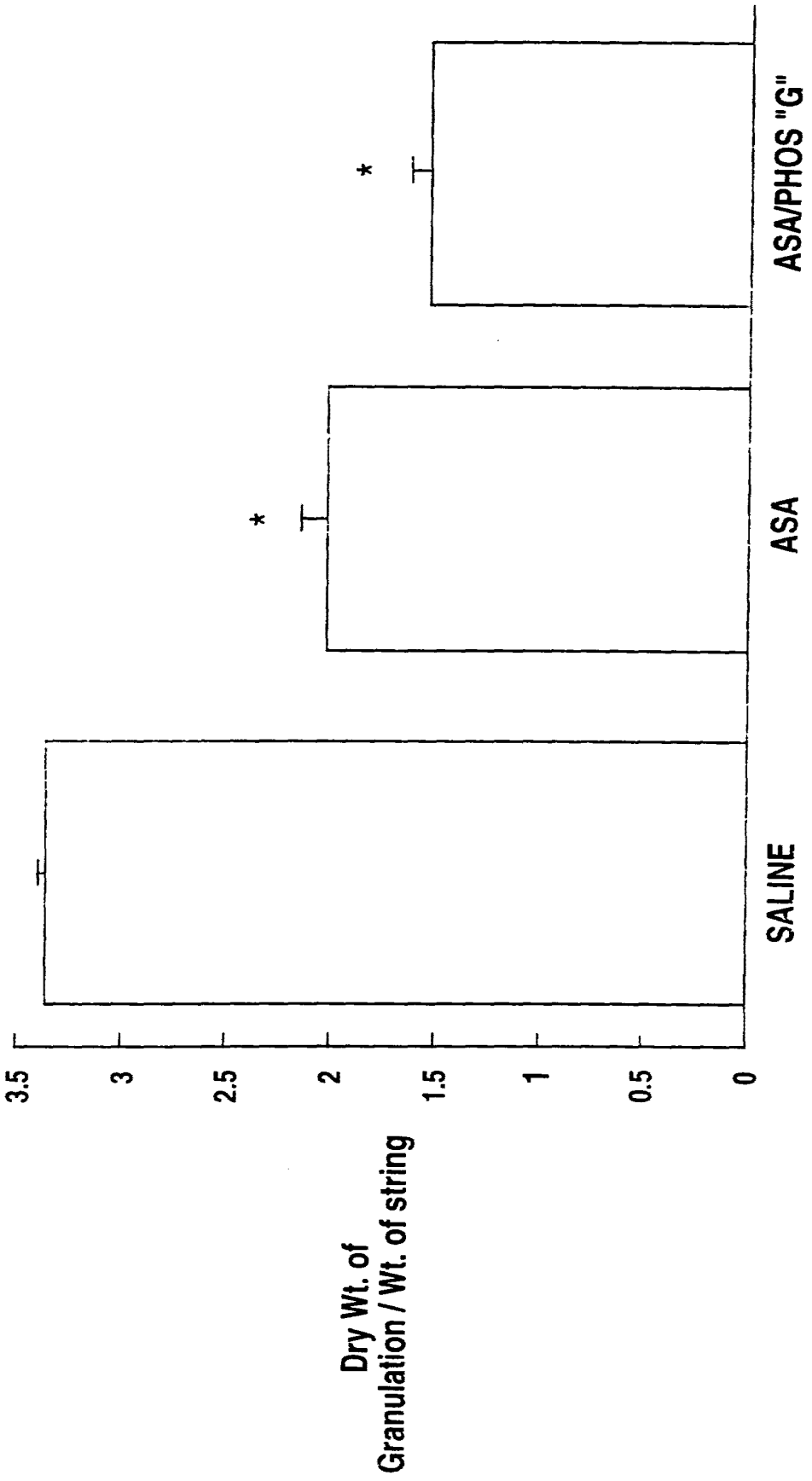
Dry Wt. of
Granulation / Wt. of string
3.5
3
2.5
2
1.5
1
0.5
0
SALINE
ASA
ASA/PHOS "G"
GROUP
*
*

Fig. 5

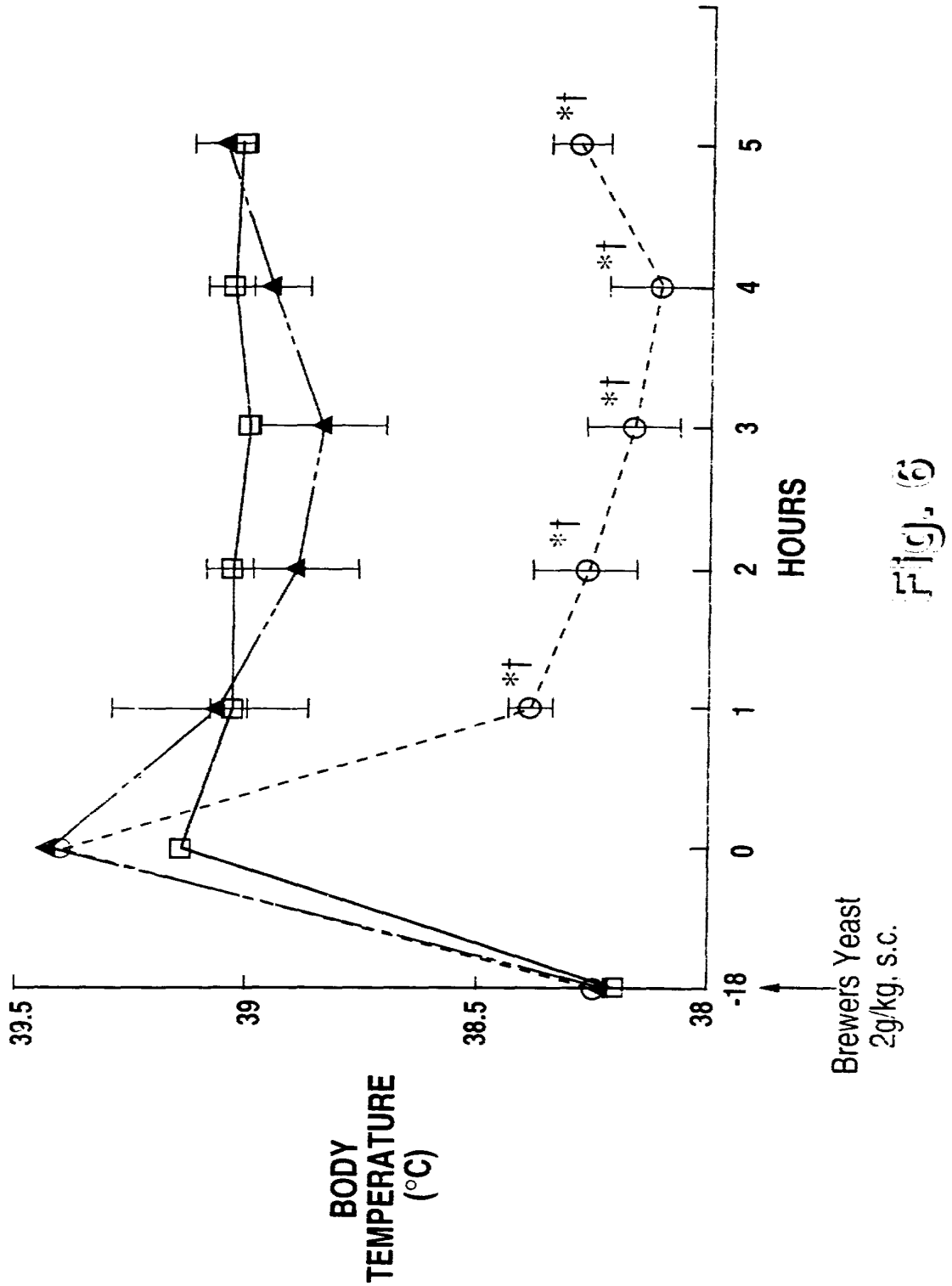
BODY TEMPERATURE (°C)
39.5
39
38.5
38
-18
0
1
2
3
4
5
HOURS
Brewers Yeast 2g/kg, s.c.
*†

Fig. 6

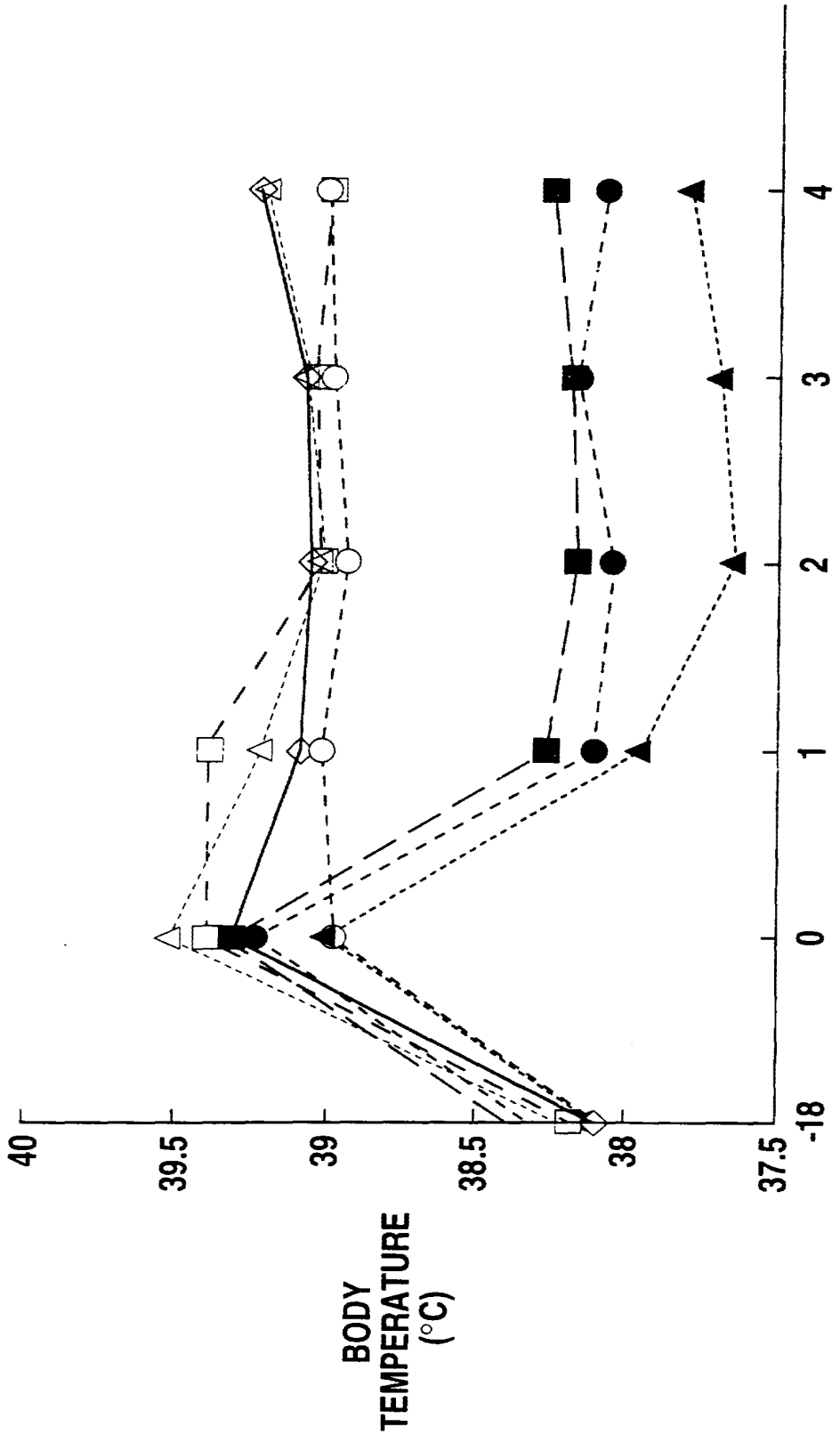

Fig. 7

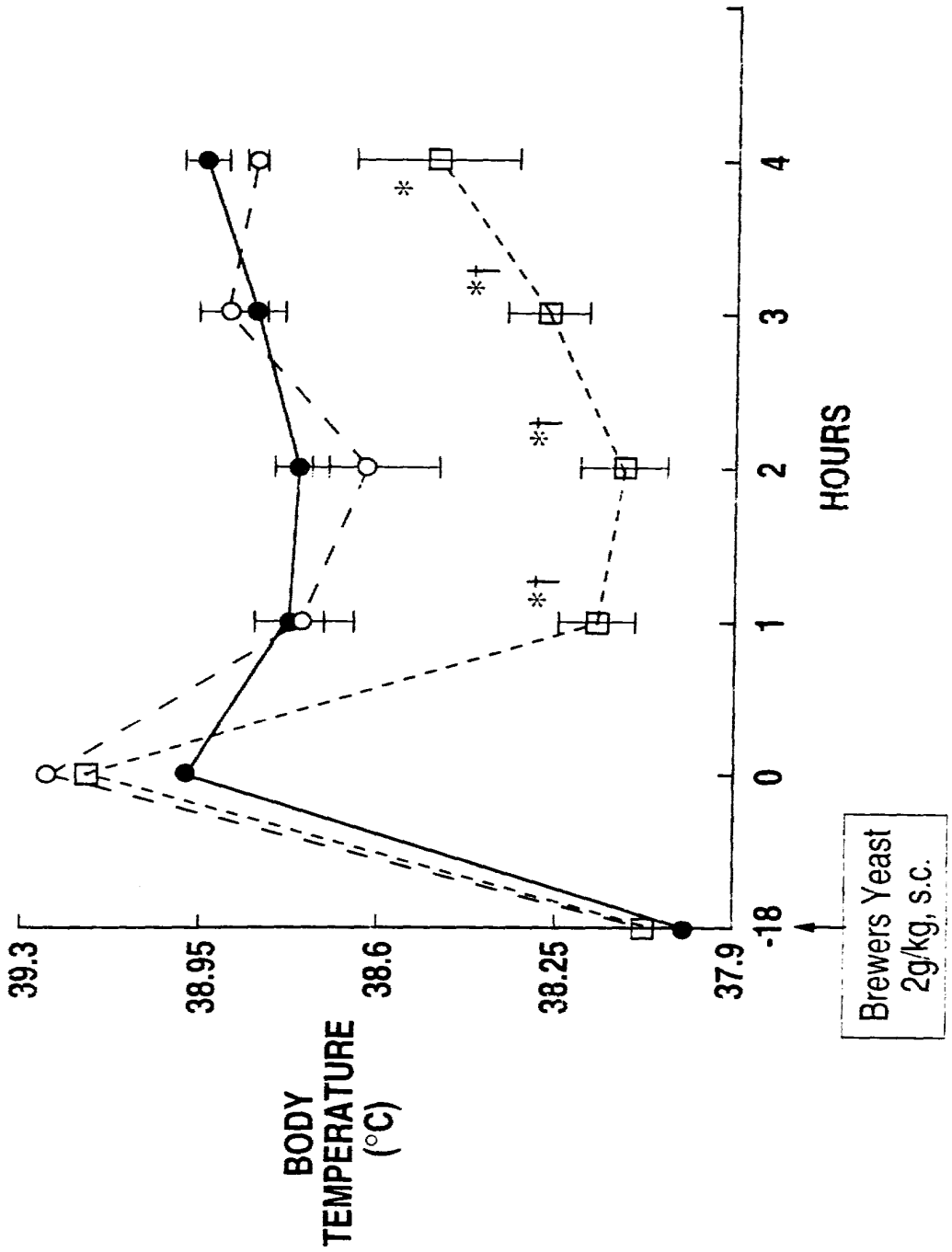
BODY TEMPERATURE (°C)
39.3
38.95
38.6
38.25
37.9
-18
0
1
2
3
4
HOURS
Brewers Yeast 2g/kg, s.c.
*
*†
*†
*†

Fig. 3

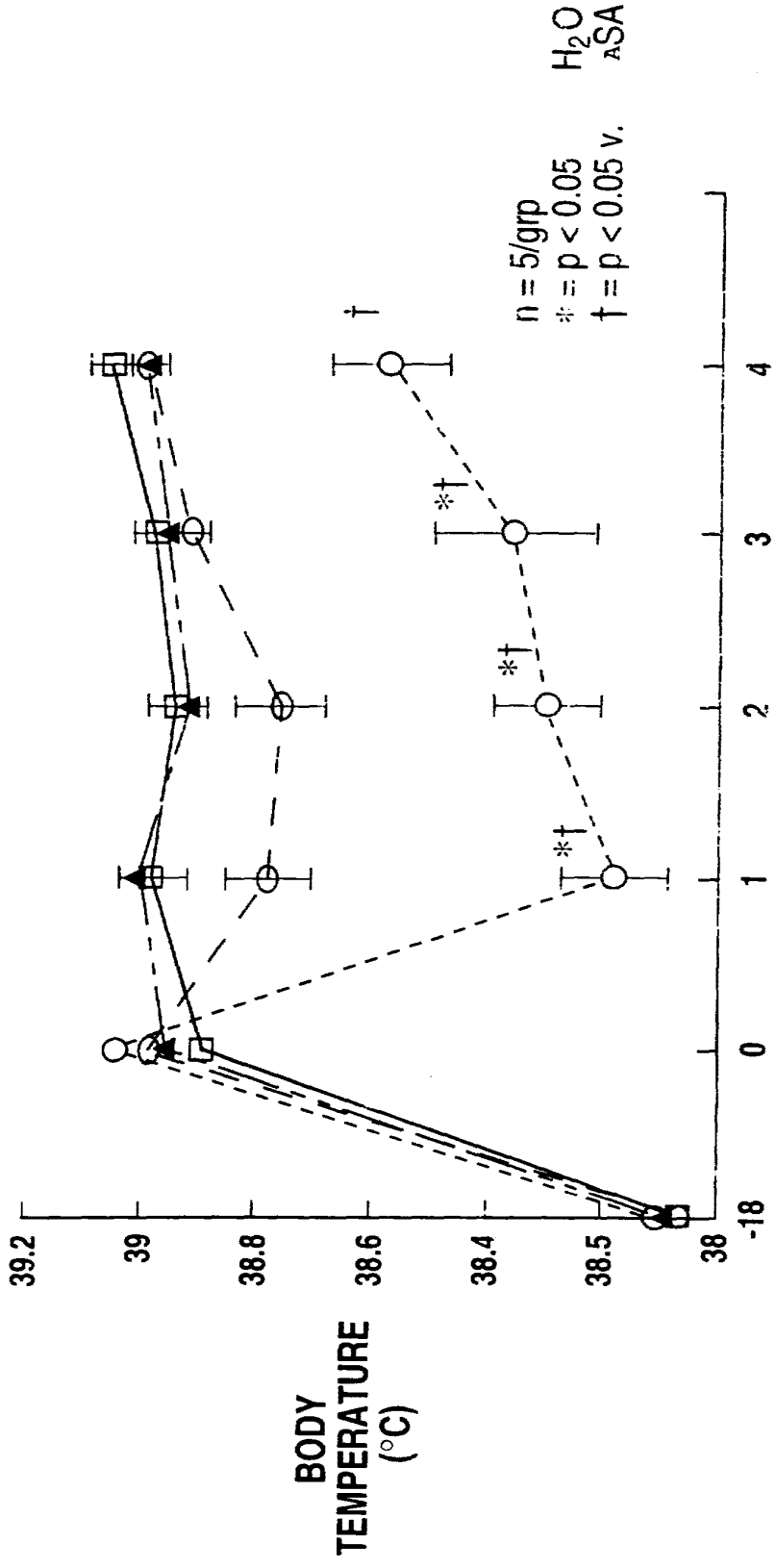
BODY TEMPERATURE (°C)
39.2
39
38.8
38.6
38.4
38.5
38
-18
0
1
2
3
4
HOURS
n = 5/grp
* = p < 0.05
† = p < 0.05 v.
H2O
ASA
*†
*†
*†
†

Fig. 9

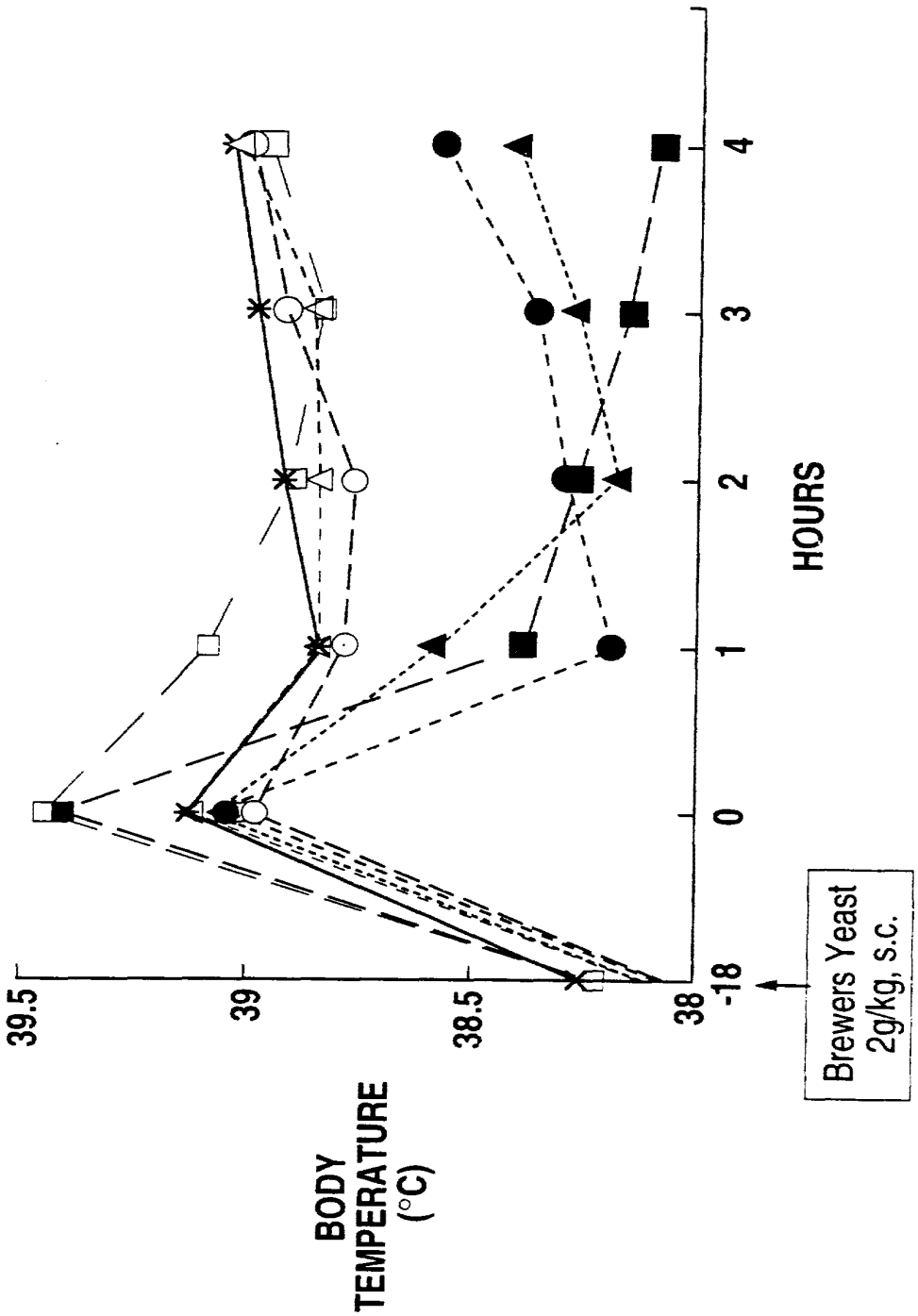
BODY TEMPERATURE (°C)
39.5
39
38.5
38
-18
0
1
2
3
4
HOURS
Brewers Yeast 2g/kg, s.c.

Fig. 10

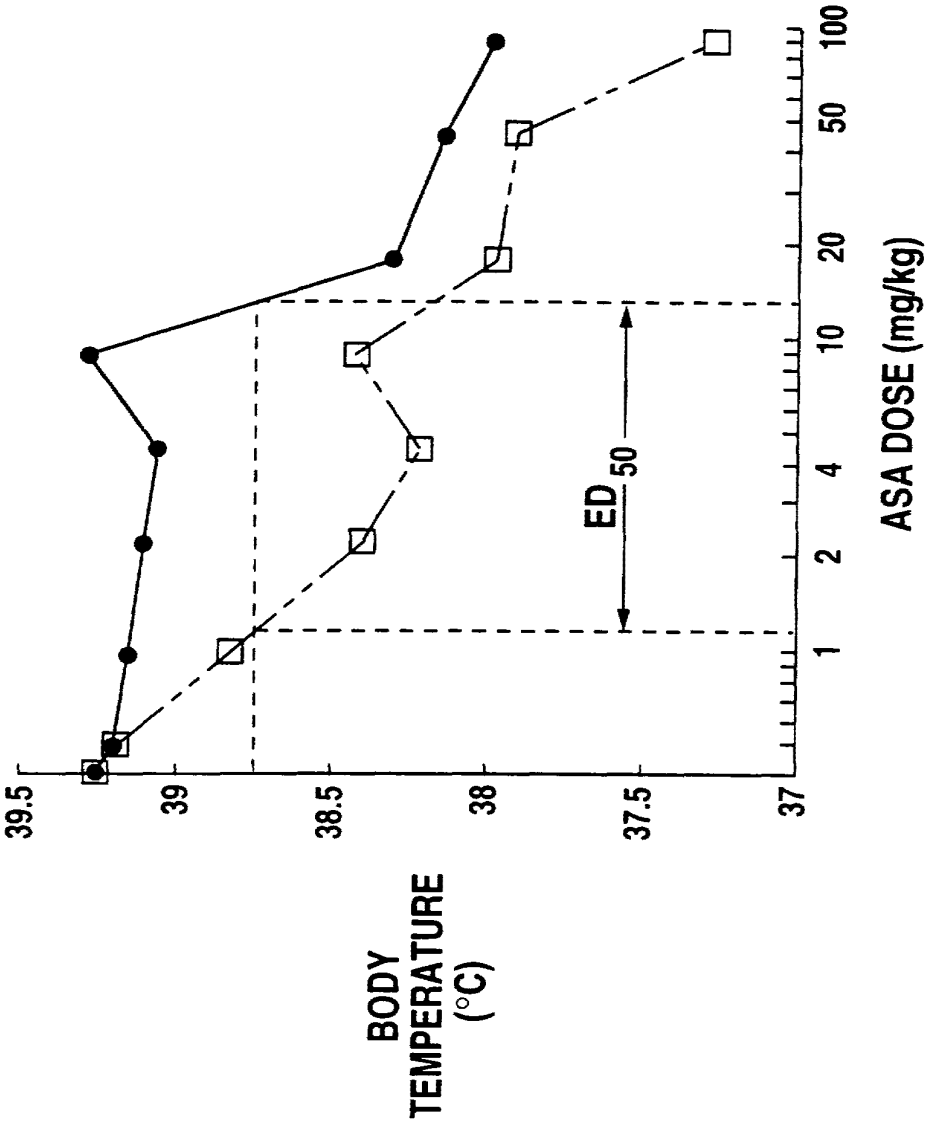
BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
ED 50
ASA DOSE (mg/kg)
1
2
4
10
20
50
100

Fig. 11

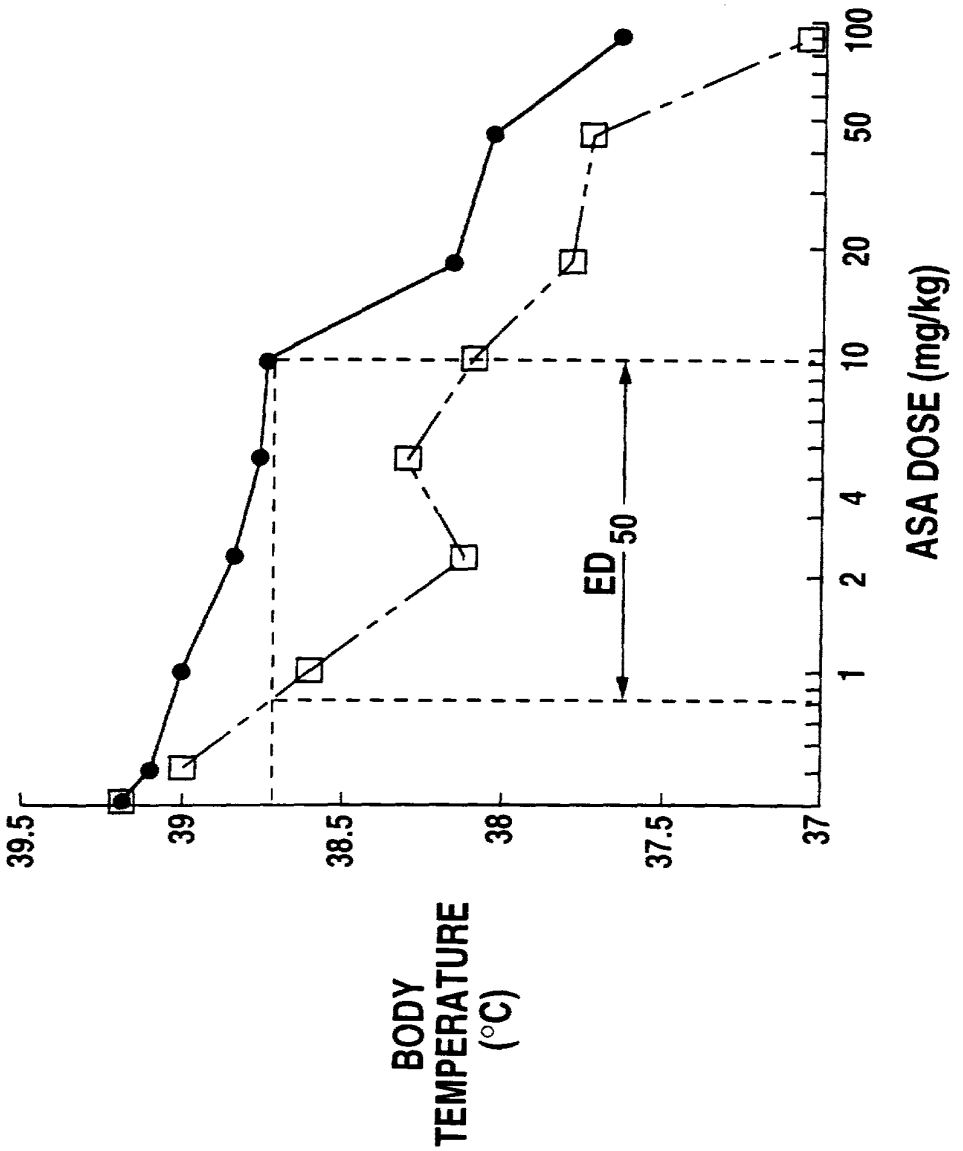
39.5
39
38.5
38
37.5
37
BODY TEMPERATURE (°C)
1
2
4
10
20
50
100
ASA DOSE (mg/kg)
ED 50

Fig. 12

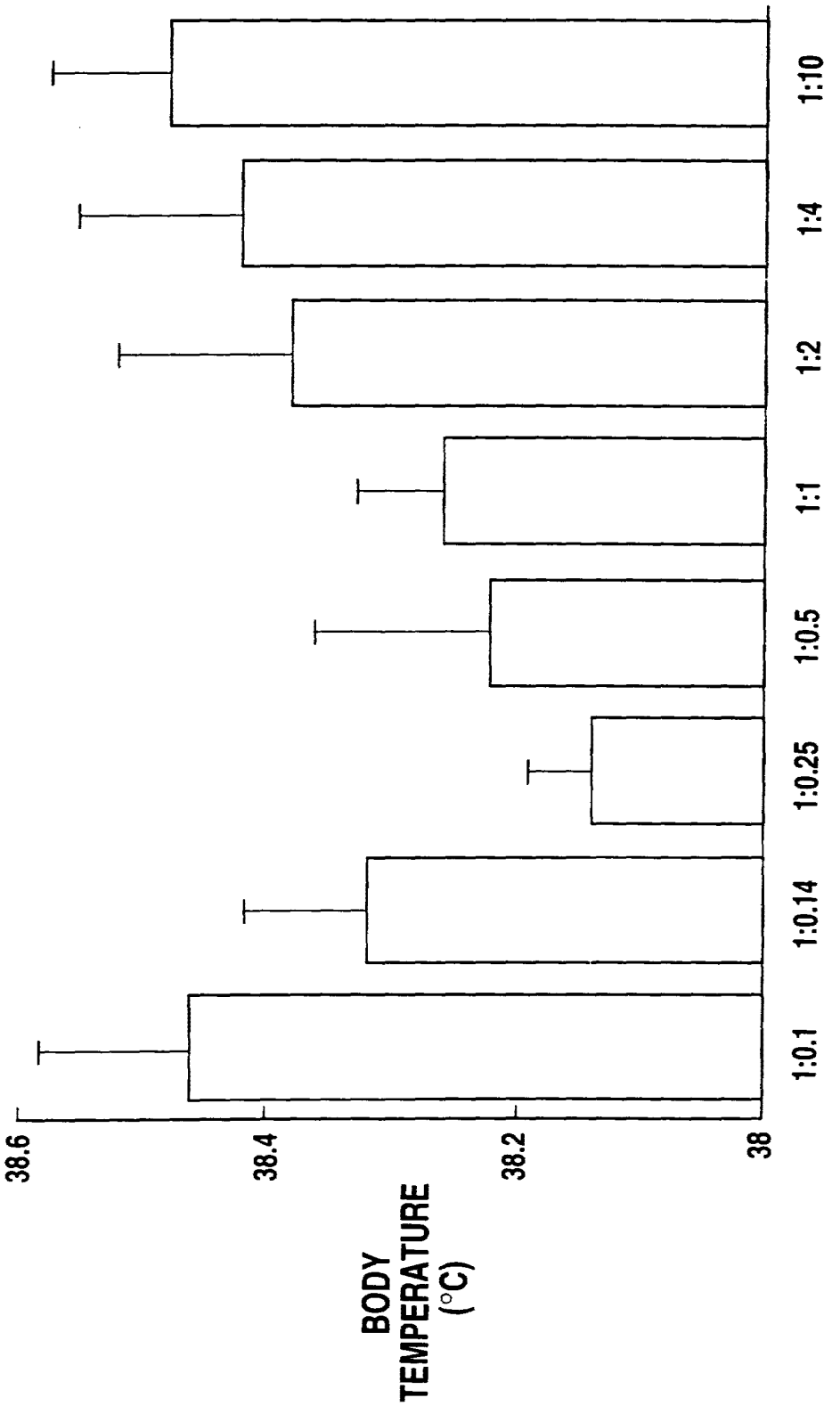
BODY TEMPERATURE (°C)
38.6
38.4
38.2
38
1:0.1
1:0.14
1:0.25
1:0.5
1:1
1:2
1:4
1:10
ASA to DPPC Ratios

Fig. 13

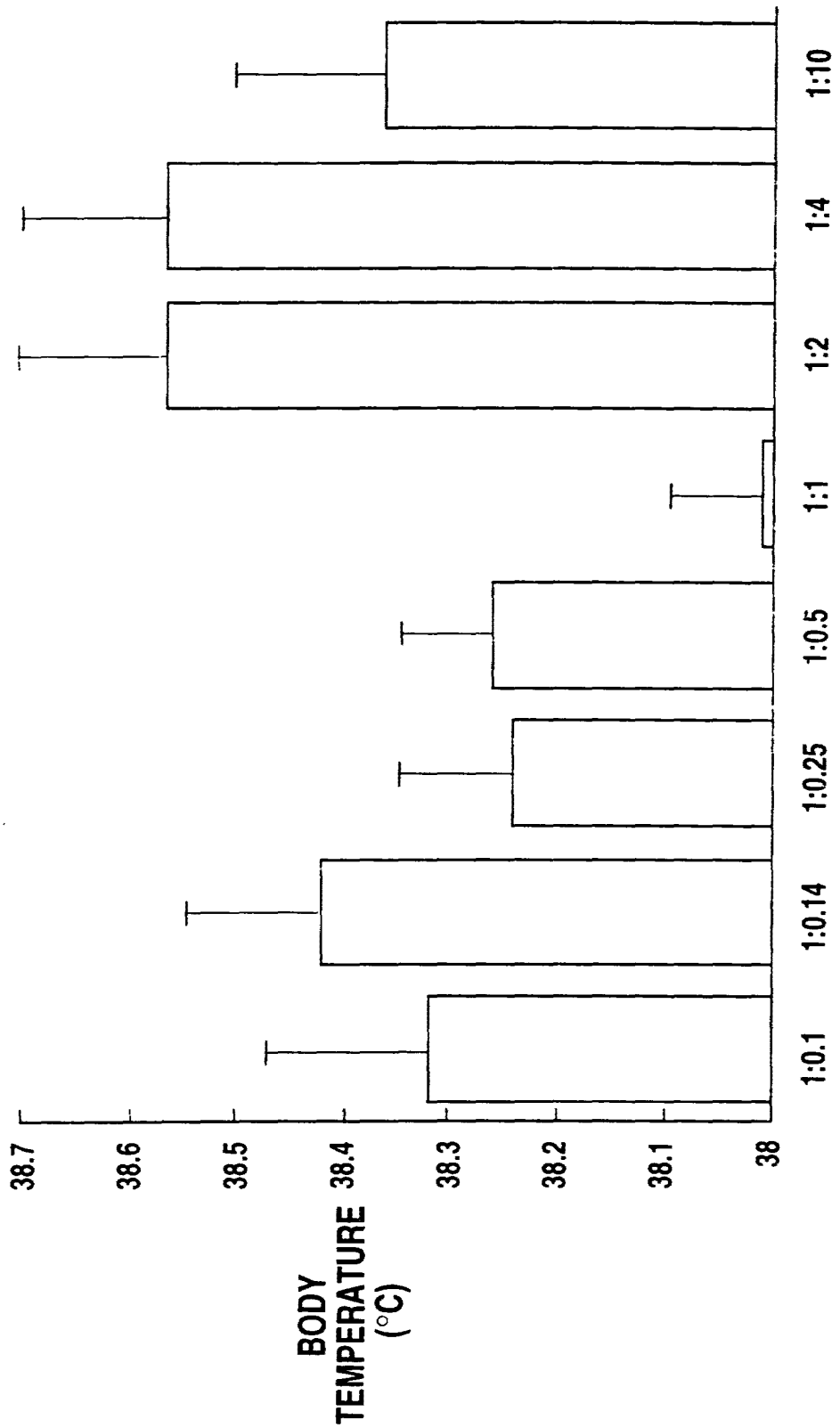
BODY TEMPERATURE (°C)
38.7
38.6
38.5
38.4
38.3
38.2
38.1
38
1:0.1
1:0.14
1:0.25
1:0.5
1:1
1:2
1:4
1:10
ASA to DPPC Ratios

Fig. 14

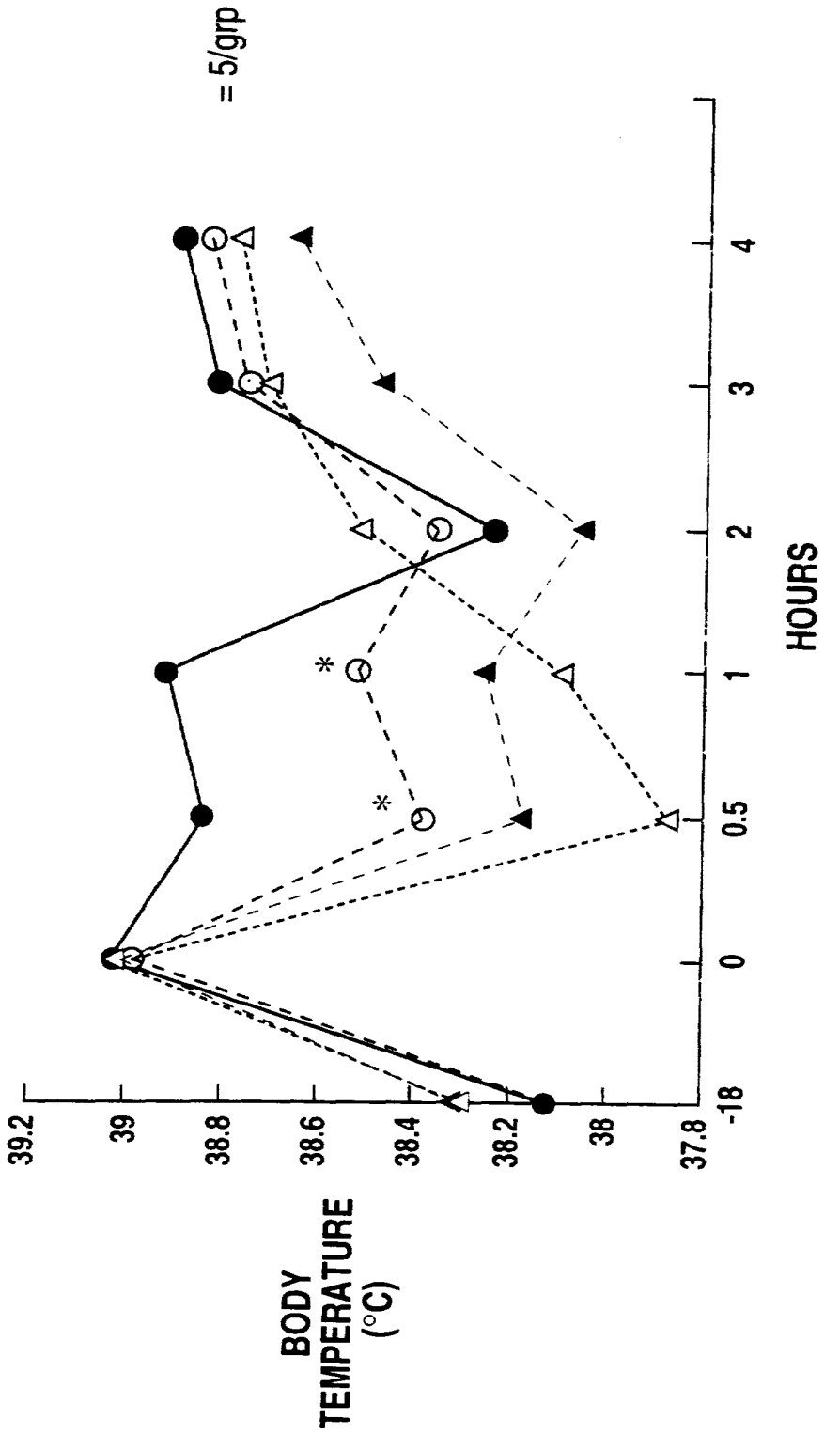
= 5/grp
BODY TEMPERATURE (°C)
39.2
39
38.8
38.6
38.4
38.2
38
37.8
-18
0
0.5
1
2
3
4
HOURS
*
*

Fig. 15

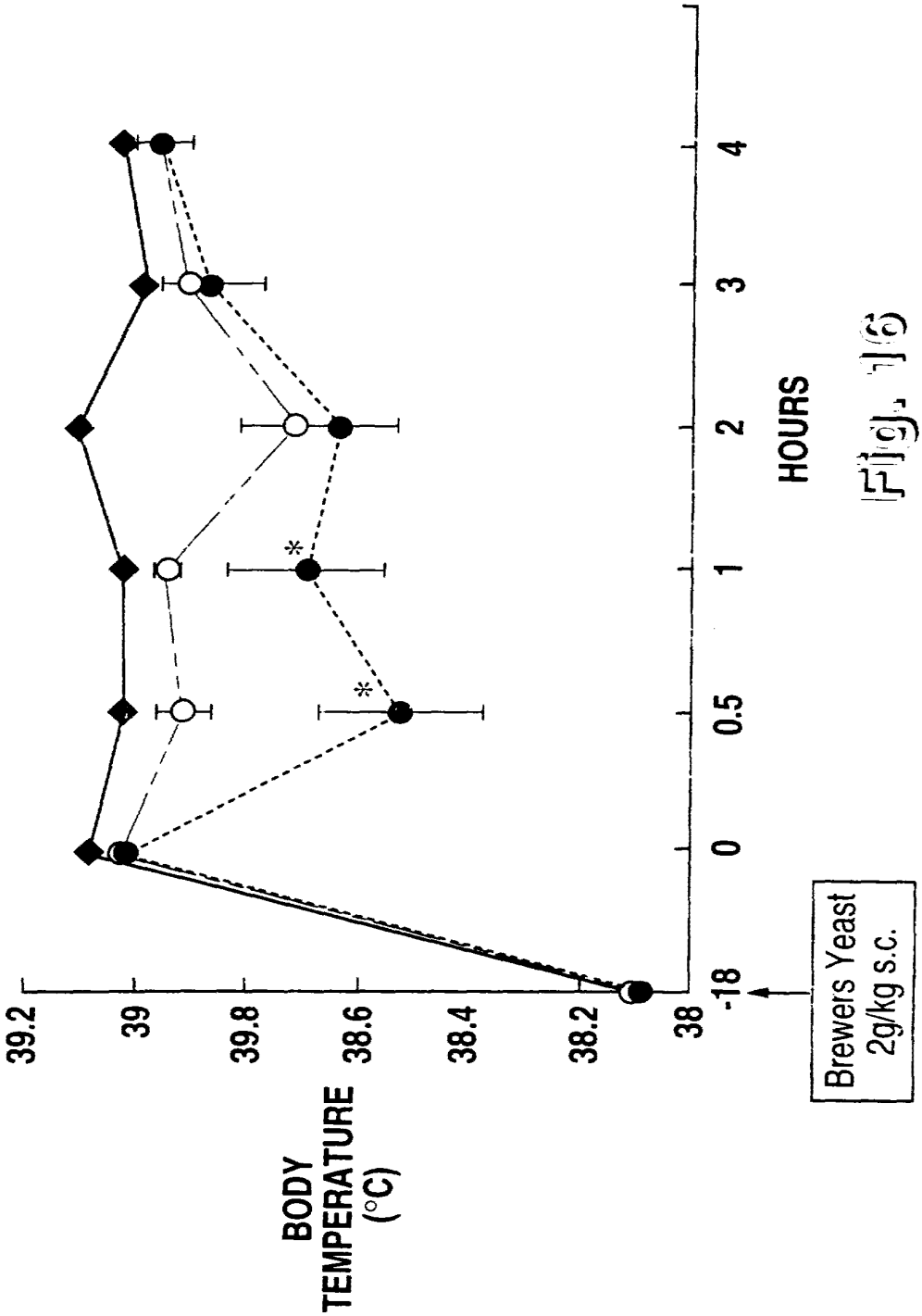
BODY TEMPERATURE (°C)
39.2
39
38.8
38.6
38.4
38.2
38
-18
0
0.5
1
2
3
4
HOURS
Brewers Yeast 2g/kg s.c.
*
*

Fig. 16

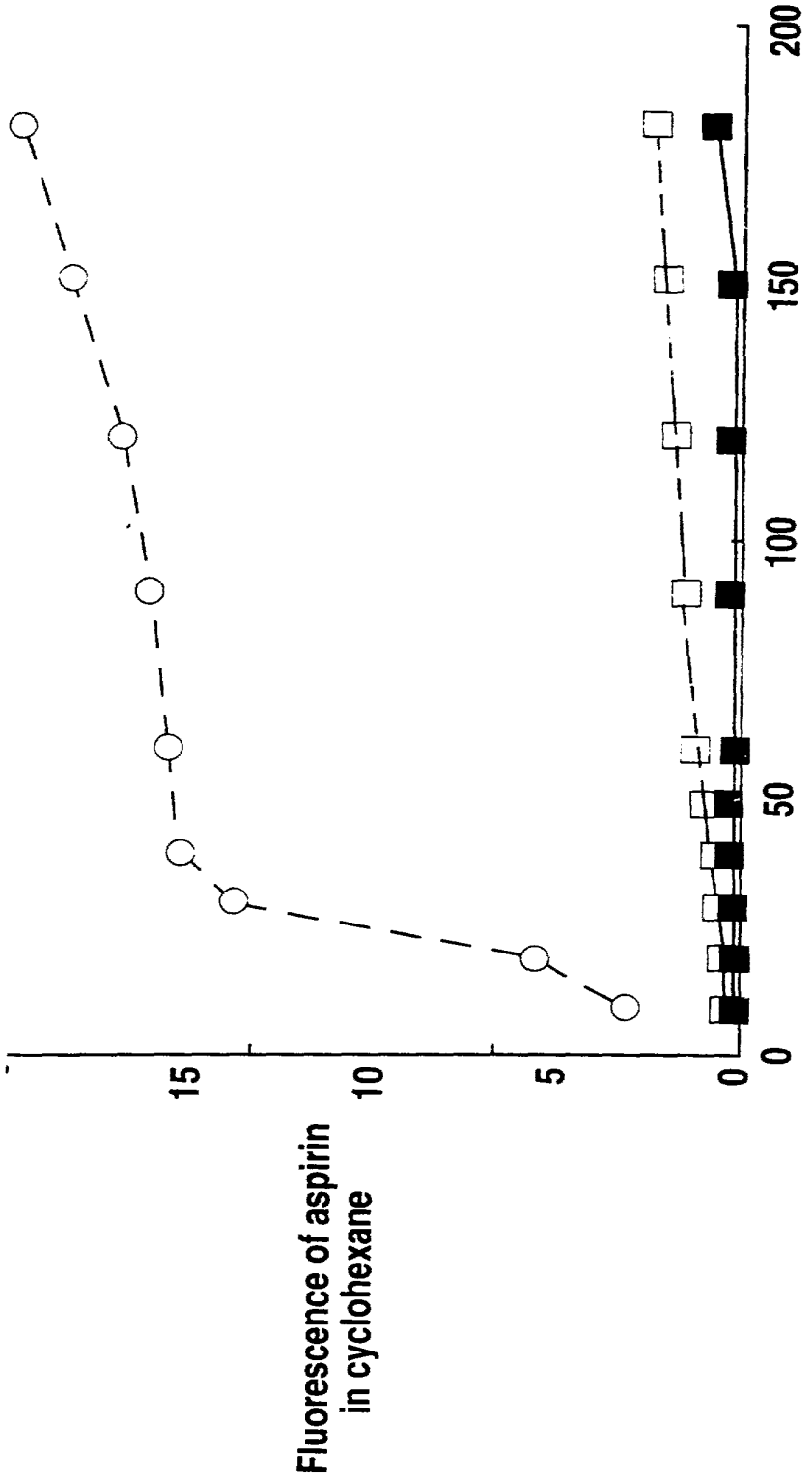
Fluorescence of aspirin in cyclohexane
0
5
10
15
0
50
100
150
200
Time (Minute)

Fig. 17

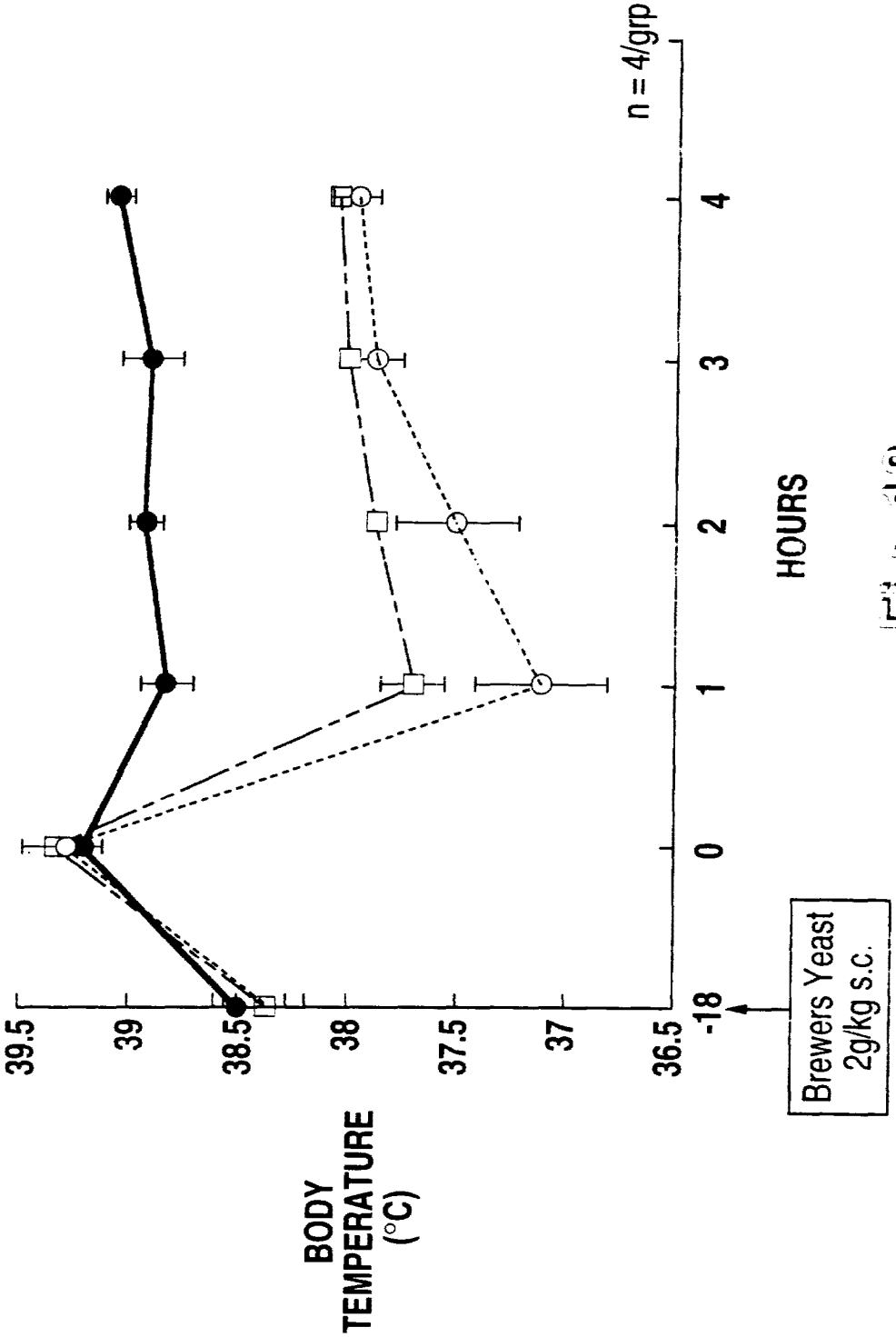
n = 4/grp
HOURS
BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
Brewers Yeast 2g/kg s.c.

Fig. 13

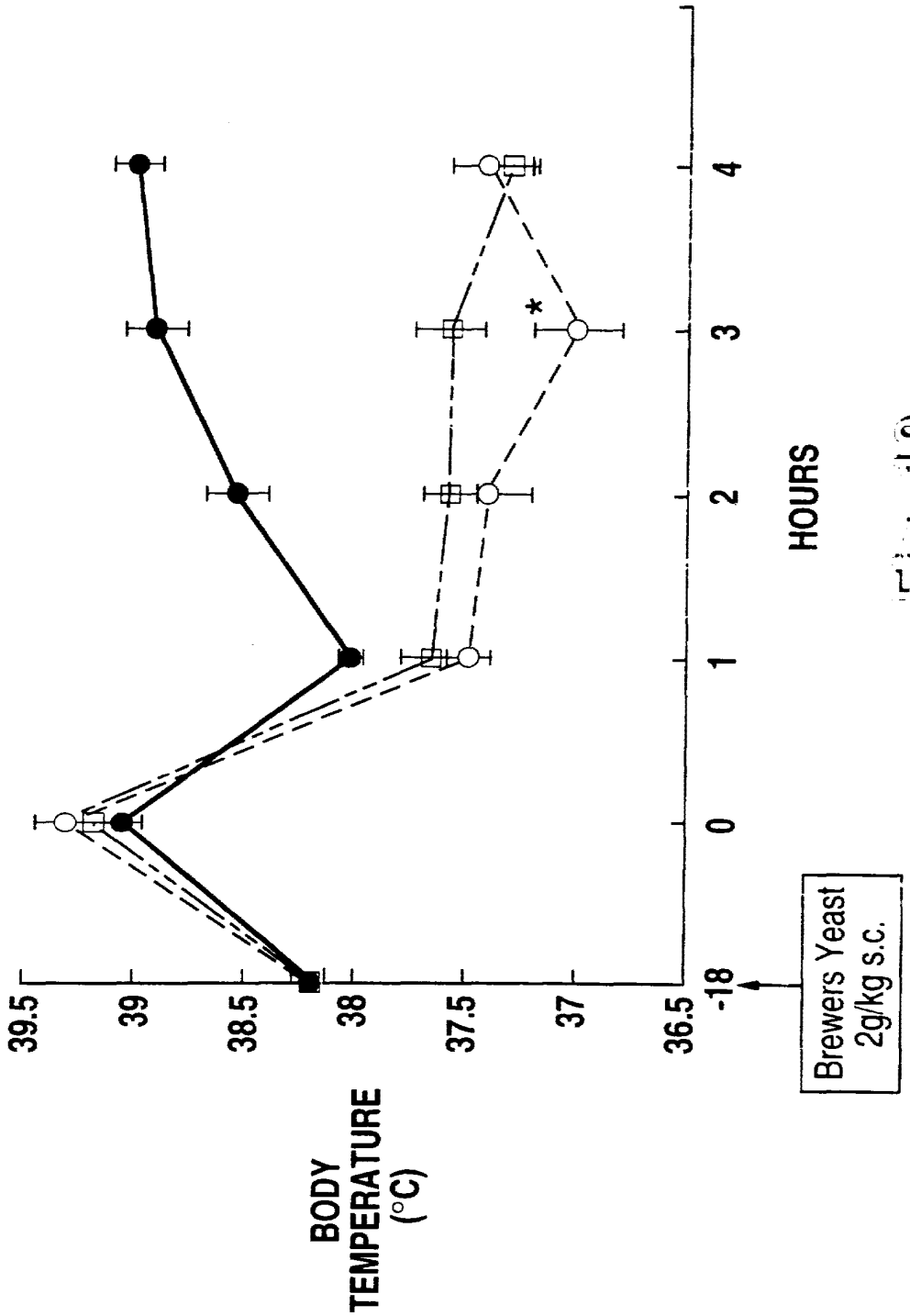
BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
HOURS
*
Brewers Yeast 2g/kg s.c.

Fig. 19

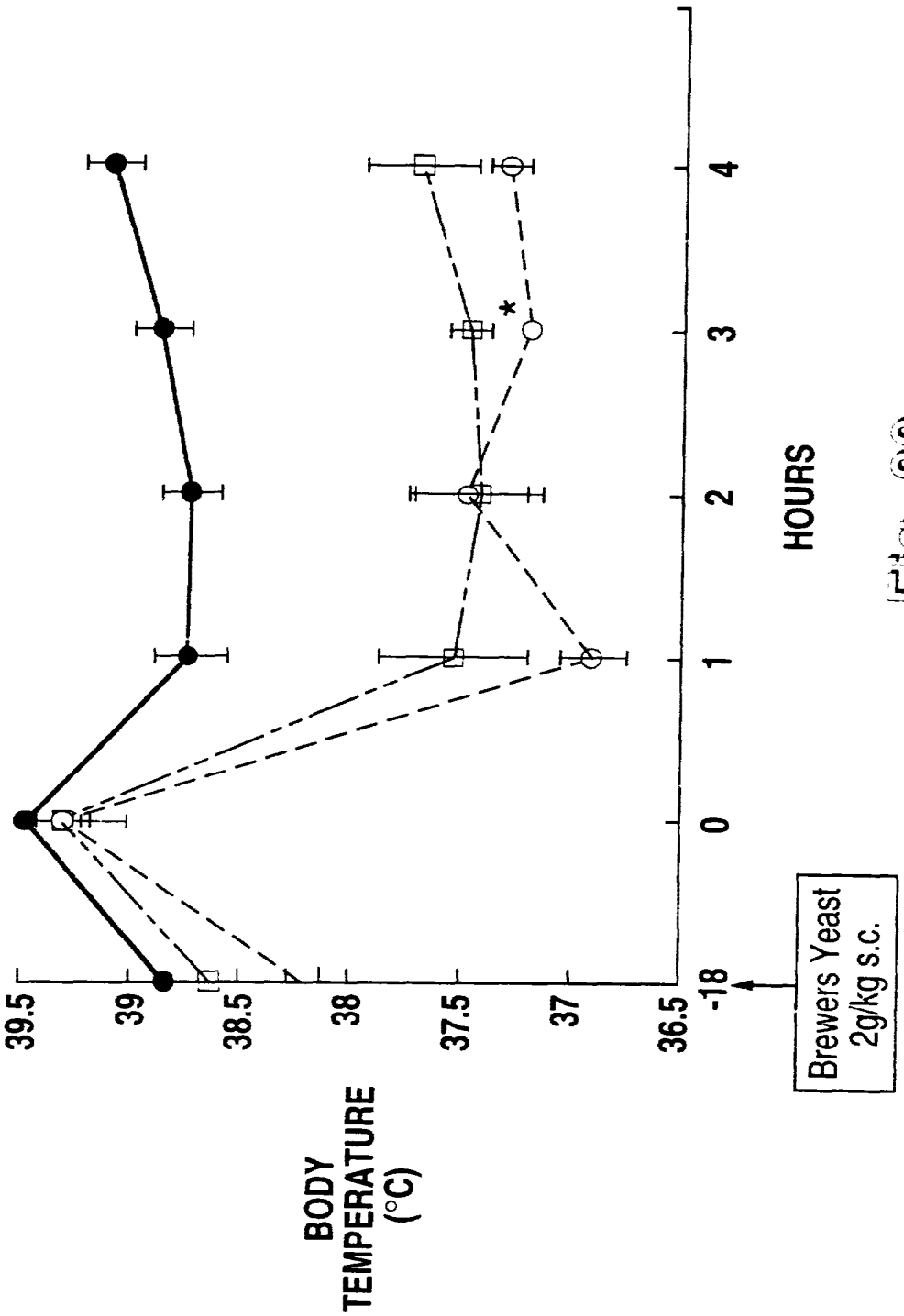
BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
HOURS
Brewers Yeast 2g/kg s.c.
*

Fig. 20

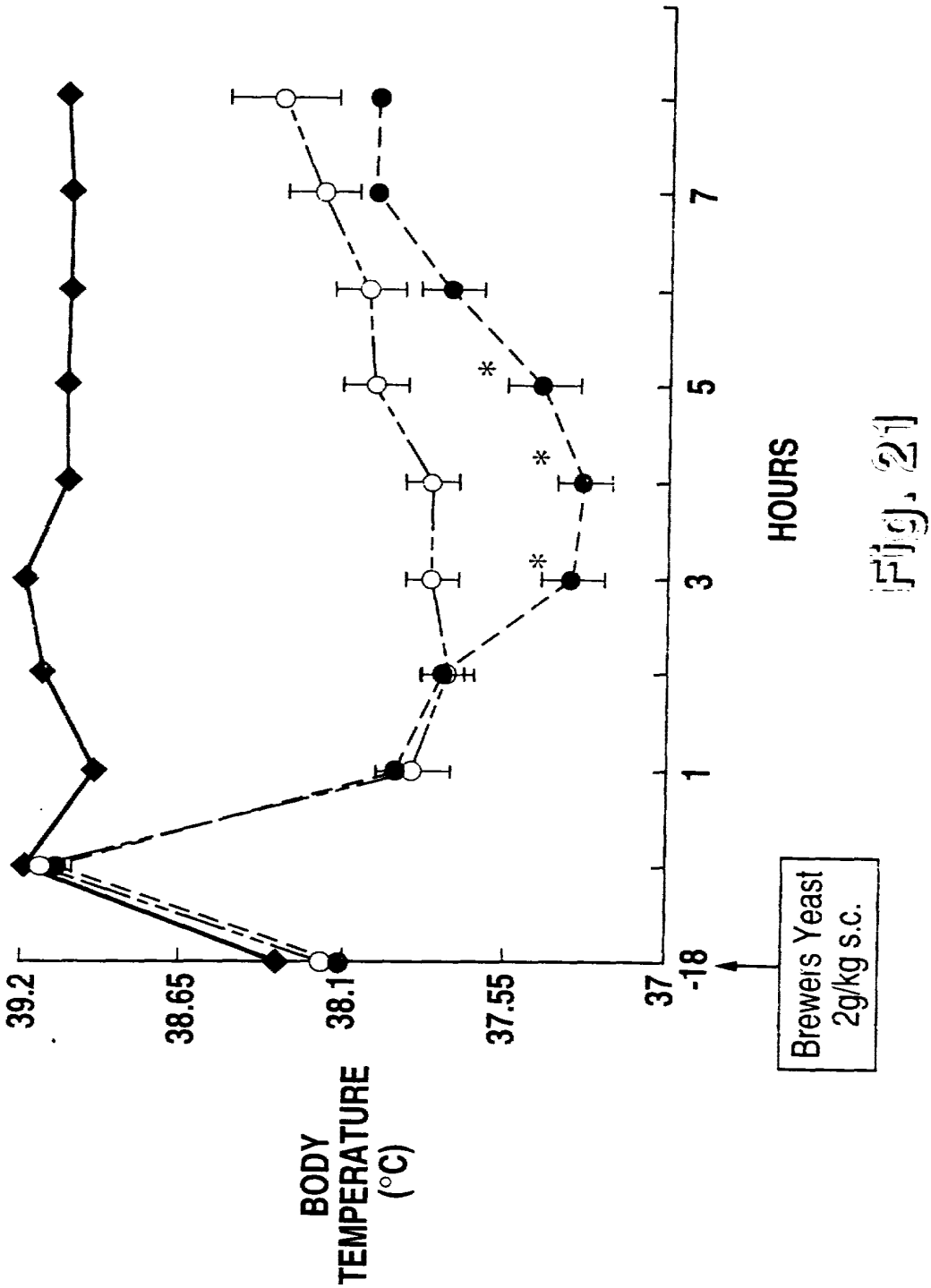
39.2
38.65
38.1
37.55
37
BODY TEMPERATURE (°C)
-18
1
3
5
7
HOURS
Brewers Yeast 2g/kg s.c.
*
*
*

Fig. 21

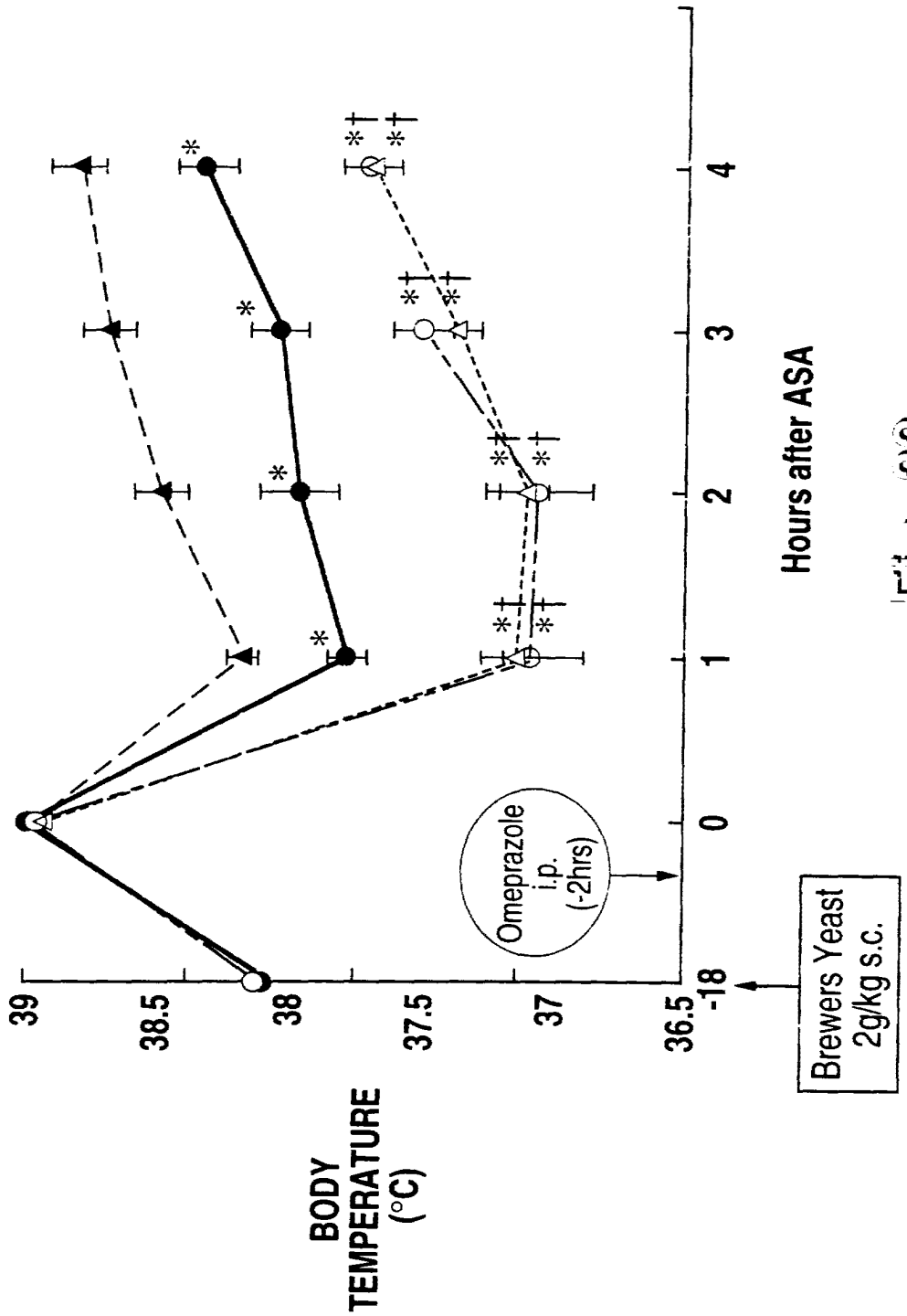
BODY TEMPERATURE (°C)
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
Hours after ASA
Omeprazole i.p. (-2hrs)
Brewers Yeast 2g/kg s.c.

Fig. 22

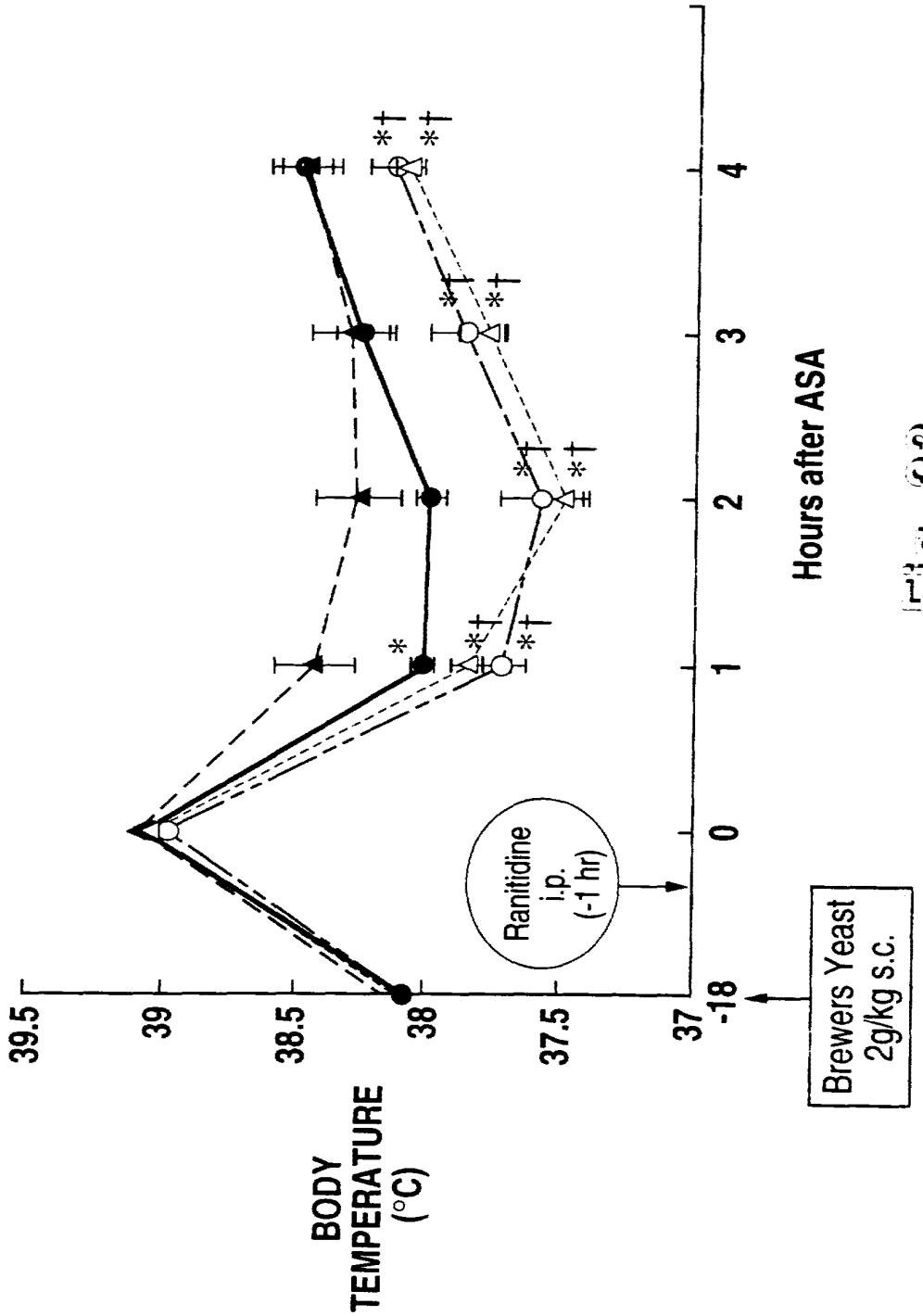
BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
-18
0
1
2
3
4
Hours after ASA
Ranitidine i.p. (-1 hr)
Brewers Yeast 2g/kg s.c.

Fig. 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/00633

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/74
US CL :424/78.05; 514/78, 171
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : A61K 31/74; 424/78.05; 514/78, 171

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

APS

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS; PHOSPHOLIPIDS; PHOSPHATIDYL-CHOLINE; SALICYLATE; ASPIRIN, NAPROXEN, INDOMETHACIN, DICLOFENAC; ANTI-PYRETIC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LICHTENBERGER et al. "Non-steroidal anti-inflammatory drugs (NSAIDS) associate with zwitterionic phospholipids: Insight into the mechanism and reversal of NSAID-induced gastrointestinal injury", NATURE MEDICINE, Vol. 1, #2, 02/1995. See entire document.	1-23
X	US, A, 4,309,420 (GHYCZY ET AL.), 05 January 1982. See entire document.	1-23
X	US, A, 4,369,182 (GHYCZY ET AL.), 18 January 1983, see entire document.	1-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 11 APRIL 1996	Date of mailing of the international search report 27 JUN 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SAMUEL A. ACQUAH aco Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/00633

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,421,747 (GHYCZY ET AI..) 20 December 1983, see entire document.	1-23
Y	JP, A, 3,176,425 (NIPPON SHINYAKU) 31 July 1991. See the abstract.	1-7, 12, 13, 16

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/74

A1

(11) International Publication Number: WO 96/22780

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(30) Priority Data:
08/379,695 27 January 1995 (27.01.95) US
08/440,417 12 May 1995 (12.05.95) US

(71) Applicant: THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 W. 7th Street, Austin, TX 78701 (US).

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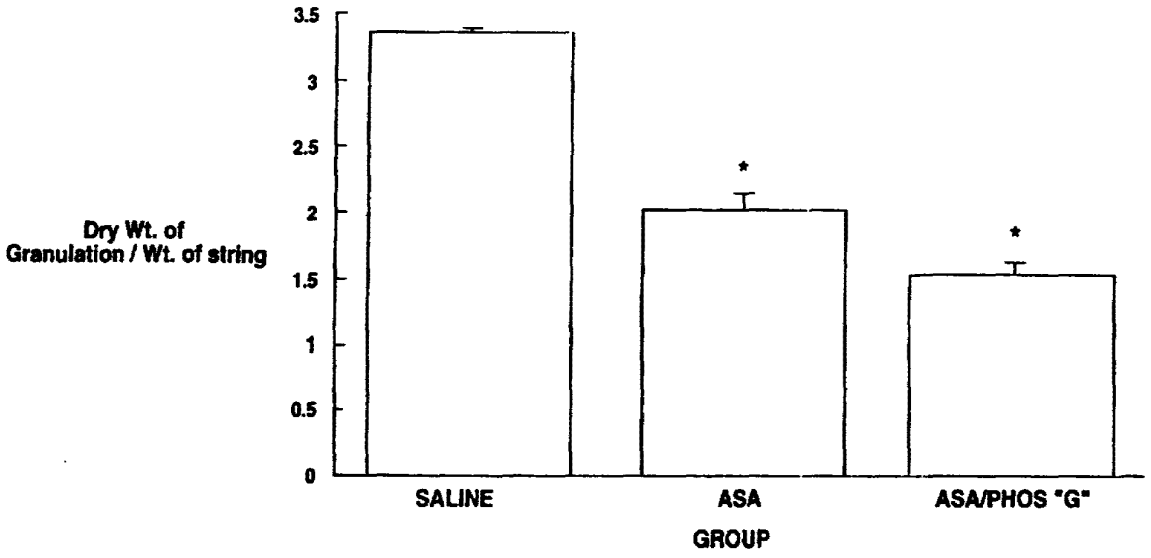
(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
With amended claims and statement.

Date of publication of the amended claims and statement:
19 September 1996 (19.09.96)

(54) Title: METHODS OF ENHANCING THE THERAPEUTIC ACTIVITY OF NSAIDS AND COMPOSITIONS OF ZWITTERIONIC PHOSPHOLIPIDS USEFUL THEREIN



(57) Abstract

Disclosed are compositions comprising non-steroid anti-inflammatory drugs (NSAID's) complexed with zwitterionic, neutral phospholipids, or both, having reduced gastrointestinal irritating effects and enhanced anti-pyretic, analgesic, and anti-inflammatory activity. Also disclosed are improved methods of using the complexes for treating fever, inflammation, and preventing platelet aggregation. In some embodiments, the anti-pyretic activity of sub-therapeutically used amounts of NSAID's are enhanced to elicit anti-pyretic activity *in vivo* when associated (noncovalently) with zwitterionic phospholipids, such as dipalmitoyl phosphatidyl choline.

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AMENDED CLAIMS
[received by the International Bureau on 5 August 1996 (05.08.96);
original claims 1-23 replaced by amended claims 1-16 (3 pages)]

1. A medicament for use as an anti-pyretic comprising a non-covalently associated composition of zwitterionic phospholipid with an equimolar amount of non-steroidal anti-inflammatory agent, wherein the amount of non-steroidal anti-inflammatory agent provides anti-pyretic activity with the zwitterionic phospholipid not observed with the nonsteroidal anti-inflammatory agent without the zwitterionic phospholipid.

2. The medicament of claim 1 wherein the non-steroidal anti-inflammatory agent is salicylate, aspirin, naproxen, indomethacin, diclofenac, or a mixture thereof.

3. The medicament of claim 1 wherein the anti-pyretic activity of the non-steroidal anti-inflammatory agent is enhanced about 2-fold to about 6-fold in the presence of the zwitterionic phospholipid compared to non-steroidal anti-inflammatory agent without the zwitterionic phospholipid.

4. The medicament of claim 1 wherein the non-steroidal anti-inflammatory agent is salicylate.

5. The medicament of claim 1 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidylcholine.

6. The medicament of claim 5 wherein the non-steroidal anti-inflammatory agent is salicylate.

7. A medicament for use in inhibiting platelet aggregation comprising a non-covalently associated combination of zwitterionic phospholipid and an equimolar amount of non-steroidal anti-inflammatory agent, wherein the amount of non-steroidal anti-inflammatory agent provides enhanced inhibition of platelet aggregation with the zwitterionic phospholipid relative to the non-steroidal anti-inflammatory agent without the zwitterionic phospholipid.

8. The medicament of claim 8 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidyl choline.

9. The medicament of claim 8 wherein the non-steroidal anti-inflammatory agent is salicylate, aspirin, naproxen, indomethacin, diclofenac, or a mixture thereof.

10. The medicament of claim 1 or 7 further comprising neutral lipid.

11. The medicament of claim 10 wherein the neutral lipid is triglyceride.

12. A medicament for use as an analgesic comprising a non-covalently associated composition of a zwitterionic phospholipid and an equimolar amount of a non-steroidal anti-inflammatory agent, wherein the amount of the non-steroidal anti-inflammatory agent provides analgesic activity with the zwitterionic phospholipid not observed without the zwitterionic phospholipid.

13. The medicament of claim 12 wherein the non-steroidal anti-inflammatory agent is salicylate, naproxen, indomethacin, diclofenac, aspirin, or a mixture thereof.

14. The medicament of claim 12 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidyl choline.

15. The medicament of claim 12 further comprising a neutral lipid.

16. The medicament of claim 15 wherein the neutral lipid is a triglyceride.

STATEMENT UNDER ARTICLE 19

The present statement is submitted to further explain the amendment of the claims and the impact thereof on the claimed invention.

The cited Lichtenberger et al (1995) article was published prior to the international filing date but later than the priority date claimed of 27 January 1995, and should be designated "P".

In the case of replacement claim 1 to a medicament for use as an anti-pyretic (fever-reducing preparation), the amount of the non-steroidal anti-inflammatory agent is further defined as an amount that provides anti-pyretic activity with the zwitterionic phospholipid not observed without the zwitterionic phospholipid.

U.S. 4,309,420, U.S. 4,369,182 and U.S. 4,421,747 (Ghyczy et al patents, collectively) relate to compositions of acetyl salicylic acid (ASA) with phospholipid in amounts of 200 mg/kg to 800 mg/kg (see '182 patent, col. 3-4, table 4). These amounts of ASA are anti-pyretic without phospholipid. The cited JP (Nippon Shinyaku) relates to a composition having low amounts of non-steroidal anti-inflammatory agents with anti-inflammatory pharmacological activity that is not affected by the presence of zwitterionic phospholipid. Applicants' specification, FIG. 7, demonstrates doses of greater than 20 mg/kg ASA alone provide anti-pyretic effects. The further definitions of the amount of NSAID in the medicaments claimed as amounts that do not provide anti-pyretic activity without the phospholipid further distance those disclosures from the invention of the replacement claims 1-16.

Replacement claims 7-11 relate to medicaments for use in inhibiting platelet aggregation. The references cited in the International Search Report do not relate to medicaments to inhibit platelet aggregation, or the enhancement of this activity by adding phospholipid to NSAID. As shown in the present specification at Example 12, pages 45-47, the inclusion of an equimolar amount of phospholipid with 1.0 mM ASA (an NSAID) results in an unexpected reduction in the amount of platelet aggregation, relative to platelet aggregation observed with ASA alone (1.0 mM ASA, no DPPC = 95% platelet aggregation vs. 1.0 mM + DPPC = 35% platelet aggregation (high ristocetin as platelet aggregating agent (1.5 mg/ml) test)); (0.01 mM ASA, no DPPC = 65% platelet aggregation vs. 0.01 mM ASA +DPPC = 12% platelet aggregation (low ristocetin as platelet aggregating agent (0.75 mg/ml)

test).

Replacement claims 12-16 relate to a medicament for use as an analgesic comprising an NSAID and a zwitterionic phospholipid at equimolar amounts. The amount of NSAID is defined as an amount that is not observed to provide analgesic activity without the zwitterionic phospholipid. The cited Ghyczy et al. patents relate to preparations of NSAID that include NSAID amounts that provide anti-inflammatory activity alone. The Nippon (JP) abstract fails to reveal or suggest medicaments having analgesic activity with amounts of NSAID that do not have observable anti-inflammatory activity alone, the preparations therein being described as including simple lipids and phospholipids that "do not affect the drug's pharmacological actions." (See ABSTRACT/USE/ADVANTAGE). As defined in the replacement claims, the amount of NSAID does not exert pharmacological activity in the absence of the phospholipid.

Favorable consideration of the replacement claims is respectfully requested.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A1

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(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
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Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMPOSITIONS FOR TREATING INFLAMMATION CONTAINING CERTAIN PROSTAGLANDINS AND A SELECTIVE CYCLOOXYGENASE-2 INHIBITOR

(57) Abstract

A method of treating cyclooxygenase mediated disease while promoting the healing of certain lesions including gastric ulcers comprising the co-administration of certain prostaglandins and a selective cyclooxygenase-2 inhibitor, or the co-administration of an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor as defined herein.

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COMPOSITIONS FOR TREATING INFLAMMATION CONTAINING CERTAIN PROSTAGLANDINS AND A SELECTIVE CYCLOOXYGENASE-2 INHIBITOR

BACKGROUND OF THE INVENTION

This invention relates to a method of treating cyclooxygenase mediated diseases in patients with ulcers. Disclosed is a method of treating cyclooxygenase mediated disease while promoting the healing of certain lesions including gastric ulcers comprising the co-administration of certain prostaglandins and a selective cyclooxygenase-2 inhibitor, or the co-administration of an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor as defined below.

Non-steroidal, anti inflammatory drugs exert most of their anti inflammatory, analgesic and antipyretic activity and inhibit hormone-induced uterine contractions and certain types of cancer growth through inhibition of prostaglandin G/H synthase, also known as cyclooxygenase. Up until recently, only one form of cyclooxygenase had been characterized, this corresponding to cyclooxygenase-1 or the constitutive enzyme, as originally identified in bovine seminal vesicles. Recently the gene for a second inducible form of cyclooxygenase (cyclooxygenase-2) has been cloned, sequenced and characterized from chicken, murine and human sources. This enzyme is distinct from the cyclooxygenase-1 which has now also been cloned, sequenced and characterized from sheep, murine and human sources. The second form of cyclooxygenase, cyclooxygenase-2, is rapidly and readily inducible by a number of agents including mitogens, endotoxin, hormones, cytokines and growth factors. As prostaglandins have both physiological and pathological roles, we have concluded that the constitutive enzyme, cyclooxygenase-1, is responsible, in large part, for endogenous basal release of prostaglandins and hence is important in their physiological functions such as the maintenance of gastrointestinal integrity and renal blood flow. In contrast, we have concluded that the inducible form, cyclooxygenase-2, is mainly responsible for the pathological effects of prostaglandins where rapid induction of the enzyme would occur in response to such agents as inflammatory agents,

hormones, growth factors, and cytokines. Thus, a selective inhibitor of cyclooxygenase-2 will have similar anti inflammatory, antipyretic and analgesic properties to a conventional non-steroidal anti inflammatory drug, and in addition would inhibit hormone-induced uterine contractions and have potential anti-cancer effects, but will have a diminished ability to induce some of the mechanism-based side effects. In particular, such a compound should have a reduced potential for gastrointestinal toxicity, a reduced potential for renal side effects, a reduced effect on bleeding times and possibly a lessened ability to induce asthma attacks in aspirin-sensitive asthmatic subjects.

US 5,015,481, issued May 14, 1991 discloses the use of defined combinations of NSAID's and prostaglandins for the prevention of NSAID induced ulcers. WO 91/16896, published November 14, 1991 discloses defined combinations of NSAID's and prostaglandins to treat mild to moderate pain. WO 91/16895, published November 14, 1991 discloses a pharmaceutical composition including a core of an NSAID selected from diclofenac and piroxacam which core is surrounded by a mantle coating of a prostaglandin, wherein an intermediate coating can be present between the NSAID core and the prostaglandin mantle coating. US 5,232,704 discloses a sustained release dosage form of prostaglandin which in combination which is said to be useful for the prevention of NSAID induced ulcers.

More recently, it has been disclosed that NSAID induced gastric ulcers are caused by the cyclooxygenase-1 activity found in most NSAID's. Accordingly, the treatment of cyclooxygenase-2 mediated diseases by administration of an NSAID that selectively inhibits cyclooxygenase-2 in substantial preference to cyclooxygenase-1 eliminates the advantage of co-administering prostaglandin with the NSAID for purposes of preventing NSAID induced ulcers. Surprisingly, we have found that in addition to its negative role in the inflammatory process, cyclooxygenase-2 also plays an important positive role in gastric mucosal protection and in promoting the healing of certain lesions including gastric ulcers. Accordingly, the applicants disclose a method of treating cyclooxygenase mediated disease while promoting

the healing of certain lesions including gastric ulcers comprising the co-administration of certain prostaglandins and a selective cycooxygenase-2 inhibitor, or the co-administration of an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor as defined below.

SUMMARY OF THE INVENTION

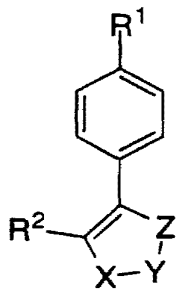
A method of treating cyclooxygenase mediated disease while promoting the healing of certain lesions including gastric ulcers and protecting the gastric mucosa comprising the co-administration of certain prostaglandin and a selective cyclooxygenase-2 inhibitor, or the co-administration of an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor as defined below.

DETAILED DESCRIPTION OF THE INVENTION

The invention encompasses a method of treating cyclooxygenase mediated disease while promoting the healing of certain lesions including gastric ulcers comprising the co-administration of a prostaglandins and a selective cycooxygenase-2 inhibitor, or the co-administration of an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor as defined below.

For purposes of this specification a compound shall be defined as a selective cyclooxygenase-2 inhibitor if the ratio of it's IC_{50} for the inhibition of cyclooxygenase-1 divided by it's IC_{50} for the inhibition of cyclooxygenase-2, as measured as described in this specification or a comparable method is 200 or greater; preferably 1000 or greater.

Accordingly, for purposes of this specification selective cyclooxygenase-2 inhibitors includes, but is not limited to compounds of Formula I



I

or a pharmaceutically acceptable salt thereof wherein:

X–Y–Z–is selected from the group consisting of:

(a) -C(O)-O-CR5(R5')-,

(b) -C(O)-CH2-CR5(R5'),

(c) -CH2-CH2-CH2-

R1 is selected from the group consisting of

(a) S(O)2CH3,

(b) S(O)2NH2,

(c) S(O)2NHC(O)CF3,

R2 is selected from the group consisting of

(a) C1-6alkyl,

(b) C3, C4, C5, C6, and C7, cycloalkyl,

(c) mono-, di- or tri-substituted phenyl or naphthyl wherein the substituent is selected from the group consisting of

(1) hydrogen,

(2) halo,

(3) C1-6alkoxy,

(4) C1-6alkylthio,

(5) CN,

(6) CF3,

(7) C1-6alkyl,

(8) N3,

(9) -CO2H,

(10) -CO2-C1-4alkyl,

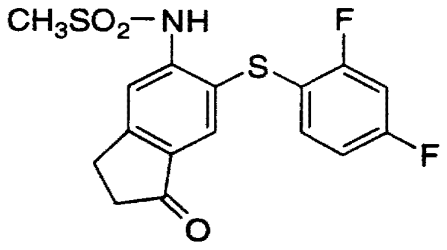
(11) -C(R^3)(R^4)-OH,
(12) -C(R^3)(R^4)-O-C_{1-4}alkyl, and
(13) -C_{1-6}alkyl-CO_2-R^3;

(d) mono-, di- or tri-substituted heteroaryl wherein the heteroaryl is a monocyclic aromatic ring of 5 atoms, said ring having one hetero atom which is S, O, or N, and optionally 1, 2, or 3 additionally N atoms; or
the heteroaryl is a monocyclic ring of 6 atoms, said ring having one hetero atom which is N, and optionally 1, 2, 3, or 4 additional N atoms; said substituents are selected from the group consisting of
(1) hydrogen,
(2) halo, including fluoro, chloro, bromo and iodo,
(3) C_{1-6}alkyl,
(4) C_{1-6}alkoxy,
(5) C_{1-6}alkylthio,
(6) CN,
(7) CF_3,
(8) N_3,
(9) -C(R^3)(R^4)-OH, and
(10) -C(R^3)(R^4)-O-C_{1-4}alkyl;

(e) benzoheteroaryl which includes the benzo fused analogs of (d);

R^3, R^4, R^5 and $R^{5'}$are each independently selected from the group consisting of
(a) hydrogen,
(b) C_{1-6}alkyl.

Additional selective cyclooxygenase-2 inhibitors within the scope of claimed method include:



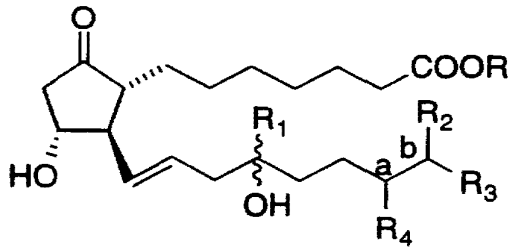
as well as compounds disclosed in WO 94/13635, published June 23, 1994; US 5,344,911, issued September 6, 1994; and WO 94/15932, published July 21 1994, all of which are hereby incorporated by reference. Additional selective cyclooxygenase-2 inhibitors within the scope of claimed method include Diclofenac, those disclosed in USSN 08/330, filed October 27, 1994; USSN 08/361,268, filed December 21, 1994 and USSN 08/443,620, filed May 18, 1995, all of which are incorporated by method.

For purposes of this specification alkyl is defined to include linear, branched, and cyclic structures, with C_{1-6}alkyl including methyl, ethyl, propyl, 2-propyl, s- and t-butyl, butyl, pentyl, hexyl, 1,1-dimethylethyl, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. Similarly, C_{1-6}alkoxy is intended to include alkoxy groups of from 1 to 6 carbon atoms of a straight, branched, or cyclic configuration. Examples of lower alkoxy groups include methoxy, ethoxy, propoxy, isopropoxy, cyclopropyloxy, cyclohexyloxy, and the like. Likewise, C_{1-6}alkylthio is intended to include alkylthio groups of from 1 to 6 carbon atoms of a straight, branched or cyclic configuration. Examples of lower alkylthio groups include methylthio, propylthio, isopropylthio, cycloheptylthio, etc. By way of illustration, the propylthio group signifies $-SCH_2CH_2CH_3$.

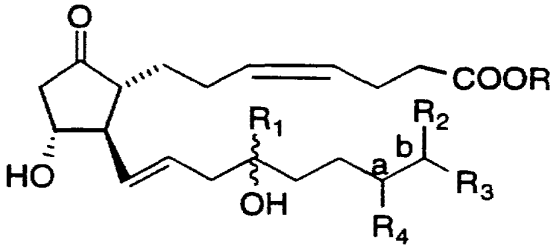
Heteroaryl includes furan, thiophene, pyrrole, isoxazole, isothiazole, pyrazole, oxazole, thiazole, imidazole, 1,2,3-oxadiazole, 1,2,3-thiadiazole, 1,2,3-triazole, 1,3,4-oxadiazole, 1,3,4-thiadiazole, 1,3,4-triazole, 1,2,5-oxadiazole, 1,2,5-thiadiazole, pyridine, pyridazine, pyrimidine, pyrazine, 1,2,4-triazine, 1,3,5-triazine, 1,2,4,5-tetrazine, and the like.

Exemplifying the invention are:
(a) 2-(4-Fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2-cyclopenten-1-one
(b) 3-(4-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(c) 3-(4-Fluorophenyl)-4-(4-(aminosulfonyl)phenyl)-2-(*5H*)-furanone,
(d) 5,5-Dimethyl-3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(e) 3-(3-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(f) 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(g) 5,5-Dimethyl-3-(3-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(h) 3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(i) 3-(3,4-Dichlorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(j) 5,5-Dimethyl-3-(3,4-difluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(k) 5,5-Dimethyl-3-(3,4-dichlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(l) 5,5-Dimethyl-3-(4-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(m) 5,5-Dimethyl-3-(2-naphthyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(n) 3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone.

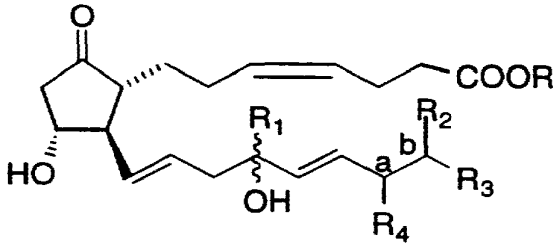
For purposes of this specification the prostaglandin suitable for the disclosed method includes the compounds of Formula II



IIa



IIb



IIc

wherein
R is hydrogen or C1-6alkyl;
R1 is hydrogen, vinyl or C1-4alkyl and the wavy line represents R or S stereochemistry;
R2, R3, and R4 are hydrogen or C1-4alkyl or R2 and R3 together with carbon b form a cycloalkenyl having 4 to 6 carbon atoms or R3 and R4 together with carbons a and b form a cycloalkenyl having 4 to 6 carbons and wherein the a-b bond can be saturated or unsaturated.

These useful prostaglandins include misoprostol, ±methyl 11α, 16-dihydroxy-16-methyl-9-oxoprost 13E-en-1-oate; enisoprost and methyl-7-[2B-[6-(1-cyclopenten-1-yl)-4-hydroxy-4-methyl-1E, 5E-hexadienyl]-3α–hydroxy-5-oxo 1R, 1α–cyclopentyl]-4Z-heptenoate. Prostaglandins within the scope of the invention also include arbaprostil,

enprostil, rioprostol, nocloprost, mexiprostil, ornoprostol, dimoxaprost, tiprostanide, and rosaprostol.

In one aspect applicants method includes the use of the prostaglandin misoprostol with Diclofenac.

With regard to the illustrated structures of formula II, the dashed line indicates the grouping being behind the plane of the paper and the solid, blackened triangular shape indicates that the group is in front of the plane of the paper.

The prostaglandins useful in the composition of the invention herein can be prepared by known reaction schemes such as by the methods taught in U.S. Pat. Nos. 3,965,143; 4,271,314; and 4,683,328 and in an article by P.W. Collins and J.W. Djurie, Chem. Rev. 1993, 93, 1533-1564.. The individual isomers can be obtained by chromatographic separation. The prostaglandin is preferably an orally available prostaglandin.

When the prostaglandin is misoprostol, (±) methyl 11alpha, 16 dihydroxy 16 methyl-9-oxoprost13E-en-1-oate, the misoprostol is present in an amount of about 100 to 200 mcg (micrograms).

For purposes of this specification, the anti-ulcer agent shall be defined to include cimetidine, famotidine, omeprazole, ranitidine and the like.

As appreciated by those of skill in the art, the co-administration of a selective cyclooxygenase-2 inhibitor with an additional active agent, such as a prostaglandin or anti-ulcer agent, includes situations wherein both active agents are provided in a single dosage form as well as situations wherein the active agents are provided in separate dosage forms. For example, while for patient compliance it may be advantageous to provide both agents in a single dosage form, depending on the particular species selected, it may be advantage to administer one of the agents three times a day and the other twice a day.

Some of the compounds described herein contain one or more asymmetric centers and may thus give rise to diastereomers and optical isomers. The present invention is meant to comprehend such possible diastereomers as well as their racemic and resolved,

enantiomerically pure forms and pharmaceutically acceptable salts thereof.

Some of the compounds described herein contain olefinic double bonds, and unless specified otherwise, are meant to include both E and Z geometric isomers.

The pharmaceutical compositions of the present invention comprise a compound of Formula I as an active ingredient or a pharmaceutically acceptable salt, thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases including inorganic bases and organic bases. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N_-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

It will be understood that in the discussion of methods of treatment which follows, references to the compounds of Formula I are meant to also include the pharmaceutically acceptable salts.

The Compound of Formula I is useful for the relief of pain, fever and inflammation of a variety of conditions including rheumatic fever, symptoms associated with influenza or other viral infections, common cold, low back and neck pain, dysmenorrhea, headache,

toothache, sprains and strains, myositis, neuralgia, synovitis, arthritis, including rheumatoid arthritis degenerative joint diseases (osteoarthritis), gout and ankylosing spondylitis, bursitis, burns, injuries, following surgical and dental procedures. In addition, such a compound may inhibit cellular neoplastic transformations and metastic tumor growth and hence can be used in the treatment of cancer. Compounds of formula I may also be useful for the treatment of dementia including pre-senile and senile dementia, and in particular, dementia associated with Alzheimer Disease (ie Alzheimer's dementia).

Compounds of formula I will also inhibit prostanoid-induced smooth muscle contraction by preventing the synthesis of contractile prostanoids and hence may be of use in the treatment of dysmenorrhea, premature labor and asthma.

By virtue of its high cyclooxygenase-2 (COX-2) activity and/or its selectivity for cyclooxygenase-2 over cyclooxygenase-1 (COX-1) as defined above, compounds of formula I will prove useful as an alternative to conventional non-steroidal antiinflammatory drugs (NSAID'S) particularly where such non-steroidal antiinflammatory drugs may be contra-indicated such as in patients with peptic ulcers, gastritis, regional enteritis, ulcerative colitis, diverticulitis or with a recurrent history of gastrointestinal lesions; GI bleeding, coagulation disorders including anemia such as hypoprothrombinemia, haemophilia or other bleeding problems (including those relating to reduced or impaired platelet function); kidney disease (eg impaired renal function); those prior to surgery or taking anticoagulants; and those susceptable to NSAID induced asthma.

Similarly, compounds of formula I, will be useful as a partial or complete substitute for conventional NSAID'S in preparations wherein they are presently co-administered with other agents or ingredients. Thus in further aspects, the invention encompasses pharmaceutical compositions for treating cyclooxygenase-2 mediated diseases as defined above comprising a non-toxic therapeutically effective amount of the compound of Formula I as defined above and one or more ingredients such as another pain reliever including

acetominophen or phenacetin; a potentiator including caffeine; an H2-antagonist, aluminum or magnesium hydroxide, simethicone, a decongestant including phenylephrine, phenylpropanolamine, pseudophedrine, oxymetazoline, ephinephrine, naphazoline, xylometazoline, propylhexedrine, or levo-desoxyephedrine; an antiitussive including codeine, hydrocodone, caramiphen, carbetapentane, or dextramethorphan; a diuretic; a sedating or non-sedating antihistamine. In addition the invention encompasses a method of treating cyclooxygenase mediated diseases comprising: administration to a patient in need of such treatment a non-toxic therapeutically effect amount of the compound of Formula I, optionally co-administered with one or more of such ingredients as listed immediately above.

As indicated above, pharmaceutical compositions for treating cyclooxygenase-2 mediated diseases as defined may include one or more ingredients as listed above as well as a compound of formula II.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by

known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the technique described in the U.S. Patent 4,256,108; 4,166,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredients is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethyl-cellulose, methylcellulose, hydroxy-propylmethycellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil,

sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed

are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of formula I may also be administered in the form of a suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compound of Formula I are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

Specific cyclooxygenase-2 inhibitor dosage levels of the order of from about 0.01 mg to about 140 mg/kg of body weight per day are useful in the treatment of the above-indicated conditions, or alternatively about 0.5 mg to about 7 g per patient per day. For example, inflammation may be effectively treated by the administration of from about 0.01 to 50 mg of the compound per kilogram of body weight per day, or alternatively about 0.5 mg to about 3.5 g per patient per day.

With regard to the prostaglandins, typical dosages by be as much as 25 to 1600 µg per day; more typically 200 to 800 µg per day (eg 200, 400, 600 or 800 µg per day. Single dosage forms may typically contain 5, 25, 50, 100, 200, 250, 400 or 500 µg per tablet.

When the prostaglandin is misoprostol, (±) methyl 11alpha, 16 dihydroxy 16 methyl-9-oxoprost13E-en-1-oate, the misoprostol is present in the dosage form (eg tablet) in an amount of about 100 to 200 µg. See, for example, a Physicans Desk Reference (PDR).

With regard to anti-ulcer agents, such as those described above, the dosages may typically range from 10 to 800 mg per day or more, with single dosages containing 10, 20, 30 ,100, 200, 400 or 800

mg of active agent. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may contain from 0.5 mg to 5 g of specific cyclooxygenase-2 inhibitor compounded with a prostaglandin of formula II and an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 percent of the total composition. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient, typically 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 800 mg, or 1000 mg of specific cyclooxygenase-2 inhibitor.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

Assays for Determining Biological Activity

The compound of Formula I can be tested using the following assays to determine their cyclooxygenase-2 inhibiting activity.

INHIBITION OF CYCLOOXYGENASE ACTIVITY

Compounds are tested as inhibitors of cyclooxygenase activity in whole cell cyclooxygenase assays. Both of these assays measure prostaglandin E_2 synthesis in response to arachidonic acid, using a radioimmunoassay. Cells used for these assays are human osteosarcoma 143 cells (which specifically express COX-2) and human U-937 cells (which specifically express COX-1). In these assays, 100% activity is defined as the difference between prostaglandin E_2 synthesis in the absence and presence of arachidonate.

Whole Cell Assays

For cyclooxygenase assays, osteosarcoma cells are cultured in 1 mL of media in 24-well multidishes (Nunclon) until confluent (1-2 x 10^5 cells/well). U-937 cells are grown in spinner flasks and resuspended to a final density of 1.5 x 10^6 cells/mL in 24-well multidishes (Nunclon). Following washing and resuspension of osteosarcoma and U-937 cells in 1 mL of HBSS, 1 µL of a DMSO solution of test compound or DMSO vehicle is added, and samples gently mixed. All assays are performed in triplicate. Samples are then incubated for 5 or 15 minutes at 37C, prior to the addition of arachidonic acid. Arachidonic acid (peroxide-free, Cayman Chemical) is prepared as a 10 mM stock solution in ethanol and further diluted 10-fold in HBSS. An aliquot of 10 µL of this diluted solution is added to the cells to give a final arachidonic acid concentration of 10 µM. Control samples are incubated with ethanol vehicle instead of arachidonic acid. Samples are again gently mixed and incubated for a further 10 min. at 37C. For osteosarcoma cells, reactions are then stopped by the addition of 100 µL of 1N HCl, with mixing and by the rapid removal of the solution from cell monolayers. For U-937 cells, reactions are stopped by the addition of 100 µL of 1N HCl, with mixing. Samples are then neutralized by the addition of 100 µL of 1N NaOH and PGE_2 levels measured by radioimmunoassay.

Whole cell assays for COX-2 and COX-1 using CHO transfected cell lines

Chinese hamster ovary (CHO) cell lines which have been stably transfected with an eukaryotic expression vector pCDNAIII containing either the human COX-1 or COX-2 cDNA's are used for the assay. These cell lines are referred to as CHO [hCOX-1] and CHO [hCOX-2], respectively. For cyclooxygenase assays, CHO[hCOX-1] cells from suspension cultures and CHO[hCOX-2] cells prepared by trypsinization of adherent cultures are harvested by centrifugation (300 x g, 10 min) and washed once in HBSS containing 15 mM HEPES, pH 7.4, and

resuspended in HBSS, 15 mM HEPES, pH 7.4, at a cell concentration of 1.5 x 10^6 cells/ml. Drugs to be tested are dissolved in DMSO to 66.7-fold the highest test drug concentration. Compounds are typically tested at 8 concentrations in duplicate using serial 3-fold serial dilutions in DMSO of the highest drug concentration. Cells (0.3 x 10^6 cells in 200 µl) are preincubated with 3 µl of the test drug or DMSO vehicle for 15 min at 37C. Working solutions of peroxide-free AA (5.5 µM and 110 µM AA for the CHO [hCOX-1] and CHO [COX-2] assays, respectively) are prepared by a 10-fold dilution of a concentrated AA solution in ethanol into HBSS containing 15 mM HEPES, pH 7.4. Cells are then challenged in the presence or absence of drug with the AA/HBSS solution to yield a final concentration of 0.5 µM AA in the CHO[hCOX-1] assay and a final concentration of 10 µM AA in the CHO[hCOX-2] assay. The reaction is terminated by the addition of 10 µl 1 N HCl followed by neutralization with 20 µl of 0.5 N NaOH. The samples are centrifuged at 300 x g at 4C for 10 min, and an aliquot of the clarified supernatant is appropriately diluted for the determination of PGE_2 levels using an enzyme-linked immunoassay for PGE_2 (Correlate PGE_2 enzyme immunoassay kit, Assay Designs, Inc.). Cyclooxygenase activity in the absence of test compounds is determined as the difference in PGE_2 levels of cells challenged with arachidonic acid versus the PGE_2 levels in cells mock-challenged with ethanol vehicle. Inhibition of PGE_2 synthesis by test compounds is calculated as a percentage of the activity in the presence of drug versus the activity in the positive control samples.

Assay of COX-1 Activity from U937 cell microsomes

U 937 cells are pelleted by centrifugation at 500 x g for 5 min and washed once with phosphate-buffered saline and repelleted. Cells are resuspended in homogenization buffer consisting of 0.1 M Tris-HCl, pH 7.4, 10 mM EDTA, 2 µg/ml leupeptin, 2 µg/ml soybean trypsin inhibitor, 2 µg/ml aprotinin and 1 mM phenyl methyl sulfonyl fluoride. The cell suspension is sonicated 4 times for 10 sec and is centrifuged at

10,000 x g for 10 min at 4° C. The supernatant is centrifuged at 100,000 x g for 1 hr at 4 ° C. The 100,000 x g microsomal pellet is resuspended in 0.1 M Tris-HCl, pH 7.4, 10 mM EDTA to approximately 7 mg protein/ml and stored at -80° C.

Microsomal preparations are thawed immediately prior to use, subjected to a brief sonication, and then diluted to a protein concentration of 125 µg/ml in 0.1 M Tris-HCl buffer, pH 7.4 containing 10 mM EDTA, 0.5 mM phenol, 1 mM reduced glutathione and 1 µM hematin. Assays are performed in duplicate in a final volume of 250 µl. Initially, 5 µl of DMSO vehicle or drug in DMSO are added to 20 µl of 0.1 M Tris-HCl buffer, pH 7.4 containing 10 mM EDTA in wells of a 96-deepwell polypropylene titre plate. 200 µl of the microsomal preparation are then added and pre-incubated for 15 min at room temperature before addition of 25 µl of 1 M arachidonic acid in 0.1 M Tris-HCl and 10 mM EDTA, pH 7.4. Samples are incubated for 40 min at room temperature and the reaction is stopped by the addition of 25 µl of 1 N HCl. Samples are neutralized with 25 µl of 1 N NaOH prior to quantitation of PGE_2 content by radioimmunoassay (Dupont-NEN or Amersham assay kits). Cyclooxygenase activity is defined as the difference between PGE_2 levels in samples incubated in the presence of arachidonic acid and ethanol vehicle.

<u>Assay of the activity of purified human COX-2</u>

The enzyme activity is measured using a chromogenic assay based on the oxidation of N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) during the reduction of PGG_2 to PGH_2 by COX-2 (Copeland et al. (1994) Proc. Natl. Acad. Sci. 91, 11202-11206).

Recombinant human COX-2 is purified from Sf9 cells as previously described (Percival et al (1994) Arch. Biochem. Biophys. 15, 111-118). The assay mixture (180 µL) contains 100 mM sodium phosphate, pH 6.5, 2 mM genapol X-100, 1 µM hematin, 1 mg/ml gelatin , 80-100

units of purified enzyme (One unit of enzyme is defined as the amount of enzyme required to produce an O.D. change of 0.001/min at 610 nm) and 4 µL of the test compound in DMSO. The mixture is pre-incubated at room temperature (22°C) for 15 minutes prior to initiation of the enzymatic reaction by the addition of 20 µL of a sonicated solution of 1 mM arachidonic acid (AA) and 1 mM TMPD in assay buffer (without enzyme or hematin). The enzymatic activity is measured by estimation of the initial velocity of TMPD oxidation over the first 36 sec of the reaction. A non-specific rate of oxidation is observed in the absence of enzyme (0.007 - 0.010 O.D. /min) and is subtracted before the calculation of the % inhibition. IC_{50} values are derived from 4-parameter least squares non-linear regression analysis of the log-dose vs % inhibition plot.

HUMAN WHOLE BLOOD ASSAY

Rationale

Human whole blood provides a protein and cell-rich milieu appropriate for the study of biochemical efficacy of anti-inflammatory compounds such as selective COX-2 inhibitors. Studies have shown that normal human blood does not contain the COX-2 enzyme. This is consistent with the observation that COX-2 inhibitors have no effect on PGE_2 production in normal blood. These inhibitors are active only after incubation of human whole blood with LPS, which induces COX-2. This assay can be used to evaluate the inhibitory effect of selective COX-2 inhibitors on PGE_2 production. As well, platelets in whole blood contain a large amount of the COX-1 enzyme. Immediately following blood clotting, platelets are activated through a thrombin-mediated mechanism. This reaction results in the production of thromboxane B_2 (TxB_2) via activation of COX-1. Thus, the effect of test compounds on TxB_2 levels following blood clotting can be examined and used as an index for COX-1 activity. Therefore, the degree of selectivity by the test compound can be determined by

measuring the levels of PGE_2 after LPS induction (COX-2) and TxB_2 following blood clotting (COX-1) in the same assay.

Method

A. COX-2 (LPS-induced PGE_2 production)

Fresh blood is collected in heparinized tubes by venipuncture from both male and female volunteers. The subjects have no apparent inflammatory conditions and have not taken any NSAIDs for at least 7 days prior to blood collection. Plasma is immediately obtained from a 2mL blood aliquot to use as blank (basal levels of PGE_2). The remaining blood is incubated with LPS (100 µg/ml final concentration, Sigma Chem, #L-2630 from E. coli; diluted in 0.1% BSA (Phosphate buffered saline) for 5 minutes at room temperature. Five hundred µL aliquots of blood are incubated with either 2µL of vehicle (DMSO) or 2µL of a test compound at final concentrations varying from 10nM to 30µM for 24 hours at 37°C. At the end of the incubation, the blood is centrifuged at 12,000 x g for 5 minutes to obtain plasma. A 100µL aliquot of plasma is mixed with 400µL of methanol for protein precipitation. The supernatant is obtained and is assayed for PGE_2 using a radioimmunoassay kit (Amersham, RPA#530) after conversion of PGE_2 to its methyl oximate derivative according to the manufacturer's procedure.

B. COX-1 (Clotting-induced TxB_2 production)

Fresh blood is collected into vacutainers containing no anticoagulants. Aliquots of 500µL are immediately transferred to siliconized microcentrifuge tubes preloaded with 2µL of either DMSO or a test compound at final concentrations varying from 10nM to 30µM. The tubes are vortexed and incubated at 37°C for 1 hour to allow blood to clot. At the end of incubation, serum is obtained by centrifugation (12,000 x g for 5 min.). A 100µL aliquot of serum is mixed with 400µL of methanol for protein precipitation. The supernatant is obtained and is assayed for TxB_2 using a enzyme immunoassay kit (Cayman, #519031) according to the manufacturer's instruction.

RAT PAW EDEMA ASSAY

Protocol

Male Sprague-Dawley rats (150-200 g) are fasted overnight and are given, po, either vehicle (1% methocel or 5% Tween 80) or a test compound. One hr later, a line is drawn using a permanent marker at the level above the ankle in one hind paw to define the area of the paw to be monitored. The paw volume (V_0) is measured using a plethysmometer (Ugo-Basile, Italy) based on the principle of water displacement. The animals are then injected subplantarly with 50 ml of 1% carrageenan solution in saline (FMC Corp, Maine) into the paw using an insulin syringe with a 25-gauge needle (i.e. 500 mg carrageenan per paw). Three hr later, the paw volume (V_3) is measured and the increases in paw volume ($V_3 - V_O$) are calculated. The animals are sacrificed by CO_2 asphyxiation and the absence or presence of stomach lesions scored. Data is compared with the vehicle-control values and percent inhibition calculated. All treatment groups are coded to eliminate observer bias.

NSAID-INDUCED GASTROPATHY IN RATS

Rationale

The major side effect of conventional NSAIDs is their ability to produce gastric lesions in man. This action is believed to be caused by inhibition of Cox-1 in the gastrointestinal tract. Rats are particularly sensitive to the actions of NSAIDs. In fact, rat models have been used commonly in the past to evaluate the gastrointestinal side effects of current conventional NSAIDs. In the present assay, NSAID-induced gastrointestinal damage is observed by measuring fecal ^{51}Cr excretion after systemic injection of ^{51}Cr-labeled red blood cells. Fecal ^{51}Cr excretion is a well-established and sensitive technique to detect gastrointestinal integrity in animals and man.

Methods

Male Sprague Dawley rats (150 - 200 g) are administered orally a test compound either once (acute dosing) or b.i.d. for 5 days (chronic dosing). Immediately after the administration of the last dose, the rats are injected via a tail vein with 0.5 mL of ^{51}Cr-labeled red blood cells from a donor rat. The animals are placed individually in metabolism cages with food and water ad *lib*. Feces are collected for a 48 h period and ^{51}Cr fecal excretion is calculated as a percent of total injected dose. ^{51}Cr-labeled red blood cells are prepared using the following procedures. Ten mL of blood is collected in heparinized tubes via the vena cava from a donor rat. Plasma is removed by centrifugation and replenished with equal volume of HBSS. The red blood cells are incubated with 400 Ci of sodium ^{51}chromate for 30 min. at 37C. At the end of the incubation, the red blood cells are washed twice with 20 mL HBSS to remove free sodium ^{51}chromate. The red blood cells are finally reconstituted in 10 mL HBSS and 0.5 mL of the solution (about 20 Ci) is injected per rat.

PROTEIN-LOSING GASTROPATHY IN SQUIRREL MONKEYS

Rationale

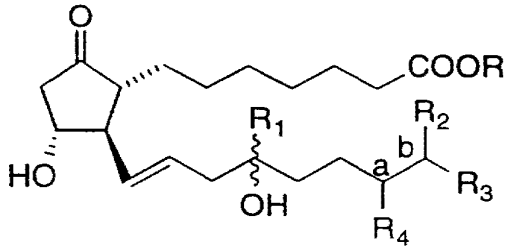
Protein-losing gastropathy (manifested as appearance of circulating cells and plasma proteins in the GI tract) is a significant and dose-limiting adverse response to standard non-steroidal anti-inflammatory drugs (NSAIDs). This can be quantitatively assessed by intravenous administration of $^{51}CrCl_3$ solution. This isotopic ion can avidly bind to cell and serum globins and cell endoplasmic reticulum. Measurement of radioactivity appearing in feces collected for 24 h after administration of the isotope thus provides a sensitive and quantitative index of protein-losing gastropathy.

Methods

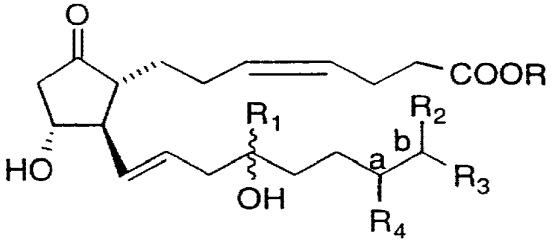
Groups of male squirrel monkeys (0.8 to 1.4 kg) are treated by gavage with either 1% methocell or 5% Tween 80 in H_2O vehicles, (3mL/kg b.i.d.) or test compounds at doses from 1 - 100 mg/kg b.i.d. for 5 days. Intravenous ^{51}Cr (5Ci/kg in 1 ml/kg phosphate buffer saline (PBS)) is administered 1 h after the last drug/vehicle dose, and feces collected for 24 h in a metabolism cage and assessed for excreted ^{51}Cr by gamma-counting. Venous blood is sampled 1 h and 8 h after the last drug dose, and plasma concentrations of drug measured by RP-HPLC.

WHAT IS CLAIMED IS:

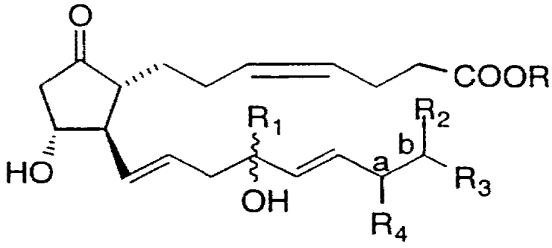
1. A pharmaceutical composition for treating cyclo-oxygenase-2 mediated disease comprising an agent selected from an anti-ulcer agent and a prostaglandin of formula II



IIa



IIb



IIc

wherein

R is hydrogen or C_{1-6}alkyl;

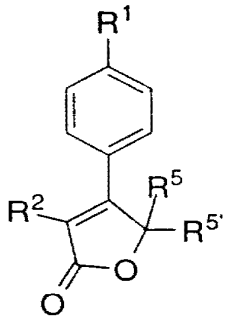
R_1 is hydrogen, vinyl or C_{1-4}alkyl and the wavy line represents R or S stereochemistry;

R_2, R_3, and R_4 are hydrogen or C_{1-4}alkyl or R_2 and R_3 together with carbon b form a cycloalkenyl having 4 to 6 carbon atoms or R_3 and R_4 together with carbons a and b form a cycloalkenyl having 4 to 6 carbons and wherein the a-b bond can be saturated or unsaturated;

together with a selective cyclooxygenase-2 inhibitor.

2. A composition of Claim 1 wherein the prostaglandin is selected from misoprostol, and methyl-7-[2B-[6-(1-cyclopenten-1-yl)-4-hydroxy-4-methyl-1E, 5E-hexadienyl]-3α–hydroxy-5-oxo 1R, 1α–cyclopentyl]-4Z-heptenoate.

3. A composition according to Claim 1 or 2 wherein the selective cyclooxygenase-2 inhibitor is a compound of Formula Ia



Ia

or pharmaceutically acceptable salts thereof wherein:

R1 is selected from the group consisting of
(a) $S(O)_2CH_3$,
(b) $S(O)_2NH_2$,
(c) $S(O)_2NHC(O)CF_3$,

R2 is selected from the group consisting of
(a) C_{1-6}alkyl,
(b) C_3, C_4, C_5, C_6, and C_7, cycloalkyl,
(c) mono-, di- or tri-substituted phenyl or naphthyl wherein the substituent is selected from the group consisting of selected from the group consisting of
(1) hydrogen,
(2) halo,
(3) C_{1-6}alkoxy,
(4) C_{1-6}alkylthio,
(5) CN,

(6) CF_3,
(7) C_{1-6}alkyl,
(8) N_3,
(9) $-CO_2H$,
(10) $-CO_2-C_{1-4}$alkyl,
(11) $-C(R^3)(R^4)-OH$,
(12) $-C(R^3)(R^4)-O-C_{1-4}$alkyl, and
(13) $-C_{1-6}$alkyl$-CO_2-R^3$;

(d) heteroaryl
(e) benzoheteroaryl

R^3, R^4, R^5 and $R^{5'}$are each independently selected from the group consisting of

(a) hydrogen,
(b) C_{1-6}alkyl.

4. A composition according to Claim 3 wherein

R^1 is selected from the group consisting of

(a) $S(O)_2CH_3$, and
(b) $S(O)_2NH_2$,

R^2 is

mono or di-substituted phenyl wherein the substituents are selected from the group consisting of

(1) hydrogen,
(2) halo, selected from the group consisting of fluoro, chloro and bromo; and

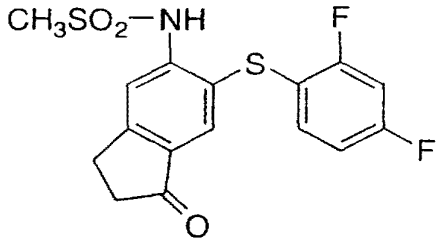
R^5 and $R^{5'}$ are each hydrogen.

5. A composition according to Claim 1 or 2 wherein the selective cyclooxygenase-2 inhibitor is

(a) 2-(4-Fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2-cyclopenten-1-one
(b) 3-(4-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,

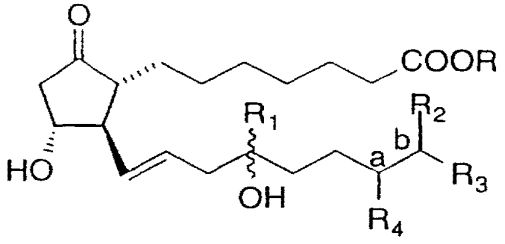
(c) 3-(4-Fluorophenyl)-4-(4-(aminosulfonyl)phenyl)-2-(*5H*)-furanone,
(d) 5,5-Dimethyl-3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(e) 3-(3-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(f) 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(g) 5,5-Dimethyl-3-(3-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(h) 3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(i) 3-(3,4-Dichlorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(j) 5,5-Dimethyl-3-(3,4-difluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(k) 5,5-Dimethyl-3-(3,4-dichlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(l) 5,5-Dimethyl-3-(4-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(m) 5,5-Dimethyl-3-(2-naphyhyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(n) 3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone.

6. A composition according to Claim 1 or 2 wherein the selective cyclooxygenase-2 inhibitor is
5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,



or a pharmaceutically acceptable salt thereof.

7. A system for treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers or protecting the gastric mucosa comprising a selective cyclooxygenase-2 inhibitor and a prostaglandin of formula II



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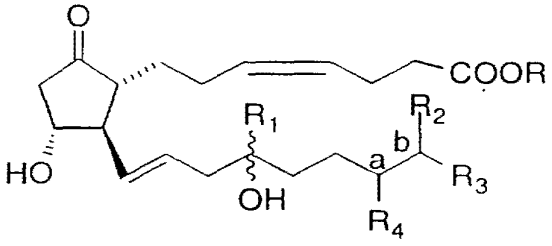
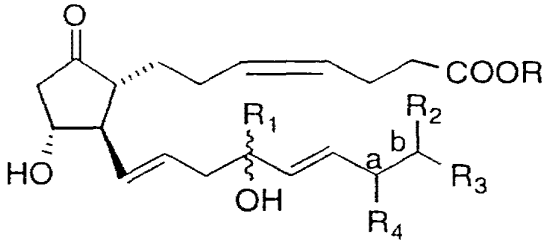


IIb



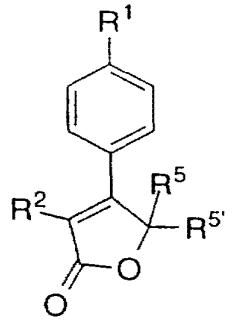
IIc

wherein

R is hydrogen or C_{1-6}alkyl;

R_1 is hydrogen, vinyl or C_{1-4}alkyl and the wavy line represents R or S stereochemistry;
R_2, R_3, and R_4 are hydrogen or C_{1-4}alkyl or R_2 and R_3 together with carbon b form a cycloalkenyl having 4 to 6 carbon atoms or R_3 and R_4 together with carbons a and b form a cycloalkenyl having 4 to 6 carbons **and wherein the a-b bond can be saturated or unsaturated, in a form for co-administration with said selective cyclooxygenase-2 inhibitor.**

8. A system according to Claim 7 wherein the selective cyclooxygenase-2 inhibitor is a compound of Formula Ia



Ia

or pharmaceutically acceptable salts thereof wherein:

R^1 is selected from the group consisting of
(a) $S(O)_2CH_3$,
(b) $S(O)_2NH_2$,
(c) $S(O)_2NHC(O)CF_3$,

R^2 is selected from the group consisting of
(a) C_{1-6}alkyl,
(b) C_3, C_4, C_5, C_6, and C_7, cycloalkyl,
(c) mono-, di- or tri-substituted phenyl or naphthyl wherein the substituent is selected from the group consisting of selected from the group consisting of
(1) hydrogen,
(2) halo,
(3) C_{1-6}alkoxy,

(4) C_{1-6}alkylthio,
(5) CN,
(6) CF_3,
(7) C_{1-6}alkyl,
(8) N_3,
(9) $-CO_2H$,
(10) $-CO_2-C_{1-4}$alkyl,
(11) $-C(R^3)(R^4)-OH$,
(12) $-C(R^3)(R^4)-O-C_{1-4}$alkyl, and
(13) $-C_{1-6}$alkyl$-CO_2-R^3$;

(d) heteroaryl,
(e) benzoheteroaryl,

R^3, R^4, R^5 and $R^{5'}$ are each independently selected from the group consisting of

(a) hydrogen,
(b) C_{1-6}alkyl.

9. A system according to Claim 8 wherein R^1 is selected from the group consisting of

(a) $S(O)_2CH_3$, and
(b) $S(O)_2NH_2$,

R^2 is

mono or di-substituted phenyl wherein the substituents are selected from the group consisting of

(1) hydrogen,
(2) halo, selected from the group consisting of fluoro, chloro and bromo; and

R^5 and $R^{5'}$ are each hydrogen.

10. A system according to Claim 7 wherein the selective cyclooxygenase-2 inhibitor is

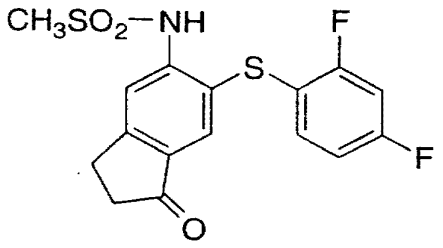
(a) 2-(4-Fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2-cyclopenten-1-one

(b) 3-(4-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(c) 3-(4-Fluorophenyl)-4-(4-(aminosulfonyl)phenyl)-2-(*5H*)-furanone,
(d) 5,5-Dimethyl-3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(e) 3-(3-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(f) 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(g) 5,5-Dimethyl-3-(3-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(h) 3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(i) 3-(3,4-Dichlorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(j) 5,5-Dimethyl-3-(3,4-difluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(k) 5,5-Dimethyl-3-(3,4-dichlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(l) 5,5-Dimethyl-3-(4-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(m) 5,5-Dimethyl-3-(2-naphthyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone, or
(n) 3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone.

11. A system according to Claim 7 wherein the selective cyclooxygenase-2 inhibitor is

5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,

3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone, or



or a pharmaceutically acceptable salt thereof.

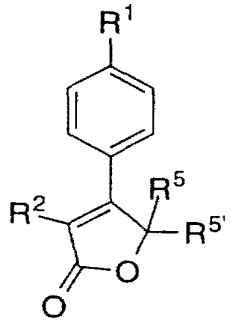
12. A co-administrable combination of a prostaglandin and a selective cyclooxygenase-2 inhibitor, said selective cyclooxygenase-2 inhibitor being at least 200 times more potent against cyclooxygenase-2 than against cyclooxygenase-2 than against cyclooxygenase-1 as measured by the ratio of said inhibitor's IC_{50} for cyclooxygenase-1 divided by said inhibitor's IC_{50} for cyclooxygenase-2, for use in treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers.

13. A co-administrable combination of a prostaglandin and a selective cyclooxygenase-2 inhibitor, said selective cyclooxygenase-2 inhibitor being at least 200 times more potent against cyclooxygenase-2 than against cyclooxygenase-2 than against cyclooxygenase-1 as measured by the ratio of said inhibitor's IC_{50} for cyclooxygenase-1 divided by said inhibitor's IC_{50} for cyclooxygenase-2, for use in treating cyclooxygenase-2 mediated disease while promoting the healing of gastric mucosa.

14. Use of an anti-ulcer agent and a selective cyclooxygenase-2 inhbitor in the manufacture of a medicament in which the agent and inhibitor are co-administrable, for treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers.

15. A use of Claim 14 wherein the anti-ulcer agent is selected from cimetidine, famotidine, omeprazole and ranitidine.

16. A use according to Claim 15 wherein the selective cyclooxygenase-2 inhibitor is a compound of Formula Ia



Ia

or pharmaceutically acceptable salts thereof wherein:

R1 is selected from the group consisting of
- (a) $S(O)_2CH_3$,
- (b) $S(O)_2NH_2$,
- (c) $S(O)_2NHC(O)CF_3$,

R2 is selected from the group consisting of
- (a) C_{1-6}alkyl,
- (b) C_3, C_4, C_5, C_6, and C_7, cycloalkyl,
- (c) mono-, di- or tri-substituted phenyl or naphthyl wherein the substituent is selected from the group consisting of selected from the group consisting of
  - (1) hydrogen,
  - (2) halo,
  - (3) C_{1-6}alkoxy,
  - (4) C_{1-6}alkylthio,
  - (5) CN,
  - (6) CF_3,
  - (7) C_{1-6}alkyl,
  - (8) N_3,
  - (9) $-CO_2H$,

(10) $-CO_2-C_{1-4}alkyl$,
(11) $-C(R^3)(R^4)-OH$,
(12) $-C(R^3)(R^4)-O-C_{1-4}alkyl$, and
(13) $-C_{1-6}alkyl-CO_2-R^3$;

(d) heteroaryl
(e) benzoheteroaryl

R^3, R^4, R^5 and $R^{5'}$are each independently selected from the group consisting of

(a) hydrogen,
(b) $C_{1-6}alkyl$.

17. A use according to Claim 16 wherein R^1 is selected from the group consisting of

(a) $S(O)_2CH_3$, and
(b) $S(O)_2NH_2$,

R^2 is

mono or di-substituted phenyl wherein the substituents are selected from the group consisting of

(1) hydrogen,
(2) halo, selected from the group consisting of fluoro, chloro and bromo; and

R^5 and $R^{5'}$ are each hydrogen.

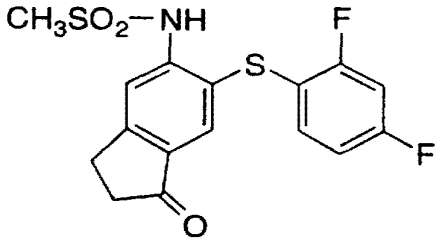
18. A use according to Claim 16 wherein the selective cyclooxygenase-2 inhibitor is

(a) 2-(4-Fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2-cyclopenten-1-one
(b) 3-(4-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(c) 3-(4-Fluorophenyl)-4-(4-(aminosulfonyl)phenyl)-2-(*5H*)-furanone,
(d) 5,5-Dimethyl-3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,

(e) 3-(3-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(f) 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(g) 5,5-Dimethyl-3-(3-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(h) 3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(i) 3-(3,4-Dichlorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(j) 5,5-Dimethyl-3-(3,4-difluorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(k) 5,5-Dimethyl-3-(3,4-dichlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(l) 5,5-Dimethyl-3-(4-chlorophenyl)-4-(4-methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(m) 5,5-Dimethyl-3-(2-naphyhyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
(n) 3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone.

19. A use according to Claim 14 wherein the selective cyclooxygenase-2 inhibitor is

5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone,
3-(3,4-Difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone, and
3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone, and



or a pharmaceutically acceptable salt thereof.

20. Use of a prostaglandin and a selective cyclooxygenase-2 inhibitor, as defined in Claim 1, 2, 3, 4, 5, 6 or 7, as co-administrable agents for treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers or protecting the gastric mucosa.

21. A pharmaceutical composition for treating cyclo-oxygenase-2 mediated disease while promoting the healing of gastric ulcers comprising an anti-ulcer agent and a selective cyclooxygenase-2 inhibitor.

22. A composition according to Claim 21, wherein the anti-ulcer agent is selected from cimetidine, famotidine, omeprazole and ranitidine.

23. A composition according to Claim 21 or 22, wherein the selective cyclooxygenase-2 inhibitor is a compound of formula Ia or a pharmaceutically acceptable salt thereof, as defined in Claim 16, 17, 18 or 19.

24. A method of treating cyclooxygenase-2 mediated disease while promoting the healing of gastric ulcers comprising the co-administration of a prostaglandin and a selective cyclooxygenase-2 inhibitor, both as defined in Claim 1, 2, 3, 4, 5, 6 or 7.

25. A method of treating cyclooxygenase-2 mediated disease while protecting the gastric mucosa comprising the co-administration of a prostaglandin and a selective cyclooxygenase-2 inhibitor, both as defined in Claim 1, 2, 3, 4, 5, 6 or 7.

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/CA 96/00638

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/557 //(A61K31/557,31:34)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 00501 A (MERCK FROSST CANADA INC ;DUCHARME YVES (CA); GAUTHIER JACQUES YVES) 5 January 1995 see abstract ---	1-25
A	US 5 015 481 A (FRANZ MICHEL ET AL) 14 May 1991 cited in the application see abstract ---	1-25
A	WO 91 16895 A (SEARLE & CO) 14 November 1991 cited in the application see abstract --- -/--	1-25

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 February 1997	11.02.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Leherte, C

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter ·onal Application No
PC1/CA 96/00638

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 16896 A (SEARLE & CO) 14 November 1991 cited in the application see abstract -----	1-25

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 96/00638

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 24,25
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-25
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

In view of the large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and compounds mentioned in the examples of the description.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l Application No
PCT/CA 96/00638

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		BG-A-	100247	28-06-96
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		CA-A-	2163888	05-01-95
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		CA-A-	2176974	25-12-94
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		EP-A-	0705254	10-04-96
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		IE-B-	62051	14-12-94

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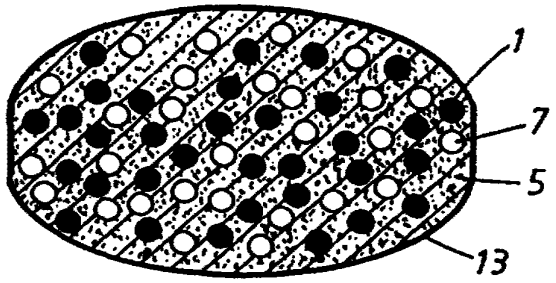
(54) Title: ORAL PHARMACEUTICAL DOSAGE FORMS COMPRISING A PROTON PUMP INHIBITOR AND A NSAID

(57) Abstract

An oral pharmaceutical dosage form comprising an acid susceptible proton pump inhibitor and one or more NSAIDs in a fixed formulation, wherein the proton pump inhibitor is protected by an enteric coating layer. The fixed formulation is in the form of an enteric coating layered tablet, a capsule or a multiple unit tableted dosage form. The multiple unit dosage forms are most preferred. The new fixed formulation is especially useful in the treatment of gastrointestinal side-effects associated with NSAID treatment.



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ORAL PHARMACEUTICAL DOSAGE FORMS COMPRISING A PROTON PUMP INHIBITOR AND A NSAID

Field of the invention

The present invention is related to new oral pharmaceutical preparations especially for use in the treatment and prophylaxis of gastrointestinal disorders associated with the use of Non Steroidal Antiinflammatory Drugs (NSAIDs). The present preparations comprise an acid susceptible proton pump inhibitor in combination with one or more NSAID(s) in a new fixed unit dosage form, especially a tableted dosage form . Furthermore, the present invention refers to a method for the manufacture of such preparations and the use of such preparations in medicine.

BACKGROUND OF THE INVENTION

NSAIDs including acetyl salicylic acid are among the most commonly prescribed and used drugs world-wide. Despite the therapeutic benefits of NSAIDs, their use is frequently limited by an increased risk of gastrointestinal side-effects, mainly upper gastrointestinal side-effects like peptic ulceration and dyspeptic symptoms.

The relative risk of developing a gastric ulcer during NSAID treatment is increased by a factor 40-50, and the relative risk of developing a duodenal ulcer is increased by a factor 8-10 (McCarty DM. Gastroenterology 1989;96:662). The relative risk of developing an ulcer complication like bleeding and perforation of the stomach is increased by a factor 1.5-5 (Hawkey C. BMJ 1990;300:278). Further, dyspeptic symptoms are experienced in 30-60% of those on NSAID treatment (Larkai EN.AmJGas 1987;82:1153).

In the UK, NSAIDs account for 25% of all reports of adverse drug reactions received by the authorities, and the corresponding figure is 21% in USA. Therefore, therapies which avoid gastrointestinal side-effect caused by NSAIDs is requested.

Attempts to modify the NSAID structure in order to prevent such side-effects have so far been less successful. The most promising solution to the problem of healing and preventing NSAID associated upper gastrointestinal problems like ulcers and dyspeptic symptoms in patients with a need for continuous NSAID treatment is to combine the NSAID treatment with an anti-ulcer drug approved for the healing and/or prophylaxis of NSAID associated gastrointestinal side-effects such as prostaglandin analogues, H_2-receptor antagonists or proton pump inhibitors.

Established risk factors for developing NSAID associated upper gastrointestinal side-effects and complications are for instance high age, previous peptic ulcer and/or bleeding, high dose of NSAID, co-therapy with steroids, and co-therapy with anticoagulants. This means, that for example fragile and elderly patients tolerating a complication like bleeding or perforation badly, should receive prophylactic treatment in connection with their NSAID treatment.

NSAIDs are mainly used for the treatment of chronic diseases like rheumatoid arthtritis and osteoarthritis, which are most often seen in the elderly population. Compliance is especially important in elderly and fragile patients, who have the highest risk of developing a life-threatening complication to NSAID treatment like bleeding or perforation. It is known that 50% of all peptic ulcer deaths occur in NSAID users and that 68% of these are >75 years old (Catford:Health Trends 1986;18:38). This is confirmed in another study concluding, that NSAID-related deaths occur primarily in those > 75 years of age (Guess. J Clin Epidemiol 1988;41:35). The importance of compliance is further supported by the finding, that a majority of peptic ulcers associated with NSAID treatment are asymptomatic until the event.

Omeprazole being a well known proton pump inhibitor has been shown to be able to prevent gastric and duodenal erosions in healthy volunteers during treatment with acetyl salicylic acid. Clinical studies have shown, that omeprazole heals gastric as well as duodenal

ulcers as fast and effectively in patients on continuous NSAID treatment as in non-NSAID users (Walan A. N Engl J Med 1989;320:69). These results have been the basis for an amendment to the dose recommendation for the use of omeprazole in healing of gastric and duodenal ulcers during continuous NSAID treatment approved by regulatory authorities in UK and Sweden.

Recent studies confirm, that omeprazole significantly reduces the risk of developing gastric ulcers, duodenal ulcers and also dyspeptic symptoms in patients on continuous NSAID treatment.

EP 0 426 479 describes tablet compositions comprising a NSAID such as ibuprofen and a gastric acid inhibiting drug, such as cimetidin etc. No specific arrangement is taken to avoid degradation if the gastric acid inhibitor is an acid susceptible compound, such as a proton pump inhibitor.

In proposed therapies comprising NSAID(s) and an acid susceptible proton pump inhibitor the different active substances are administred separately. It is well known that patient compliance is a main factor in receiving a good result in medical treatments. Therefore, administration of two or even more different tablets to the patient is not convenient or satisfactory to achieve the most optimal results. The present invention now provides new oral dosage forms comprising two or more different active substances combined in one fixed unit dosage form, preferably a tablet.

Some anti-ulcer drugs such as proton pump inhibitors are susceptible to degradation/transformation in acid reacting and neutral media as mentioned above. In respect of the stability properties, it is obvious that the one of the active substances being a proton pump inhibitor must be protected from contact with acidic gastric juice by an enteric coating layer. There are different enteric coating layered preparations of proton pump inhibitors described in the prior art, see for example US-A 4,786,505 (AB Hässle) comprising omeprazole.

There are problems to produce a fixed unit dosage form comprising a rather high amount of active substance. Active substances with different physical properties combined in the same preparation give further problems. Preparation of a multiple unit tableted dosage form arises specific problems when enteric coating layered pellets containing the acid susceptible proton pump inhibitor are compressed into tablets. If the enteric coating layer does not withstand the compression of the pellets into a tablet, the susceptible active substance will be destroyed upon administration by penetrating acidic gastric juice, i.e. the acid resistance of the enteric coating layer of the pellets will not be sufficient in the tablet after compression.

Summary of the invention

The present invention provides oral, fixed unit dosage forms, i.e. multiple unit tableted dosage forms, enteric coating layered tablets, multilayered tablets or capsules filled with more than one pharmaceutically active compound. The active compounds are preferably an acid susceptible proton pump inhibitor in combination with one or more NSAIDs and wherein at least the proton pump inhibitor is protected by an enteric coated layer. These new dosage forms will simplify the regimen and improve the patient compliance.

Description of the Figures

Fig. 1 illustrates a cross-section of a multiple unit tableted dosage form comprising an acid susceptible proton pump inhibitor in the form of enteric coating layered pellets (1) in admixture with a fast disintegrating granulate comprising a NSAID (2). The tablet is covered by an filmcoating layer (13).

Fig. 2 illustrates a cross-section of a multiple unit tableted dosage form comprising an acid susceptible proton pump inhibitor in the form of enteric coating layered pellets (1) and a NSAID in the form of cyclodextrin complex (3) included in a fast disintegrating granulate (4). The tablet is covered by a filmcoating layer (13).

Fig. 3 illustrates a cross-section of a tablet with two separate layers, one layer comprises an acid susceptible proton pump inhibitor in the form of enteric coating layered pellets (1) in admixture with excipients (5) and the other layer comprises a NSAID (6) included in a gelling matrix giving extended release. The separate layers are optionally separated by a separating layer (12) and the tablet is covered by a filmcoating layer (13).

Fig. 4 illustrates a cross-section of a multiple unit tableted dosage form comprising an acid susceptible proton pump inhibitor in the form of enteric coating layered pellets (1) and a NSAID in the form of enteric coating layered pellets (7) in admixture with excipients (5). The tablet is covered by a filmcoating layer (13).

Fig. 5 illustrates a cross-section of an enteric coating layered tablet comprising an acid susceptible proton pump inhibitor (8) in admixture with one or more NSAID(s) (9) and excipients (5). The tablet is covered by an enteric coating layer (11) and optionally a separating layer (10) is layered in between the tablet core and the enteric coating layer.

Fig. 6 illustrates a tablet comprising an acid susceptible proton pump inhibitor in the form of enteric coating layered pellets (1) in admixture with a fast disintegrating granulate (4) in a tablet core, surrounded by a coating layer comprising a NSAID substance/granulation (2). The tablet is covered by a pigmented filmcoating layer (13).

Detailed description of the invention

One object of the invention is to provide an oral, multiple unit tableted dosage form comprising an anti-ulcer drug, preferably an acid susceptible proton pump inhibitor in the form of individually enteric coating layered units, together with one or more NSAIDs and tablet excipients compressed into a tablet. The enteric coating layer(s) covering the individual units of the acid susceptible proton pump inhibitor has properties such that the compression of the units into a tablet does not significantly affect the acid resistance of the

individually enteric coating layered units. Furthermore, the multiple unit tableted dosage form provides a good stability to the active substances during long-term storage.

Alternatively, the prepared tablet has separate layers, one layer that comprises the acid susceptible proton pump inhibitor in the form of compressed enteric coated layered units and another layer that comprises the NSAID(s).

The new fixed dosage form is preferably in the form of a multiple unit tableted dosage form comprising enteric coating layered units of the acid susceptible substance and the other active substance(s) in the granulated material constituting the rest of the compressed tablet, as shown in Fig. 1.

Alternatively, the different active substances may be intimately mixed with each other and compressed into a conventional tablet, which is enteric coating layered, see Fig. 5, or both active substances are in the form of enteric coating layered pellets compressed into a multiple unit tableted formulation together with preferably fast disintegrating granules of inactive excipients, as exemplified in Fig. 4.

Further alternatives are exemplified as multiple unit dosage forms wherein the proton pump inhibitor is in the form of individually enteric coating layered units and the NSAID(s) in the form of a) a complex to obtain improved bioavailability, see Fig. 2, or b) in the form of a gelling matrix resulting in a preparation with extended release of the NSAID(s), see Fig. 3. A further alternative is a multiple dosage form with the proton pump inhibitor in the form of individually enteric coating layered units compressed into a tablet and thereupon a separate layer of the NSAID(s) is applied by spray layering on the tablet. The tablet is covered by a pigmented filmcoating layer to protect the NSAID(s), see Fig. 6, because some NSAID(s) are light sensitive and require a light protecting layer.

In still another alternative, the different active substances are dry mixed and filled into a capsule. In the latter preparation the acid susceptible proton pump inhibitor is in the form of

enteric coating layered units and the NSAID(s) is/are in the form of granules or alternatively in the form of modified release formulated units such as enteric coating layered units or units layered with a controlled release layer.

The NSAID(s) may be formulated in instant release, sustained release or extended release formulations. Alternatively, the components may be formulated in an effervescent formulation. Furthermore, as some NSAID(s) are light sensitive the formulation is preferably light protected by a pigmented tablet filmcoating layer, as exemplified in Fig. 6, or by including a pigment in one of the coating layers to be applied on the tableted dosage form.

A further object of the invention is to provide a dosage form which is divisible, such as divisible tablets.

Still a further object of the invention is to provide a multiple unit tableted dosage form, which is divisible and easy to handle. Some of the multiple unit tableted dosage forms may be dispersed in a slightly acidic aqueous liquid and can be given to patients with swallowing disorders and in pediatrics. Such a suspension of dispersed units/pellets of appropriate size can be used for oral administration and also for feeding through a naso-gastric tube.

The different active components used in the present dosage forms are defined below.

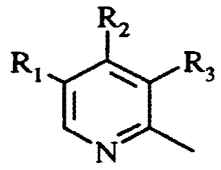
Active substances

The anti-ulcer drug is preferably an acid susceptible proton pump inhibitor. Such proton pump inhibitors are for example compounds of the general formula I

I

wherein

Het_1 is



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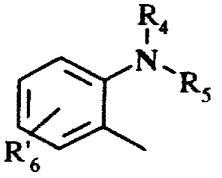

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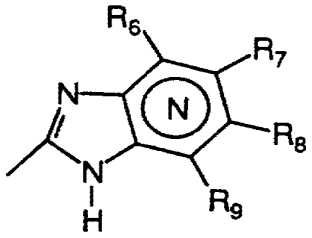

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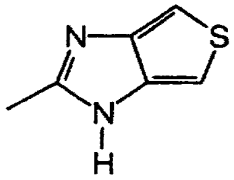

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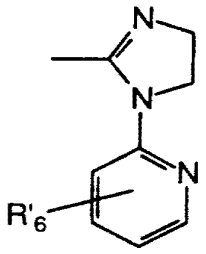

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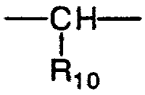

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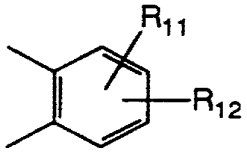


wherein

N in the benzimidazole moiety means that one of the carbon atoms substituted by R_6-R_9 optionally may be exchanged for a nitrogen atom without any substituents;

R_1, R_2 and R_3 are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenyl and phenylalkoxy;

R_4 and R_5 are the same or different and selected from hydrogen, alkyl and aralkyl;

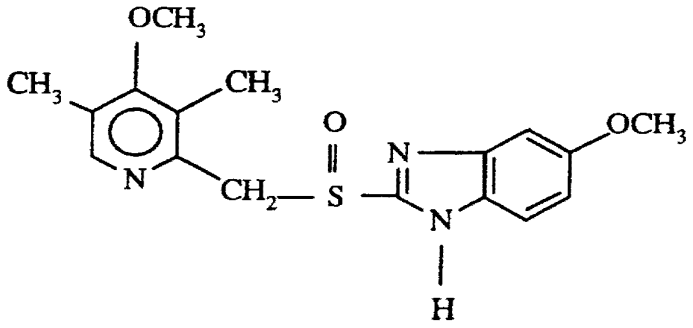
R_6' is hydrogen, halogen, trifluoromethyl, alkyl and alkoxy;

R_6-R_9 are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxycarbonyl, oxazolyl, trifluoroalkyl, or adjacent groups R_6-R_9 form ring structures which may be further substituted;

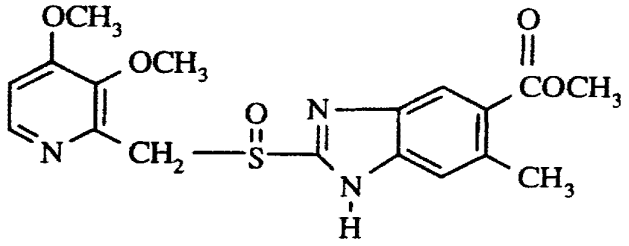
R_{10} is hydrogen or forms an alkylene chain together with R_3 and

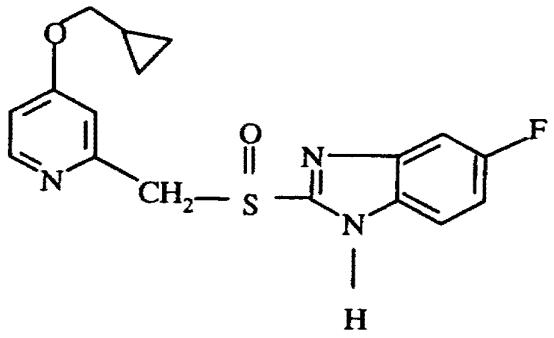
R_{11} and R_{12} are the same or different and selected from hydrogen, halogen or alkyl, alkyl groups, alkoxy groups and moities thereof , they may be branched or straight C_1 - C_9 - chains or comprise cyclic alkyl groups, such as cycloalkyl-alkyl.

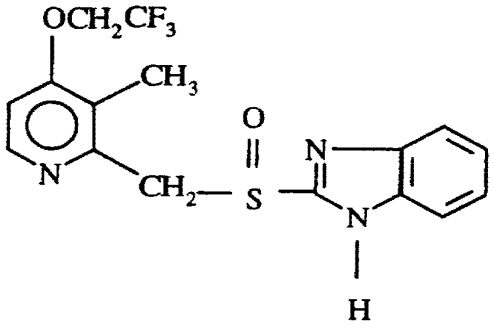
Examples of proton pump inhibitors according to formula I are



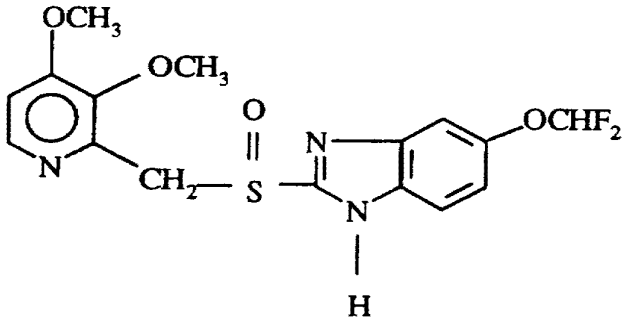
Omeprazole



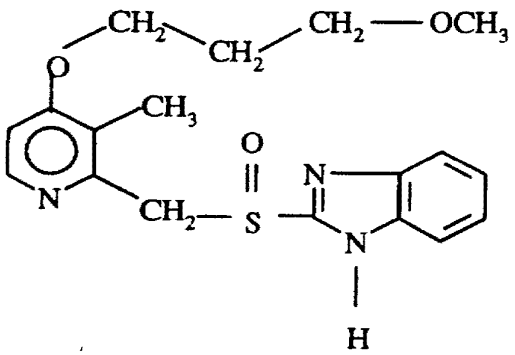




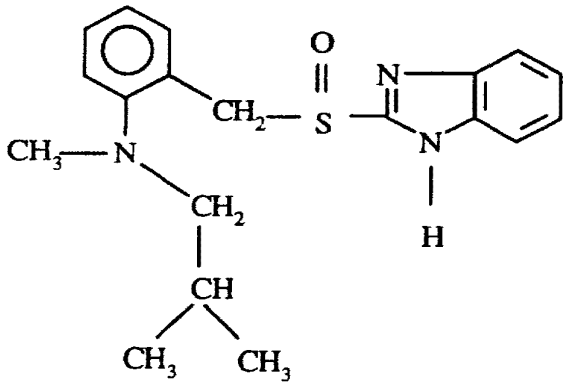
Lansoprazole



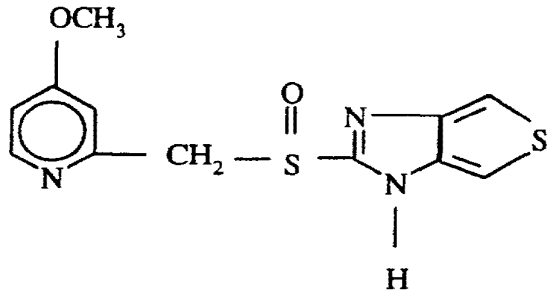
Pantoprazole

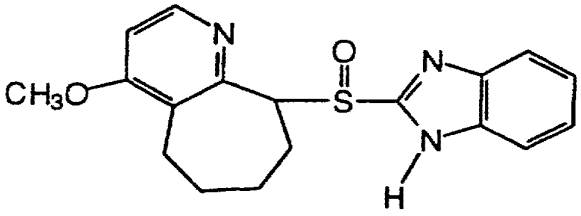


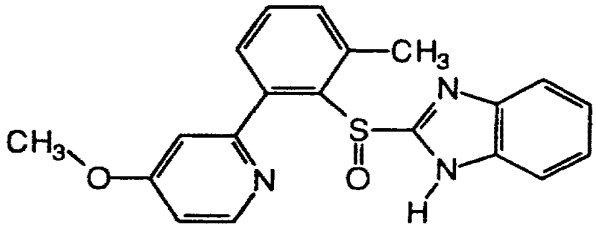
Pariprazole

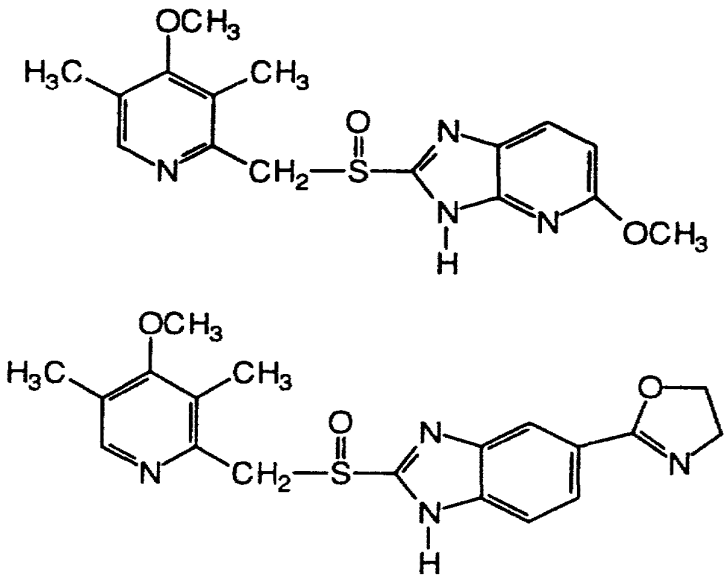


Leminoprazole









The acid susceptible proton pump inhibitors used in the dosage forms of the invention may be used in their neutral form or in the form of an alkaline salt, such as for instance the Mg^{2+},Ca^{2+},Na^{+} , K^{+} or Li^{+}salts, preferably the Mg^{2+} salts. Further where applicable, the compounds listed above may be used in racemic form or in the form of the substantially pure enantiomer thereof, or alkaline salts of the single enantiomers.

Suitable proton pump inhibitors are for example disclosed in EP-A1-0005129, EP-A1-174 726, EP-A1-166 287, GB 2 163 747 and WO90/06925, WO91/19711, WO91/19712, and further especially suitable compounds are described in WO95/01977 and WO94/27988.

A wide variety of NSAIDs may be used in combination with a suitable proton pump inhibitor and optional pharmaceutically acceptable excipients in the fixed unit dosage form according to the present invention. Such NSAIDs include for example propionic acid derivatives, oxicams, acetic acid and acetamide derivatives, salicylic acid derivatives and pyrazolidine derivatives.

Also future NSAIDs like cyclooxygenase (COX) 2 selective NSAIDs and NO-releasing NSAIDs (de Soldato P, NO-releasing NSAID:s, A new class of safer anti-inflammatory analgesic and anti-pyrretic agents; The IV International meeting on side-effects of anti-inflammatory drugs August 7 - 9, 1995) may be included.

In the following examples of some suitable NSAIDs are listed: Acetyl salicylic acid, indometacin, diclofenac, piroxicam, tenoxicam, ibuprofen, naproxen, ketoprofen, nabumetone, ketorolac, azapropazone, mefenamic acid, tolfenamic acid, sulindac, diflunisal, tiaprofenic acid, podophyllotoxin derivatives, acemetacin, aceclofenac, droxicam, oxaprozin, floctafenine, phenylbutazone, proglumetacin, flurbiprofen, tolmetin and fenbufen.

The active NSAIDs could be in standard forms or used as salts, hydrates, esters etc. A combination of two or more of the above listed drugs may be used. Preferable NSAIDs for the new fixed dosage form are diclofenac, ibuprofen, naproxen and piroxicam.

The preferred multiple unit tableted dosage form comprising a proton pump inhibitor (in the form of a racemat, an alkaline salt or one of its single enantiomers) and one or more NSAIDs, is characterized in the following way. Individually enteric coating layered units (small beads, granules or pellets) containing the proton pump inhibitor and optionally containing alkaline reacting substances, are mixed with the NSAID(s) and conventional tablet excipients. Preferably, the NSAID(s) and tablet excipients are in the form of a granulation. The dry mixture of enteric coating layered units, NSAID granules and optional excipients are compressed into multiple unit tableted dosage forms. With the expression "individual units" is meant small beads, granules or pellets, in the following referred to as pellets of the acid susceptible proton pump inhibitor.

The compaction process (compression) for formulating the multiple unit tableted dosage form must not significantly affect the acid resistance of the enteric coating layered pellets comprising the acid susceptible proton pump inhibitor. In other words the mechanical properties, such as the flexibility and hardness as well as the thickness of the enteric coating

layer(s), must secure that the requirements on enteric coated articles in the United States Pharmacopeia are accomplished in that the acid resistance does not decrease more than 10% during the compression of the pellets into tablets.

The acid resistance is defined as the amount of proton pump inhibitor in the tablets or pellets after being exposed to simulated gastric fluid USP, or to 0,1 M HCl (aq) relative to that of unexposed tablets and pellets, respectively. The test is accomplished in the following way. Individual tablets or pellets are exposed to simulated gastric fluid of a temperature of 37°C. The tablets disintegrate rapidly and release the enteric coating layered pellets to the medium. After two hours the enteric coating layered pellets are removed and analyzed for content of the proton pump inhibitor using High Performance Liquid Chromatography (HPLC).

Further specific components which may be used in the fixed unit dosage forms of the present invention are defined below.

Core material - for enteric coating layered pellets/units

The core material for the individually enteric coating layered pellets can be constituted according to different principles. Seeds layered with the proton pump inhibitor, optionally mixed with alkaline substances, can be used as the core material for the further processing.

The seeds which are to be layered with the proton pump inhibitor can be water insoluble seeds comprising different oxides, celluloses, organic polymers and other materials, alone or in mixtures or water-soluble seeds comprising different inorganic salts, sugars, non-pareils and other materials, alone or in mixtures. Further, the seeds may comprise the proton pump inhibitor in the form of crystals, agglomerates, compacts etc. The size of the seeds is not essential for the present invention but may vary between approximately 0.1 and 2 mm. The seeds layered with the proton pump inhibitor are produced either by powder or

solution/suspension layering using for instance granulation or spray coating layering equipment.

Before the seeds are layered, the proton pump inhibitor may be mixed with further components. Such components can be binders, surfactants fillers, disintegrating agents, alkaline additives or other and/or pharmaceutically acceptable ingredients alone or in mixtures. The binders are for example polymers such as hydroxypropyl methylcellulose (HPMC), hydroxypropyl-cellulose (HPC), carboxymethylcellulose sodium, polyvinyl pyrrolidone (PVP), or sugars, starches or other pharmaceutically acceptable substances with cohesive properties. Suitable surfactants are found in the groups of pharmaceutically acceptable non-ionic or ionic surfactants such as for instance sodium lauryl sulfate.

Alternatively, the proton pump inhibitor optionally mixed with alkaline substances and further mixed with suitable constituents can be formulated into a core material. Said core material may be produced by extrusion/spheronization, balling or compression utilizing conventional process equipment. The size of the formulated core material is approximately between 0.1 and 4 mm and preferably between 0.1 and 2 mm. The manufactured core material can further be layered with additional ingredients comprising the proton pump inhibitor and/or be used for further processing.

The proton pump inhibitor is mixed with pharmaceutical constituents to obtain preferred handling and processing properties and a suitable concentration of the proton pump inhibitor in the final preparation. Pharmaceutical constituents such as fillers, binders, lubricants, disintegrating agents, surfactants and other pharmaceutically acceptable additives may be used.

Further, the proton pump inhibitor may also be mixed with an alkaline, pharmaceutically acceptable substance (or substances). Such substances can be chosen among, but are not restricted to substances such as the sodium, potassium, calcium, magnesium and aluminium salts of phosphoric acid, carbonic acid, citric acid or other suitable weak inorganic or

organic acids; aluminium hydroxide/sodium bicarbonate coprecipitate; substances normally used in antacid preparations such as aluminium, calcium and magnesium hydroxides; magnesium oxide or composite substances, such as $Al_2O_3.6MgO.CO_2.12H_2O$, ($Mg_6Al_2(OH)_{16}CO_3.4H_2O$), $MgO.Al_2O_3.2SiO_2.nH_2O$ or similar compounds; organic pH-buffering substances such as trihydroxymethylaminomethane, basic amino acids and their salts or other similar, pharmaceutically acceptable pH-buffering substances.

Alternatively, the aforementioned core material can be prepared by using spray drying or spray congealing technique.

Enteric coating layer(s)

Before applying the enteric coating layer(s) onto the core material in the form of individual pellets, the pellets may optionally be covered with one or more separating layer(s) comprising pharmaceutical excipients optionally including alkaline compounds such as pH-buffering compounds. This/these separating layer(s), separate(s) the core material from the outer layers being enteric coating layer(s). This/these separating layer(s) protecting the core material of proton pump inhibitor should be water soluble or rapidly disintegrating in water.

The separating layer(s) can be applied to the core material by coating or layering procedures in suitable equipments such as coating pan, coating granulator or in a fluidized bed apparatus using water and/or organic solvents for the coating process. As an alternative the separating layer(s) can be applied to the core material by using powder coating technique. The materials for the separating layers are pharmaceutically acceptable compounds such as, for instance, sugar, polyethylene glycol, polyvinylpyrrolidone, polyvinyl alcohol, polyvinyl acetate, hydroxypropyl cellulose, methylcellulose, ethylcellulose, hydroxypropyl methyl cellulose, carboxymethylcellulose sodium, water soluble salts of enteric coating polymers and others, used alone or in mixtures. Additives such as plasticizers, colorants, pigments, fillers anti-tacking and anti-static agents, such as for instance magnesium stearate, titanium dioxide, talc and other additives may also be included into the separating layer(s).

When the optional separating layer, is applied to the core material it may constitute a variable thickness. The maximum thickness of the separating layer(s) is normally only limited by processing conditions. The separating layer may serve as a diffusion barrier and may act as a pH-buffering zone. The pH-buffering properties of the separating layer(s) can be further strengthened by introducing into the layer(s) substances chosen from a group of compounds usually used in antacid formulations such as, for instance, magnesium oxide, hydroxide or carbonate, aluminium or calcium hydroxide, carbonate or silicate; composite aluminium/magnesium compounds such as, for instance $Al_2O_3.6MgO.CO_2.12H_2O$, ($Mg_6Al_2(OH)_{16}CO_3.4H_2O$), $MgO.Al_2O_3.2SiO_2.nH_2O$, aluminium hydroxide/sodium bicarbonate coprecipitate or similar compounds; or other pharmaceutically acceptable pH-buffering compounds such as, for instance the sodium, potassium, calcium, magnesium and aluminium salts of phosphoric, carbonic, citric or other suitable, weak, inorganic or organic acids; or suitable organic bases, including basic amino acids and salts thereof. Talc or other compounds may be added to increase the thickness of the layer(s) and thereby strenghten the diffusion barrier. The optionally applied separating layer(s) is not essential for the invention. However, the separating layer(s) may improve the chemical stability of the active substance and/or the physical properties of the novel multiple unit tableted dosage form.

Alternatively, the separating layer may be formed in situ by a reaction between an enteric coating polymer layer applied on the core material and an alkaline reacting compound in the core material. Thus, the separating layer formed comprises a water soluble salt formed between the enteric coating layer polymer(s) and an alkaline reacting compound which is in the position to form a salt.

One or more enteric coating layers are applied onto the core material or onto the core material covered with separating layer(s) by using a suitable coating technique. The enteric coating layer material may be dispersed or dissolved in either water or in suitable organic solvents.As enteric coating layer polymers one or more, separately or in combination, of the following can be used, e.g. solutions or dispersions of methacrylic acid copolymers,

cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, cellulose acetate trimellitate, carboxymethylethylcellulose, shellac or other suitable enteric coating polymer(s).

The enteric coating layers contain pharmaceutically acceptable plasticizers to obtain the desired mechanical properties, such as flexibility and hardness of the enteric coating layers. Such plasticizers are for instance, but not restricted to triacetin, citric acid esters, phthalic acid esters, dibutyl sebacate, cetyl alcohol, polyethylene glycols, polysorbates or other plasticizers.

The amount of plasticizer is optimized for each enteric coating layer formula, in relation to selected enteric coating layer polymer(s), selected plasticizer(s) and the applied amount of said polymer(s), in such a way that the mechanical properties, i.e. flexibility and hardness of the enteric coating layer(s), for instance exemplified as Vickers hardness, are adjusted so that the acid resistance of the pellets covered with enteric coating layer(s) does not decrease significantly during compression of pellets into tablets. The amount of plasticizer is usually above 10 % by weight of the enteric coating layer polymer(s), preferably 15 - 50 % and more preferably 20 - 50 %. Additives such as dispersants, colorants, pigments polymers e.g. poly (ethylacrylat, methylmethacrylat), anti-tacking and anti-foaming agents may also be included into the enteric coating layer(s). Other compounds may be added to increase film thickness and to decrease diffusion of acidic gastric juices into the acid susceptible material. To protect the acid susceptible substance, the proton pump inhibitor, and to obtain an acceptable acid resistance of the dosage form according to the invention, the enteric coating layer(s) constitutes a thickness of approximately at least 10 µm, preferably more than 20 µm. The maximum thickness of the applied enteric coating is normally only limited by processing conditions and the desired dissolution profile.

The enteric coating layer may also be used for layering of the NSAID(s). Alternatively, the enteric coating layer described above may also be used for an enteric coating layer of conventional tablets comprising a composition of a proton pump inhibitor and one or more

NSAIDs, optionally the prepared tablet core also is covered by one of the separating layers described above to separate the tablet core from the enteric coating layer.

Over-coating layer

Pellets covered with enteric coating layer(s) may further be covered with one or more over-coating layer(s). The over-coating layer(s) should be water soluble or rapidly disintegrating in water. The over-coating layer(s) can be applied to the enteric coating layered pellets by coating or layering procedures in suitable equipments such as coating pan, coating granulator or in a fluidized bed apparatus using water and/or organic solvents for the coating or layering process. The materials for over-coating layers are chosen among pharmaceutically acceptable compounds such as, for instance sugar, polyethylene glycol, polyvinylpyrrolidone, polyvinyl alcohol, polyvinyl acetate, hydroxypropyl cellulose, methylcellulose, ethylcellulose, hydroxypropyl methyl cellulose, carboxymethylcellulose sodium and others, used alone or in mixtures. Additives such as plasticizers, colorants, pigments, fillers, anti-tacking and anti-static agents, such for instance magnesium stearate, titanium dioxide, talc and other additives may also be included into the over-coating layer(s). Said over-coating layer may further prevent potential agglomeration of enteric coating layered pellets, further it may protect the enteric coating layer towards cracking during the compaction process and enhance the tableting process. The maximum thickness of the applied over-coating layer(s) is normally limited by processing conditions and the desired dissolution profile. The over-coating layer may also be used as a tablet filmcoating layer.

NSAID preparation

The active substance(s) in the form of one or more NSAID substances is dry mixed with inactive excipients, wherein one or more of the excipients optionally is a disintegrant. The mixture is wet massed with a granulation liquid. The wet mass is dried preferably to a loss on drying of less than 3% by weight. Thereafter the dry mass is milled to a suitable size for

the granules, such as smaller than 4 mm, and preferably smaller than 1 mm. Suitable inactive excipients for the NSAID granulation are for instance, sodium starch glycolate, corn starch, crosslinked polyvinylpyrrolidone, low substituted hydroxypropyl cellulose, microcrystalline cellulose, mannitol and colloidal silicon dioxide anhydrous (Aerosil®) and the like. The dry mixture comprising NSAID(s) is mixed with a suitable granulation liquid comprising for instance, polyvinyl pyrrolidone, hydroxypropyl cellulose, polyethylene glycol, hydroxypropyl cellulose and optionally wetting agents, such as sodium lauryl sulphate, dissolved in purified water or a suitable alcohol or a mixture thereof.

Mechanical treatment may in some cases be used to form a complex between the NSAID(s) and a complex forming agent, such as beta-hydroxypropyl cyclodextrin like in Example 3 below. Cyclodextrin complexes of NSAID(s) are shown to have an increased bioavailability of the NSAID(s), see for instance Drug Dev. Ind. Pharm. 19(7), 843-852,(1993).

Further, the NSAID may be mixed with a gelling agent during the granulation, such as hydrophilic polymer(s). Suitable gelling hydrophilic polymers are for instance hydroxypropylmethylcellulose, polyoxyethylen (polyethylene glycol), hydroxypropylcellulose, hydroxyethylcellulose and xantan. The granules may also comprise buffering substances. See for instance Example 4 below. Some NSAIDs irritate the gastric mucosa and benefit from a protecting enteric coating layer and may be formulated as enteric coating layered pellets.

Multiple unit tablets

The enteric coating layered pellets comprising a proton pump inhibitor are mixed with the granules comprising NSAID(s) and tablet excipients. The mixture is compressed into a multiple unit tableted dosage form. The compressed tablet is optionally covered with a filmforming agent(s) to obtain a smooth surface of the tablet and further enhance the stability of the tablet during packaging and transport. Such a tablet filmcoating layer may further comprise additives such as anti-tacking agents, colorants and pigments or other

additives to obtain a tablet of good appearance and with a light-protection for light sensitive components.

The enteric coated pellets with or without an over-coat and the NSAID granules are mixed with tablet excipients such as fillers, binders, disintegrants, lubricants and other pharmaceutically acceptable additives and compressed into tablets. Suitable lubricants for the tableting process are for instance sodium stearyl fumarate, magnesium stearate and talc.

Alternatively, the NSAID(s) may be dry mixed with the enteric coating layered pellets comprising the proton pump inhibitor optionally together with inactive excipients and compressed into tablets (direct compression), or the different active substances may be formulated in different layers, optionally the NSAID(s) in the form of a layer with a controlled release.

Further, both the NSAID(s) and the proton pump inhibitor in the form of enteric coating layered pellets may be mixed with inactive tablet excipients and compressed into a tablet. The compressed tablet is optionally covered by a tablet filmcoating layer to obtain a tablet of good appearance.

As a further alternative a multiple unit tableted dosage form comprising the proton pump inhibitor is spray coating layered by a suspension or solution comprising the NSAID(s). The prepared tablet is thereafter covered by a pigmented tablet filmcoating layer.

The fraction of enteric coating layered pellets constitutes less than 75 % by weight of the total tablet weight and preferably less than 60 %. By increasing the amount of the granules comprising the NSAID(s) the fraction of enteric coating layered proton pump inhibitor pellets in the multiple unit dosage form may be reduced. By choosing small enteric coating layered pellets in the formulation according to the present invention, the number of pellets in each tablet can be held high which in turn makes the tablet divisible with retained dosing accuracy.

Thus, the preferred multiple unit tablet formulation consists of enteric coating layered pellets containing one active substance in the form of an acid susceptible proton pump inhibitor, optionally mixed with alkaline reacting compound(s), compressed into tablet together with granules containing NSAID(s) and optionally tablet excipients. The addition of an alkaline reacting material to the proton pump inhibitor is not necessary, in any sense but such a substance may further enhance the stability of the proton pump inhibitor or some of the alkaline reacting compounds may react in situ with the enteric coating material to form a separating layer. The enteric coating layer(s) is making the pellets of the dosage form insoluble in acidic media, but disintegrating/dissolving in near neutral to alkaline media such as, for instance the liquids present in the proximal part of the small intestine, where dissolution of the proton pump inhibitor is desired. The NSAID(s) may be released in the stomach. The enteric coating layered pellets may further be covered with an overcoating layer before being formulated into the tablet and they may also contain one or more separating layer(s) in between the core material and the enteric coating layer.

Process

The process for the manufacture of the dosage form represents a further aspect of the invention. After formulation of the pellets by spray coating or layering of the proton pump inhibitor onto seeds, or by extrusion/spheronization or granulation, e.g. rotor granulation of homogeneous pellets, the pellets are first optionally covered with the separating layer(s) and then with the enteric coating layer(s) or a separating layer is spontaneously developed in situ between an alkaline core material and the enteric coating layer material. The coating is carried out as described above and in the accompanying examples. The preparation of the granules comprising the NSAID(s) and enteric coating layered NSAID pellets are also described above and in the examples. The pharmaceutical processes can preferably be completely water-based.

The enteric coating layered pellets, with or without an over-coat, are mixed with the prepared granules, tablet excipients and other pharmaceutical acceptable additives and compressed into tablets. Alternatively, the different active substances in the form of powders may be intimately dry mixed with tablet excipients, wet massed and compressed into conventional tablets before applying an optional separating layer and an enteric coating layer. The NSAID(s) may also be incorporated in a coating layer applied onto a multiple unit dosage form comprising the proton pump inhibitor, or the NSAID(s) and proton pump inhibitor in the form of enteric coating layered pellets are mixed with inactive tablet excipients and compressed into a multiple unit tableted dosage form.

The different active substances may also be formulated into different layers, wherein the layer comprising the NSAID(s) may be in the form of a control release preparation. As a further alternative, the acid susceptible proton pump inhibitor in the form of enteric coating layered pellets may be filled in a capsule together with the NSAID(s) in the form of granules or enteric coating layered pellets, and optionally mixed with pharmaceutical excipients.

Use of the preparation

The dosage forms according to the invention are especially advantageous in the treatment of gastrointestinal side-effects caused by NSAID(s), such as in a continuous treatment with NSAID(s). The new dosage forms are administered one to several times a day, preferably once or twice daily. The typical daily dose of the active substances varies and will depend on various factors such as the individual requirements of the patients, the mode of administration and disease. In general each dosage form will comprise 0,1-200 mg of the proton pump inhibitor and 0,1 - 1 000 mg of the NSAID(s). Preferably, each dosage form will comprise 10-80 mg of the proton pump inhibitor and 10-800 mg of the NSAID(s), and more preferably 10-40 mg of proton pump inhibitor and 10-500 mg of the NSAID(s), respectively. Especially preferred combinations comprise for instance 10 mg omeprazole together with 50 mg diclofenac, 10 mg omeprazole and 250 mg naproxen, 10 mg omeprazole and 10 mg piroxicam, or 10 mg omeprazole and 400 mg ibuprofen.

The multiple unit tablet preparation may also be suitable for dispersion in an aqueous liquid with slightly acidic pH-value before being orally administered or fed through a naso-gastric tube.

The invention is illustrated more in detail in the following examples.

Examples

Example 1:

Fast disintegrating multiple unit tableted dosage form comprising magnesium omeprazole and ibuprofen.

Core material	
Magnesium omeprazole	12.00 kg
Non-pareil cores	12.00 kg
Hydroxypropyl methylcellulose	1.8 kg
Water purified	35.4 kg
Separating layer	
Core material (acc. to above)	23.50 kg
Hydroxypropyl cellulose	2.35 kg
Talc	4.03 kg
Magnesium Stearate	0.34 kg
Water purified	48.00 kg
Enteric coating layer	
Pellets with sep layer (acc. to above)	29.00 kg
Methacrylic acid copolymer (30% suspension)	38.70 kg

Triethyl citrate	3.48 kg
Mono- and diglycerides (NF)	0.58 kg
Polysorbate 80	0.06 kg
Water purified	22.68 kg

Over-coating layer

Enteric coating layered pellets (acc. to above)	44.7 kg
Hydroxypropyl methylcellulose	0.58 kg
Mg-Stearate	0.017 kg
Water purified	11.6 kg

Tablets	mg/tablet
Over-coated pellets comprising omeprazole	47.85
Ibuprofen	400
Microcrystalline cellulose (MCC)	273.6
Polyvinylpyrrolidone cross-linked	100.4
Polyvinylpyrrolidone K-25	33.3
Sodium laurylsulphate	26.7
Water purified	297
Sodium stearyl fumarate	4.0

Suspension layering was performed in a fluid bed apparatus. Magnesium omeprazole was sprayed onto inert non-pareil cores from a water suspension containing the dissolved binder.

The prepared core material was coating layered with a separating layer in a fluid bed apparatus with a hydroxypropyl cellulose solution containing talc and magnesium stearate. The enteric coating layer consisting of methacrylic acid copolymer, mono- and diglycerides, triethylcitrate and polysorbate was sprayed onto the pellets (layered with a separating layer) in a fluid bed apparatus. In the same type of apparatus the enteric coating layered pellets

were coated with hydroxypropyl methylcellulose/Mg-Stearate suspension. The obtained pellets were classified by sieving.

Tablet granulation liquid was made by dissolving 26.7 parts of sodium laurylsulphate and 33.3 parts of polyvinylpyrrolidone K-25 in 267 parts of purified water. 400 parts of ibuprofen, 226 parts of the MCC and 10.4 parts of the cross-linked polyvinylpyrrolidone were dry-mixed. The granulating liquid was added to the powder mixture and the mass wet-mixed. 30 parts of water was added as quantum satis.

The wet mass was dried in an oven at 60°C for approx. 6 hrs. The dried granules were milled to pass a 0.8 mm sieve.

The enteric coating layered omeprazole pellets, the milled ibuprofen granules, 47.6 parts of MCC, 4.0 parts sodium stearylfumarate and 90 parts of crosslinked polyvinylpyrrolidone were mixed and compressed to tablets on a tableting machine equipped with 15 mm diameter punches. Hardness of the 886 mg tablets tested with a Schleuniger apparatus varied between 5.3 and 5.9 kP. Disintegration time tested in simulated gastric juice (USP, without enzymes) was 49-52 sec (n=2).

Example 2

Fast disintegrating multiple unit tableted dosage form comprising S-omeprazole magnesium salt and naproxen.

Core material

S-omeprazole magnesium	120 g
Non-pareil cores	150 g
Polysorbat 80	2.4 g
Hydroxypropyl methylcellulose	18 g

Water purified	562 g
Separating layer	
Core material (acc. to above)	200 g
Hydroxypropyl cellulose	30 g
Talc	51.4 g
Magnesium Stearate	4.3 g
Water purified	600 g
Enteric coating layer	
Pellets with sep layer (acc. to above)	250 g
Methacrylic acid copolymer 30% suspension	333.7 g
Triethyl citrate	30 g
Mono- and diglycerides (NF)	5.0 g
Polysorbate 80 (=Tween 80)	0.5 g
Water purified	195.8 g
Over-coating layer	
Enteric coating layered pellets	371 g
Carboxymethylcellulose-sodium	5.0 g
Water purified	191 g

Tablets	mg/tablet
Over-coated pellets comprising	
S-omeprazole Mg-salt	55
Naproxen	250
Microcrystalline cellulose (MCC)	150
Hydroxypropylcellulose, low substituted	40
Polyvinylpyrrolidone K-90	5.0
Water purified	250

Suspension layering was performed in a fluid bed apparatus. S-omeprazole magnesium salt was sprayed onto inert sugar seeds (non-pareil cores) from a water suspension containing the dissolved binder and polysorbat 80.

The prepared core material was coating layered by a separating layer in a fluid bed apparatus with a hydroxypropyl cellulose solution containing talc and magnesium stearate. The enteric coating layer consisting of methacrylic acid copolymer, mono- and diglycerides, triethylcitrate and polysorbate was sprayed onto the pellets (with separating layer) in a fluid bed apparatus. In the same type of apparatus the enteric coating layered pellets were covered with carboxymethylcellulose-sodium solution. The over-coating layered pellets were classified by sieving.

5 parts of polyvinylpyrrolidone K-90 was dissolved in 150 parts of purified water to form the granulation liquid. Naproxen, MCC, and low-substituted hydroxypropyl cellulose were dry-mixed. The granulating liquid was added to the powder mixture and the mass wet-mixed. 100 parts of water was added as quantum satis.

The wet mass was dried in an oven at 60°C for approx. 5-6 hrs. The dried granules were milled to pass a 1.0 mm sieve.

The enteric coating layered pellets and the milled granules were mixed and compressed to tablets on a tableting machine equipped with 18x8.5 mm punches. Average hardness for the 500 mg tablets tested (across the longest axis) with a Schleuniger apparatus was 9.4 kP. Disintegration time tested in purified water at 37 °C was 15-30 sec (n=2).

Example 3

Fast disintegrating multiple unit tableted dosage form comprising pantoprazole and piroxicam-β-hydroxypropyl-cyclodextrin.

Core material	
Pantoprazole	100 g
Non-pareil cores	200 g
Hydroxypropylcellulose LF	25 g
Water purified	607 g
Separating layer	
Core material (acc. to above)	200 g
Hydroxypropyl cellulose LF	20 g
Talc	34.3 g
Magnesium Stearate	2.9 g
Water purified	400 g
Enteric coating layer	
Pellets with sep layer (acc. to above)	200 g
Methacrylic acid copolymer, 30% suspension	333 g
Triethyl citrate	30 g
Mono- and diglycerides (NF)	5 g
Polysorbate 80	0.5 g
Water purified	281.5 g

Tablets	mg/tablet
Pellets comprising pantoprazole	133
Piroxicam	20
β-hydroxypropyl-cyclodextrin, (90%)	72
Microcrystalline cellulose (MCC)	276
Polyvinylpyrrolidone cross-linked	36.8
Water purified	≤ 2

Sodium stearylfumarate (SSF)	3.9

Suspension layering was performed in a fluid bed apparatus. Pantoprazole was sprayed onto inert sugar seeds (non-pareil cores) from a water suspension containing the dissolved binder.

The prepared core material was coating layered by a separating layer in a fluid bed apparatus with a hydroxypropyl cellulose solution containing talc and magnesium stearate. The enteric coating layer consisting of methacrylic acid copolymer, mono- and diglycerides, triethylcitrate and polysorbate was sprayed onto the pellets (with a separating layer) in a fluid bed apparatus. The pellets were classified by sieving.

The piroxicam was added to β-hydroxypropyl-cyclodextrin during mechanical treatment and moisturization with the water. The mass was dried in a drying oven at 50°C and then milled to pass a 0.8 mm sieve.

The piroxicam-β-hydroxypropyl-cyclodextrin , the MCC, the cross-linked polyvinylpyrrolidone and the SSF were dry-mixed and thereafter this mixture was mixed with the pantoprazole pellets .

Compression to tablets was done on a tableting machine equipped with 18x8.5 mm punches. Average hardness for the 577 mg tablets tested with a Schleuniger apparatus was 16.7 kP with variation between 14.8 and 18.7 kP, measurement taken along the longest axis. Disintegration time tested in water was approx. 4 minutes.

The tablets were coated with a pigmented dispersion like in Ex. 7.

Example 4

Two-layered tablet dosage form with fast disintegrating part having 20 mg of lansoprazole in the form of enteric coated pellets comprised in one layer, and the other layer being an extended release part designed as a hydrophilic gel matrix comprising 250 mg of naproxen.

Lansoprazole enteric coated pellets

Core material

Lansoprazole	400 g
Non-pareil cores	400 g
Hydroxypropyl methylcellulose	80 g
Sodium laurylsulphate	3 g
Water purified	1360 g

Sub-coating

Core material (acc. to above)	100 g
Hydroxypropyl methylcellulos	9 g
Polyethyleneglycol 6000	1 g
Talc	18 g
Ethanol 95%	250 g
Water purified	250 g

Enteric coating

Sub-coated pellets (acc. to above)	100 g
Hydroxypropyl methylcellulose phtalate	39.9 g
Acetyltributyl citrate	8 g
Cetanol	2.1 g
Ethanol 95%	162 g
Acetone	378 g

Suspension layering was performed in a fluid bed apparatus. Lansoprazole was sprayed onto inert non-pareil cores from a water suspension containing the dissolved binder and the wetting agent.

The prepared core material was sub-coated in a Wurster equipped fluid bed apparatus with the talc suspended in a HPMC/PEG- solution. PEG also have a function as plasticizer for the HPMC.

Enteric coating was performed in the same equipment with a solution in organic solvents of the materials forming the enteric layer.

Tablets	mg/tablet
Pellets comprising lansoprazole	94
Microcrystalline cellulose	181.8
Polyvinyl pyrrolidone cross-linked	18.2
Naproxen	250
Polyoxyethylene (mwt appr. 4000000)	200
Sodium aluminium silicate	50
L-Arginine	190
Ethanol 95% (w/v) approx.	280

Naproxen, Polyox WSR 301®, L-Arginin and sodium aluminium silicate were dry-mixed. The granulating liquid, ethanol, was added to the powder mixture and the mass wet-mixed. The wet mass was dried in an oven at 60°C for approx. 8 hrs. The dried granules were milled to pass a 1.0 mm sieve.

Tablet compression was made by first pre-compressing 690 mg of the naproxen-containing granules and then filling 281 mg of a mixture consisting of 81 mg lansoprazole pellets plus 181.8 mg of MCC and 18.2 mg of crosslinked polyvinylpyrrolidone per tablet, on top. These materials were then compressed together to give the two-layered tablets on a Diaf tableting machine equipped with 9x20 mm punches. Tablet hardness tested with a Schleuniger apparatus over the longest axis was approximately 14 kP.

Naproxen dissolution was tested in phosphate buffer pH 6.8. Obtained results;

1 hrs	14%	dissolved
3 hrs	34%	"
5 hrs	58%	"
7 hrs	79%	"
24 hrs	102%	"

Example 5

Fast disintegrating multiple unit tableted dosage form comprising magnesium omeprazole and piroxicam.

Core material (omeprazole)

Magnesium omeprazole	5.00 kg
Non-pareil cores	10.00 kg
Hydroxypropyl methylcellulose	0.75 kg
Water purified	19.65 kg

Separating layer (omeprazole)	
Core material (acc. to above)	14.60 kg
Hydroxypropyl cellulose	1.46 kg
Talc	2.5 kg
Magnesium Stearate	0.21 kg
Water purified	29.2 kg
Enteric coating layer (omeprazole)	
Pellets with sep layer(acc. to above)	9.00 kg
Methacrylic acid copolymer (30% suspension)	15.00 kg
Triethyl citrate	1.35 kg
Mono- and diglycerides (NF)	0.22 kg
Polysorbate 80	0.02 kg
Water purified	8.8 kg
Over-coating layer (omeprazole)	
Enteric coating layered pellets	9.0 kg
Hydroxypropyl methylcellulose	0.18 kg
Mg-Stearate	0.005 kg
Water purified	3.6 kg

Suspension layering was performed in a fluid bed apparatus. Magnesium omeprazole was sprayed onto inert sugar seeds (non-pareil cores) from a water suspension containing the dissolved binder.

The prepared core material was coating layered by a separating layer in a fluid bed apparatus with a hydroxypropyl cellulose solution containing talc and magnesium stearate. The enteric coating layer consisting of methacrylic acid copolymer, mono- and diglycerides, triethylcitrate and polysorbate was sprayed onto the sub-coated pellets in a fluid bed apparatus. In the same type of apparatus the enteric coating layered pellets were covered

with hydroxypropyl methylcellulose/Mg-Stearate suspension. The over-coating layered pellets were classified by sieving.

Core material (piroxicam)	
Piroxicam micronized	35 g
Sugar seeds	100 g
Hydroxypropyl methylcellulose 6 cps	25 g
Water purified	250 g
Ethanol 99% (w/v)	250 g

Enteric coating layer (piroxicam)	
Piroxicam pellets (acc. to above)	100 g

were coated with a suspension of the following composition to give a product with a content of 163 mg/g;

Hydroxypropyl methylcellulose acetatesuccinate LF	14.38 parts
Triethyl citrate	2.87 parts
Sodium laurylsulphate	0.43 parts
Talc	4.32 parts
Water purified	183.3 parts

Suspension layering was performed in a fluid bed apparatus. Micronized piroxicam was sprayed onto inert non-pareil cores from a water suspension containing the dissolved binder.

The enteric coating layer consisting of hydroxypropyl methylcellulose acetatesuccinate, triethylcitrate, sodium laurylsulphate and talc was sprayed onto the piroxicam pellets in a fluid bed apparatus.

Tablets (for 1000 pcs)

pellets comprising omeprazole	95.7 g
pellets containing piroxicam	122.7 g
Microcrystalline cellulose (MCC)	240 g
Polyvinylpyrrolidone cross-linked (PVP-XL)	20 g
Hydroxypropylcellulose, low-substituted (L-HPC)	40 g
Sodium stearylfumarate (SSF)	4.6 g

MCC, L-HPC and PVP-XL were mixed together until homogenity. The two kind of enteric coating layered pellets were admixed thereafter. Finally the lubricant SSF was admixed and this mixture was compressed to tablets on a tableting machine equipped with 8.5x16 mm punches. Hardness of the 523 mg tablets tested with a Schleuniger apparatus varied between 8 and 9 kP. Disintegration time tested in water 37°C was less than 1 minute

The tablets were coated with a pigmented dispersion like in Example 7.

Example 6

Fast disintegrating enteric coating layered tablet comprising magnesium omeprazole and diclofenac.

Tablets (for 2000 pcs)

Omeprazole magnesium (corr. 20 mg omeprazole)		45.0 g
Diclofenac sodium (corr. 20 mg diclofenac)		43.2 g
Microcrystalline cellulose (MCC)		110 g
Polyvinylpyrrolidone cross-linked (PVP-XL)		50 g
Hydroxypropylcellulose, low-substituted (L-HPC)		50 g
Sodium stearylfumarate (SSF)		8.6 g
Water purified	approx.	170 g

The omeprazole, diclofenac, MCC, L-HPC, 30 grams of PVP-XL and 5.6 grams of SSF were mixed and then the water was added during continuously mixing. The granulate was dried in a drying oven at 60°C for approx. 1.5 hours. The dry granulate was milled to pass sieve 1.0 mm.

The milled granules were mixed with 20 grams of PVP-XL and 3.0 grams of SSF. This mixture was compressed to 153 mg tablets on a tableting machine using 7 mm diameter punches. Average tablet hardness was 7.4 kP (n=6). Disintegration time in water 37°C was 1 minute 20 seconds (n=1).

The tablets were coating layered with a separating layer consisting of hydroxypropyl methylcellulose (HPMC) and talc in a Wurster equipped fluidized bed.

Application of separating layer

Tablets 7 mm	100.1 g
coating dispersion;	
HPMC 6 cps	5.5 g
Talc	1.15 g
EtOH 99%(w/v)	46.7 g
Water purified	46.7 g

The obtained coating layered tablets were further coating layered by an enteric coating layer in the same apparatus.

Application of enteric coating layer

Tablets with separating layer	100 g
coating dispersion;	
Methacrylic acid copolymer as 30% suspension	26.4 g (7.92 g dry mtrl.)
Polyethyleneglycole 400	0.9 g
Titanium dioxide	0.83 g
Iron oxide reddish brown	0.28 g
Water purified	55.1 g

The weight increase of the tablets in the enteric coating step was approx. 11 mg/tablet, corresponding to approx. 7% of the weigth of charged tablets.

The pigments in the enteric coating layer provides protection against light.

Example 7

Fast disintegrating multiple unit tableted dosage form comprising magnesium omeprazole and an inner coating layer comprising diclofenac-sodium and an outer pigmented coating layer providing light protection.

Magnesium omeprazole enteric coating layered pellets from Ex. 5.

Tablets	mg/tablet
Pellets comprising omeprazole	83.3
Microcrystalline cellulose (MCC)	181.4
Polyvinylpyrrolidone cross-linked	3.7
Sodium stearyl fumarate (SSF)	0.4

Pellets were prepared as in Example 5.

The MCC, the cross-linked polyvinylpyrrolidone and the omeprazole containing pellets were dry-mixed. Thereafter the SSF was admixed.

The mixture was compressed to tablets on a tableting machine equipped with 9 mm diameter punches. Hardness of the 269 mg tablets tested with a Schleuniger apparatus varied between 8 and 9 kP.

The tablets were coated in a fluidized bed with the solution below, until average tablet weight was 298 mg.

Diclofenac-sodium	20.0 parts by weight
HPMC 6 cps	11.4 parts by weight
EtOH 99%(w/v)	113.6 parts by weight
Water purified	113.6 parts by weight

Finally these tablets were covered with pigmented suspension in the same equipment. The composition of the coating suspension was;

HPMC 6 cps	10 parts by weight
Polyethylene glycol mwt 6000	2.5 parts by weight
TiO_2	1.83 parts by weight
Iron oxide yellow	0.40 parts by weight
EtOH 99%(w/v)	85 parts by weight
Water purified	85 parts by weight

Obtained average tablet weight was 303 mg. Disintegration time tested in water 37°C was less than 4 minutes (n=4).

Example 8

A capsule formulation comprising magnesium omeprazole and piroxicam.

Capsules

Enteric coating layered omeprazole pellets	
(manufacturing and composition as in Ex. 5)	95.7mg/cap
Enteric coating layered piroxicam pellets	
(manufacturing and composition as in Ex. 5)	122.7mg/cap

Prepared pellets are filled into hard gelatine capsules, size 3. Optionally a small amount of lubricant is added before filling into capsules. The amount of omeprazole in each capsule is approx. 20 mg and the amount of piroxicam is approx. 20 mg.

Example 9

A capsule formulation comprising S-omeprazole magnesium salt and naproxen.

Capsules

Enteric coating layered pellets	
(manufacturing and composition as in Ex. 2)	55.2mg/cap
Naproxen granulation	
(manufacturing and composition as in Ex. 2)	445mg/cap

Prepared granules and enteric coating layered pellets are filled into hard gelatine capsules, size 00. Optionally a small amount of lubricant is added before filling into capsules. The amount of S-omeprazole in each capsule is approx. 10 mg and the amount of naproxen is approx. 250 mg.

Example 10:

Fast disintegrating multiple unit tableted dosage form comprising magnesium omeprazole and diclofenac-Na.

Core material	
Magnesium omeprazole	5 kg
Sugar sphere seeds	10 kg
Hydroxypropyl methylcellulose	0.75 kg
Water purified	19.7 kg
Separating layer	
Core material	10.2 kg
Hydroxypropyl cellulose	1.02 kg
Talc	1.75 kg
Magnesium stearate	0.146 kg
Water purified	21.4 kg
Enteric coating layer	
Pellets covered with separating layer	11.9 kg
Methacrylic acid copolymer (30 % suspension)	19.8 kg
Triethyl citrate	1.79 kg
Mono- and diglycerides (NF)	0.297 kg
Polysorbate 80	0.03 kg
Water purified	11.64 kg
Over-coating layer	
Enteric coating layered pellets	20.0 kg
Hydroxypropyl methylcellulose	0.238 kg
Magnesium stearate	0.007 kg

Water purified	6.56 kg

Tablets	mg/tablet
Overcoated pellets comprising omeprazole	82.4
Diclofenac-Na	50.0
Microcrystalline cellulose (MCC)	261
Polyvinylpyrrolidone cross-linked	5.6
Sodium stearyl fumarate	0.56

Suspension layering was performed in a fluid bed apparatus. Magnesium omeprazole was sprayed onto sugar sphere seeds from a water suspension containing the dissolved binder. The size of sugar sphere seeds were in the range of 0.25 to 0.35 mm.
The prepared core material was covered with a hydroxypropyl cellulose solution containing talc and magnesium stearate. The enteric coating layer consisting of methacrylic acid copolymer, mono- and diglycerides, triethyl citrate and polysorbate was sprayed onto the pellets covered with a separating layer in a fluid bed apparatus. In a fluid bed apparatus enteric coating layered pellets were coated with a hydroxypropyl methylcellulose solution containing magnesium stearate. The over-coating layered pellets were classified by sieving.

The enteric coating layered pellets with an over-coating layer, diclofenac-Na, MCC, polyvinylpyrrolidone cross-linked and sodium stearyl fumarate were dry mixed and compressed into tablets using an excenter tableting machine equipped with 11 mm punches. The amount of omeprazole in each tablet was approx. 10 mg and the amount of diclofenac-Na was approx. 50 mg. The tablet hardness was measured to 80 N.

Example 11:

Fast disintegrating multipe unit tableted dosage form comprising magnesium omeprazole and piroxicam.

Core material	
Magnesium omeprazole	10.0 kg
Sugar sphere seed	10.0 kg
Hydroxypropyl methylcellulose	1.5 kg
Water purified	29.9 kg
Separating layer	
Core material	20.0 kg
Hydroxypropyl cellulose	2.0 kg
Talc	3.43 kg
Magnesium stearate	0.287 kg
Water purified	41.0 kg
Enteric coating layer	
Pellets covered with separating layer	24.5 kg
Methacrylic acid copolymer (30 % suspension)	32.7 kg
Triethyl citrate	2.94 kg
Mono- and diglycerides (NF)	0.49 kg
Polysorbate 80	0.049 kg
Water purified	19.19 kg
Over-coating layer	
Enteric coating layered pellets	37.8 kg
Hydroxypropyl methylcellulose	0.49 kg
Magnesium stearate	0.0245 kg
Water purified	11.6 kg
Tablets	mg/tablet
Overcoated pellets comprising omeprazole	94.9
Piroxicam	20.0

Microcrystalline cellulose (MCC)	280
Polyvinylpyrrolidone cross-linked	5.6
Sodium stearyl fumarate	0.56

Enteric coating layered pellets of magnesium omeprazole with an overcoating layer were prepared as in Example 10.

The enteric coating layered pellets with an over-coating layer, piroxicam, MCC, polyvinylpyrrolidone cross-linked and sodium stearyl fumarate were dry mixed and compressed into tablets using an excenter tableting machine equipped with 11 mm punches. The amount of omeprazole in each tablet was approx. 20 mg and the amount of piroxicam was approx. 20 mg. The tablet hardness was measured to 110 N.

Results

"Acid resistance" i.e. % left after exposure to 0.1 N HCl for 2 hrs	
	Tablets
Ex 1	95%
Ex 2	95%
Ex 3	99%
Ex 4	91%
Ex 5	92%
Ex 6	96%
Ex 7	93%
Ex 10	91%
Ex 11	91%

The best mode to practice the present invention is according to the dosage forms of the types described in examples 5, 7 and 10.

The enteric coating layered pellets comprising a proton pump inhibitor may also be prepared as described in the following examples.

Example 12

Preparation of enteric coating layered pellets by extrusion/spheronization.

Core material	
Magnesium omeprazole	600 g
Mannitol	1000 g
Microcrystalline cellulose	300 g
Hydroxypropyl cellulose	100 g
Sodium lauryl sulphate	6 g
Water purified	802 g
Separating layer	
Core material (acc. to above)	400 g
Hydroxypropyl methylcellulose	48 g
Water purified	960 g
Enteric coating layer	
Pellets covered with separating layer (acc. to above)	200 g
Methacrylic acid copolymer	100 g
Triethyl citrate	30 g
Mono- and diglycerides (NF)	5 g

Polysorbate 80	0.5 g
Water purified	309 g

Sodium lauryl sulphate is dissolved in purified water to form the granulation liquid. Magnesium omeprazole, mannitol, microcrystalline cellulose and hydroxypropyl cellulose are dry-mixed. The granulation liquid is added to the powder mixture and the mass is wet-mixed.

The wet mass is forced through an extruder equipped with screens of size 0.5 mm. The extrudate is spheronized on a friction plate in a spheronizing apparatus. The core material is dried in a fluid bed dryer and classified. The prepared core material is covered by a separating layer in a fluid bed apparatus with a hydroxypropyl methylcellulose/water solution.

The enteric coating layer is applied to the pellets covered with separating layer from an aqueous dispersion of methacrylic acid copolymer plasticized with triethyl citrate to which a mono- and diglycerides/polysorbate dispersion has been added. The pellets are dried in a fluid bed apparatus.

Example 13

Preparation of enteric coating layered pellets by powder layering of sugar sphere seeds.

Core material

Magnesium omeprazole	1 500 g
Sugar sphere seeds	1 500 g
Hydroxypropyl methylcellulose	420 g
Aerosil®	8 g
Water purified	4 230 g

Separating layer	
Core material (acc. to above)	500 g
Hydroxypropyl cellulose	40 g
Talc	67 g
Magnesium stearate	6 g
Water purified	800 g

Enteric coating layer	
Pellets covered with separating layer (acc. to above)	500 g
Methacrylic acid copolymer	200 g
Triethyl citrate	60 g
Water purified	392 g

Magnesium omeprazole, part of the hydroxypropyl methylcellulose and Aerosil® are dry-mixed forming a powder. Sugar sphere seeds (0.25-0.40 mm) are layered with the powder in a centrifugal fluidized coating granulator while spraying a hydroxypropyl methylcellulose solution (6 %, w/w).

The prepared core material is dried and covered by a separating layer in a centrifugal fluidized coating-granulator. A fluid bed apparatus is used for enteric coating layereing.

Example 14

Preparation of enteric coating layered pellets with cores of silicon dioxide seeds.

Core material	
Magnesium omeprazole	8.00 kg
Silicon dioxide	8.00 kg
Hydroxypropyl methylcellulose	1.41 kg
Sodium lauryl sulphate	0.08 kg

Water purified	28.00 kg

Separating layer

Core material (acc. to above)	10.00 kg
Hydroxypropyl methylcellulose	0.80 kg
Water purified	10.00 kg

Enteric coating layer

Pellets covered with separating layer (acc. to above)	300 g
Methacrylic acid copolymer	124 g
Polyethylene glycol 400	25 g
Mono- and diglycerides (NF)	3 g
Polysorbate 80	1 g
Water purified	463 g

Suspension layering is performed in a fluid bed apparatus. Magnesium omeprazole is sprayed onto the silicon dioxide seeds from a water suspension containing the dissolved binder and a surface active ingredient.

The prepared core material is covered with a separating layer in a fluid bed apparatus with a hydroxypropyl methylcellulose solution. The enteric coating layer consisting of methacrylic acid copolymer, mono- and diglycerides, polyethylene glycol 400 and polysorbate is sprayed onto the pellets covered with separating layer in a fluid bed apparatus.

Example 15

Preparation of enteric coating layered pellets.

Enteric coating layer

Pellets covered with separating layer

(manufacturing and composition	
as in example 12)	500 g
Methacrylic acid copolymer	250 g
Polyethylene glycol 6000	75 g
Mono- and diglycerides (NF)	12.5 g
Polysorbate 80	1.2 g
Water purified	490 g

Example 16

Preparation of enteric coating layered pellets.

Enteric coating	
Pellets covered with separating layer	500 g
(manufacturing and composition as in example 1)	
Hydroxypropyl methylcellulose phthalate	250 g
Cetanol	50 g
Ethanol (95%)	1000 g
Acetone	2500 g

Example 17

Preparation of enteric coating layered pellets.

Core material	
Omeprazole	225 g
Mannitol	1425 g
Hydroxypropyl cellulose	60 g
Microcrystalline cellulose	40 g
Lactose anhydrous	80 g

Sodium lauryl sulphate	5 g
Disodium hydrogen phosphate dihydrate	8 g
Water purified	350 g

Separating layer

Core material (acc. to above)	300 g
Hydroxypropyl cellulose	30 g
Talc	51 g
Magnesium stearate	4 g

Enteric coating layer

Pellets covered with separating layer (acc. to above)	300 g
Methacrylic acid copolymer	140 g
Triethyl citrate	42 g
Mono- and diglycerides (NF)	7 g
Polysorbate 80	0.7 g

The dry ingredients for producing the core material are well mixed in a mixer. Addition of granulation liquid is made and the mixture is kneeded and granulated to a proper consistency. The wet mass is pressed through an extruder screen and the granules are converted into a spherical form in a spheronizer. The core material is dried in a fluid bed apparatus and classified into a suitable particle size range, e.g. 0.5 - 1.0 mm.The prepared core material is covered with a separating layer and enteric coating layered as described in previous examples.

Preparation of active substance.

Magnesium omeprazole used in some of the examples is produced according to the process described in WO/95/01977, the single enantiomers of omeprazole salts are prepared as

described in WO/94/27988 and omeprazole is produced according to the process disclosed in EP-A1 0005129. These documents are hereby incorporated in a whole by reference.

CLAIMS

1. An oral pharmaceutical dosage form comprising an acid susceptible proton pump inhibitor together with at least one Non Steroidal Antiinflammatory Drug (NSAID) and optionally pharmaceutically acceptable excipients, characterized in that the dosage form is in the form of a fixed unit dosage form comprising at least two pharmaceutically active components, and wherein at least the proton pump inhibitor is protected by an enteric coating layer.

2. A dosage form according to claim 1, wherein the dosage form is a tablet formulation.

3. A dosage form according to claim 1, wherein the dosage form is a capsule formulation.

4. A dosage form according to claim 1, wherein the proton pump inhibitor is protected by two layers, an enteric coating layer and a layer separating the enteric coating from the proton pump inhibitor.

5. A dosage form according to claim 1, wherein the dosage form comprises a proton pump inhibitor and one NSAID.

6. A dosage form according to claim 1, wherein the proton pump inhibitor is omeprazole, an alkaline salt thereof, one of its single enantiomer or an alkaline salt thereof.

7. A dosage form according to claim 6, wherein the proton pump inhibitor is S-omeprazole magnesium salt.

8. A dosage form according to claim 1, wherein the proton pump inhibitor is lansoprazole, or one of its single enantiomers or a pharmaceutically acceptable salt thereof.

9. A dosage form according to claim 1, wherein the proton pump inhibitor is pantoprazole, or one of its single enantiomers or a pharmaceutically acceptable salt thereof.

10. A dosage form according to one of claims 6 - 9, wherein the NSAID is ibuprofen, diclofenac, piroxicam or naproxen, or a pharmaceutical acceptable salt thereof.

11. A dosage form according to one of claims 6 - 9, wherein the NSAID is diclofenac or piroxicam, or pharmaceutically acceptable salt thereof.

12. A dosage form according to claim 1, wherein the amount of proton pump inhibitor is in the range of 10-80 mg and the amount of NSAID(s) is in the range of 10-800 mg.

13. A dosage form according to claim 1, wherein the amount of proton pump inhibitor is in the range of 10-40 mg and the amount of NSAID(s) is in the range of 10-500 mg.

14. A tableted dosage form according to claim 2, wherein the tablet consists of two separate layers, one layer comprising a proton pump inhibitor and the other layer comprising one or more NSAIDs.

15. A tableted dosage form according to claim 2, wherein the tablet formulation is a multiple unit tableted dosage form comprising the proton pump inhibitor in the form of individually enteric coating layered pellets compressed together with NSAID comprising granules into a tablet, whereby the enteric coating layer covering the individual pellets has mechanical properties such that the tableting of the pellets together with the NSAID comprising granules and optionally pharmaceutically acceptable excipients does not significantly affect the acid resistance of the individually enteric coating layered pellets.

16. A tableted dosage form according to claim 15, wherein the acid resistance of the enteric coating layered pellets is in coherence with the requirements on enteric coating layered articles defined in the United States Pharmacopeia.

17. A tableted dosage form according to claim 15, wherein the acid resistance of the enteric coating layered pellets does not decrease more than 10 % during the compression of the pellets into the multiple unit tableted dosage form.

18. A tableted dosage form according to claim 15, wherein the enteric coating of the individual pellets comprises a plasticized enteric coating layer material.

19. A tableted dosage form according to claim 15, wherein the enteric coating layered pellets are further covered with an over-coating layer comprising pharmaceutically acceptable excipients.

20. A tableted dosage form according to claim 15, wherein the tablet is divisible.

21. A tableted dosage form according to claim 20, wherein the tablet is dispersible to an aqueous suspension comprising NSAID(s) and enteric coating layered pellets of a proton pump inhibitor.

22. A tablet dosage form according to claim 2, wherein the tablet consists of two separate layers, one layer comprising the proton pump inhibitor in the form of enteric coating layered pellets compressed with tablet excipients into a layer, and the other layer gives an extended release of the incorporated NSAID(s).

23. A tablet dosage form according to claim 22, wherein the layer comprising the NSAID(s) is a gelling matrix giving extended release.

24. A tableted dosage form according to claim 2, wherein the tablet is an enteric coating layered tablet comprising a mixture of the proton pump inhibitor and the NSAID comprising granules, optionally comprising a water soluble or in water rapidly disintegrating separating layer in between the tablet core and the enteric coating layer.

25. A tableted dosage form according to claim 2, wherein the tablet comprising enteric coating layered pellets of the proton pump inhibitor compressed into a tablet, which tablet is covered by a separate layer comprising the NSAID(s).

26. A tableted dosage form according to claim 25, wherein the tablet is covered by a pigmented tablet filmcoating layer.

27. A tablet dosage form according to claim 2, wherein the tablet consists of two types of enteric coating layered pellets, one type comprises the proton pump inhibitor, and the other type comprises NSAID(s), together compressed with tablet excipients into a tablet.

28. A tablet dosage form according to claim 2, wherein the tablet consists of enteric coating layered pellets comprising the proton pump inhibitor, and pellets comprising the NSAID(s) coating layered with an extended release film, and these coating layered pellets are compressed with tablet excipients into a tablet.

29. A process for the manufacture of a fixed dosage form comprising a proton pump inhibitor and one or more NSAIDs in a capsule, characterized in that the proton pump inhibitor is prepared in the form of enteric coating layered pellets and that the pellets are filled into a capsule together with prepared NSAID granules or enteric coating layered NSAID pellets, or NSAID pellets coating layered with an extended release film, optionally the mixture of pellets or granules are mixed with pharmaceutically acceptable excipients, and filled in a capsule.

30. A process for the manufacture of a fixed dosage form comprising a proton pump inhibitor and one or more NSAIDs in a multiple unit tableted dosage form, characterized in that the proton pump inhibitor is prepared in the form of enteric coating layered pellets and these pellets are mixed with prepared NSAID granules and optionally pharmaceutically acceptable tablets excipients whereafter the dry mixture is compressed into a multiple unit tablet without giving any significant change of the acid resistance of the enteric coating layer.

31. A process for the manufacture of a fixed dosage form comprising a proton pump inhibitor and one or more NSAIDs in a multiple unit tableted dosage form, characterized in that the proton pump inhibitor is prepared in the form of enteric coating layered pellets and the NSAID(s) is prepared in the form of coating layered pellets wherein the coating layer is an extended release layer or an enteric coating layer, and the prepared pellets are mixed with tablet excipients and compressed into a tablet.

32. A process for the manufacture of a fixed dosage form comprising a proton pump inhibitor and one or more NSAID(s) in an enteric coating layered tablet characterized in that the proton pump inhibitor is admixed with the NSAID(s) and pharmaceutically acceptable excipients whereafter the mixture is compressed into a tablet, and the tablet is covered with an enteric coating layer and optionally covered with a separating layer before the enteric coating layer is applied.

33. A method for the treatment of gastrointestinal side-effects associated with NSAID treatment in mammals and man by administering to a host in need thereof a therapeutically effective dose of a multiple unit tableted dosage form according to any of claims 1 to 28.

34. A method according to claim 33, wherein the disorder is an upper gastrointestinal disorder associated with NSAID treatment.

35. Use of a dosage form according to any of claims 1 to 28 for the manufacture of a medicament for treatment or prevention of gastro intestinal side-effects associated with NSAID(s) treatment disorders.

36. Use according to claim 35 wherein the disorder is an upper gastrointestinal disorder associated with NSAID treatment.

Fig 1

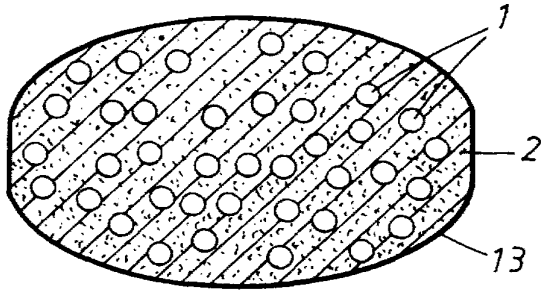


Fig 2

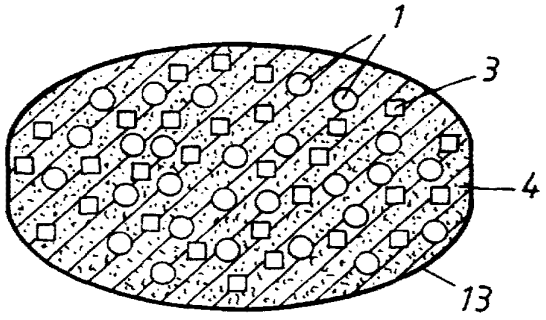


Fig 3

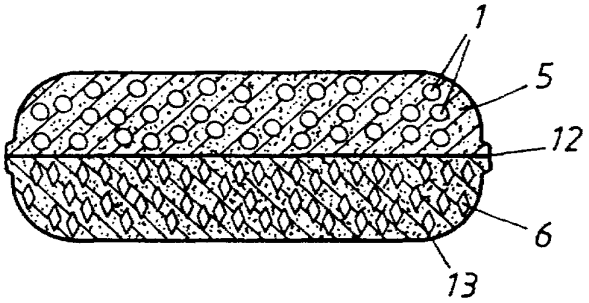


Fig. 4

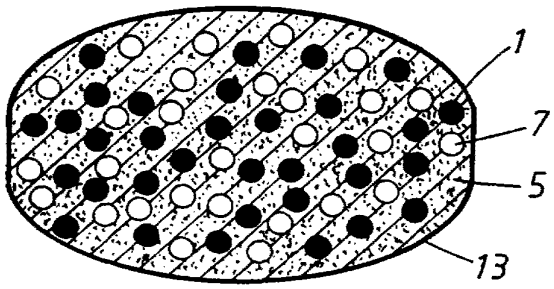


Fig. 5

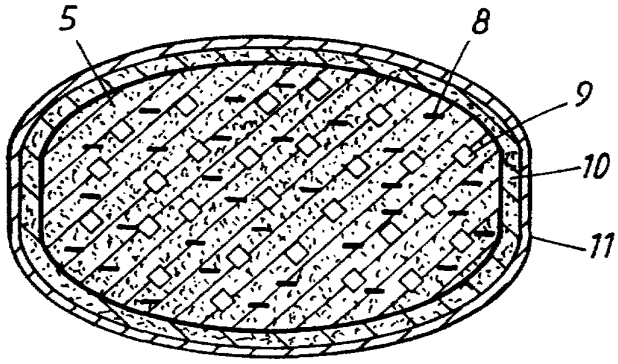
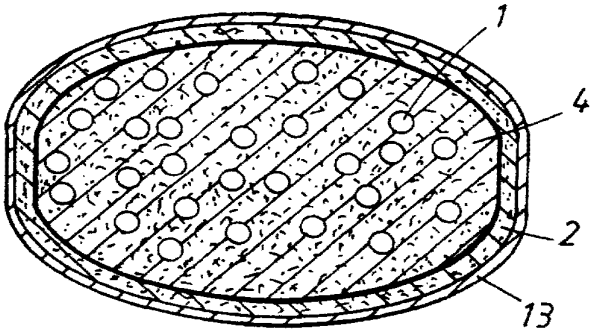


Fig. 6



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/01735

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 45/06, A61K 31/44, A61K 31/19, A61K 31/54, A61K 9/26, A61K 9/54
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, US FULLTEXT, WPI, WPIL, CLAIMS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0426479 A1 (MCNEIL-PPC, INC.), 8 May 1991 (08.05.91) --	1-36
A	STN International, File CAPLUS, CAPLUS accession no. 1992:187668, Scheiman, James M. "Pathogenesis of gastroduodenal injury due to nonsteroidal antiinflammatory drugs", Semin. Arthritis Rheum. (1992), 21(4), 201-10 --	1-36
A	EP 0247983 A2 (AKTIEBOLAGET HÄSSLE), 2 December 1987 (02.12.87), page 4, line 25 - page 5, line 2; page 8, line 22 - line 32 --	12-36

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 1997	22.04.97
Name and mailing address of the ISA: Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Anneli Jönsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/01735

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0365947 A1 (PHARMACIA AB), 2 May 1990 (02.05.90), page 3, line 41 - line 46; page 4, line 42 - line 57	12-36

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/01735

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 33-34
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Claims 33-34 are directed to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/Rule 39.1(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

04/03/97

International application No.
PCT/SE 96/01735

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0426479 A1	08/05/91	AU 646230 B	17/02/94
		AU 6568990 A	09/05/91
		CA 2028746 A,C	03/05/91
		DE 69006684 D,T	09/06/94
		ES 2057439 T	16/10/94
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		IE 64953 B	20/09/95
		JP 3206052 A	09/09/91
		US 5204118 A	20/04/93
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EP 0247983 A2	02/12/87	SE 0247983 T3	
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		AT 140387 T	15/08/96
		AU 601974 B	27/09/90
		AU 7191287 A	05/11/87
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INTERNATIONAL SEARCH REPORT

Information on patent family members

04/03/97

International application No.
PCT/SE 96/01735

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		IE 62640 B	22/02/95
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		US 5178868 A	12/01/93

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(30) Priority Data:
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(74) Agent: MAYFIELD, Denise, L.; Vinson & Elkins, L.L.P., 2300 First City Tower, 1001 Fanin Street, Houston, TX 77002-6760 (US).

(81) Designated States: CA, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) Title: METHODS OF ENHANCING THE THERAPEUTIC ACTIVITY OF NSAIDs AND COMPOSITIONS OF ZWITTERIONIC PHOSPHOLIPIDS USEFUL THEREIN

(57) Abstract

Disclosed are compositions comprising non-steroid anti-inflammatory drugs (NSAIDs) complexed with zwitterionic, neutral phospholipids, or both, having reduced gastrointestinal irritating effects and enhanced anti-pyretic, analgesic, and anti-inflammatory activity. Also disclosed are improved methods of using the complexes for treating fever, inflammation, and preventing platelet aggregation. In some embodiments, the anti-pyretic activity of sub-therapeutically used amounts of NSAIDs are enhanced to elicit anti-pyretic activity *in vivo* when associated (Noncovalently) with zwitterionic phospholipids, such as dipalmitoyl phosphatidyl choline. Methods and compositions useful for enhancing the therapeutic activity of non-steroidal anti-inflammatory agents in the presence of anti-secretory agents are also discussed.

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METHODS OF ENHANCING THE THERAPEUTIC ACTIVITY OF NSAIDS AND COMPOSITIONS OF ZWITTERIONIC PHOSPHOLIPIDS USEFUL THEREIN

This application claims priority to U.S. Serial No. 08/719,134 filed September 24, 1996.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the fields of pharmacology and medicine and more particularly, it concerns the treatment and prevention of fever, pain and inflammation with non-steroidal anti-inflammatory drugs (NSAIDs) complexed with phospholipids, and in other embodiments in further combination with neutral lipids. The invention also provides methods for retarding platelet aggregation, and the application of these methods in treating cardiovascular and vascular diseases as it relates to platelet activity.

2. Description of the Related Art

The consumption of NSAIDs among the general populace is unparalleled by any other drug class due to their great efficacy in the treatment of pain, inflammation and fever (Rainsford, 1985). The widespread usage of these drugs is anticipated to increase even further due to their efficacy in the treatment of osteoarthritic and generalized aches and pain as the elderly increase as a percentage of the population (Alexander *et al.*, 1985; Jolobe and Montgomery, 1984), and as NSAIDs are employed in the treatment/prevention of stroke and cardiovascular disease.

The major concern with these developments relates to the tendency of NSAIDs to induce gastrointestinal (GI) mucosal lesions, perforations and bleeding resulting in significant morbidity and mortality, even in occasional NSAID users (Rainsford, 1989; Graham, 1989; Allison *et al.*, 1992). Strategies to reduce the gastroduodenal injurious effects of these drugs with enteric coatings, have had limited success due to the delayed therapeutic actions of these specially packaged NSAIDs (Alpsten *et al.*, 1982; Mojaverian *et al.*, 1987).

bleeding by a prostaglandin-independent mechanism (Rainsford, 1989; Whittle *et al.*, 1980; Whittle, 1981; Ligumsky *et al.*, 1982; McCormack and Brune, 1987).

The present inventor and others have obtained evidence that the mucosa of the stomach and other regions of the GI tract have hydrophobic, non-wettable properties, that protect the underlying epithelium from gastric acid and other luminal toxins (Hills *et al.*, 1983; Goddard *et al.*, 1987; Goddard *et al.*, 1990; Kao *et al.*, 1990). This biophysical characteristic, which can be quantified by contact angle analysis, appears to be attributable to the presence of an extracellular lining of surfactant-like phospholipid on the luminal aspects of the mucus gel layer (Goddard *et al.*, 1990; Kao *et al.*, 1990). Evidence has also come forth that these zwitterionic phospholipids are synthesized in surface mucus cells of the stomach, as well as those present in discrete submucosal glands of the GI tract, where they are stored in specific organelles and secreted by a prostaglandin-dependent pathway (Kao and Lichtenberger, 1991). It has also been reported that aspirin and other NSAIDs have the ability to rapidly transform the gastric mucosa from a non-wettable to a wettable state within minutes after luminal administration, thereby increasing the tissue's susceptibility to the corrosive actions of gastric acid (Hills *et al.*, 1983; Goddard *et al.*, 1987; Goddard *et al.*, 1990; Kao *et al.*, 1990).

One solution to this problem has been to formulate injectable solutions of NSAIDs and thus bypass the GI tract completely. The low water solubility of these drugs, however, has caused problems with this technique. Stable, injectable solutions of indoleacetic and indanacetic acid derivatives have been developed to address this problem, by complexing these NSAIDs with phosphatidylcholine and phosphatidylethanolamine derivatives (See US Patent 4,309,420).

US Patent 4,421,747 describes NSAIDs complexed with phospholipids for oral administration. These complexes were shown to retain their anti-inflammatory action and to have reduced ulcer formation in rats. However, no enhancement of therapeutic effects was reported with these preparations.

WO 91/16920 (Vical Inc.) relates to phospholipid prodrug derivatives of a salicylate or non-steroidal, anti-inflammatory drug. These preparations are made by combining salicylic acid or NSAID with a phospholipid in the presence of a coupling agent, thereby producing a covalently linked NSAID-phospholipid compound. These prodrugs are

described as useful in reducing the toxicity of high dose, long term usage of NSAID preparations.

JP 3176425 (Nippon Shinyaku KK) relates to compositions including nonsteroidal, anti-inflammatory drugs together with neutral lipids and phospholipids in a fat and oil emulsion. Although the method of preparation is not described in the abstract, these compositions appear to be encapsulated in lipid, such as in a micelle. The combination of the drug with the neutral lipids and the phospholipids is described as not affecting the drug's pharmacological actions.

JP 63048228 (Toe Eiyo KK) relates to topically applied compositions that include non-steroidal anti-inflammatory drug together with phospholipid and a "disintegrator". The disintegrator is described as providing for a preparation with improved dispersability and increased absorptivity. JP 63048226 (Ono Pharmaceutical KK) relates to compositions that include a phospholipid base (such as phosphatidylcholine) and an anti-inflammatory agent (such as acetylsalicylic acid and indomethacin). KK JP 58150508 (Ono Pharmaceutical) relates to topical compositions that include a phospholipid base (such as phosphatidylcholine) and an anti-inflammatory agent (such as acetylsalicylic acid and indomethacin).

US Patent 4,369,182 (Nattermann & CIE), relates to inflammation-preventing pharmaceutical compositions for oral administration. The compositions are prepared and then lyophilized into powder form. The described compositions include natural and synthetic phospholipids (dipalmitoylphosphatidylcholine (DPPC)), in combination with nonsteroidal agents including salicylic acid, acetyl-salicylic acid, diflunisal, indomethacin, glucametacine, acemetacin, sulindac, ibuprofen, naproxen, tolmetin and other NSAID's. Also described are NSAID's in combination with phosphatidylcholine preparations, named phospholipons. US Patent 4,421,747 relates to methods of alleviating inflammation with compositions as described in the '182 patent.

Despite the extensive work in the area of NSAIDs, a need continues to exist in the art for preparations that include reduced amounts of this useful class of drug without loss of therapeutic efficacy. Methods and compositions that provide for similar or enhanced anti-pyretic, anti-inflammatory, anti-platelet and analgesic activity at lower doses than currently prescribed for pharmacological activity would also render this very valuable class

of drugs available to those previously unable to tolerate standard and/or prolonged therapeutic regimens of NSAID.

SUMMARY OF THE INVENTION

The present invention seeks to overcome these and other drawbacks inherent in the prior art by providing compositions and methods capable of maintaining and/or improving the pharmacological activity of non-steroidal anti-inflammatory drugs by noncovalent association with zwitterionic phospholipids. In some embodiments, these preparations may further include neutral lipids, such as the triglycerides.

The present invention illustrates the ability of one or more zwitterionic phospholipids to enhance the fever-reducing potential of an NSAID. Pharmacological activity of low dose NSAID to reduce inflammation and pain may also be observed, and in some cases enhanced by chemically associating the NSAID with zwitterionic phospholipid, such as phosphatidyl choline (PC), dipalmitoylphosphatidylcholine (DPPC), and other disaturated phosphatidyl cholines, and the like. In some embodiments, the association of NSAID and zwitterionic phospholipid is of a non-covalent nature. In still other embodiments, the NSAID and zwitterionic phospholipid compositions may be further described as including more or less equimolar amounts of these ingredients. The compositions may also, of course, comprise a pharmaceutically acceptable carrier in any form, such as solid powder, gel or liquid form.

The present invention focuses techniques that are demonstrated in some cases to enhance the therapeutic activities of NSAIDs. This is accomplished without the disadvantage of hindering the pharmacological activity or therapeutic bioavailability of the drug rendering the preparations effective even at low doses.

Accordingly, the present invention provides in one aspect a method for enhancing the antipyretic activity of a nonsteroidal anti-inflammatory drug (NSAID). This method comprises providing a non-covalently associated combination of a zwitterionic phospholipid with an amount of a nonsteroidal anti-inflammatory drug that provides reduced anti-pyretic activity in the absence of the zwitterionic phospholipid. Some embodiments of the present preparations are further described as being essentially free of

anionic phospholipid, or as including an amount of anionic phospholipid that is biologically inert and/or not an active component, of the preparation.

The term "essentially free" as used in the description of the present invention, is understood to mean compositions that include less than about 0.10% of anionic phospholipid, and in even further defined embodiments, less than 0.01% anionic phospholipid. As used in the description of the present invention, the term zwitterionic phospholipid embraces a wide range of phospholipids, including but not limited to phosphatidylcholine, phosphatidylserine, phosphalidylethanolamine, sphingomyelin and other ceramides, as well as various other zwitterionic phospholipids. In some embodiments, these compositions are essentially free of anionic phospholipid.

In other embodiments of the described method, the amount of nonsteroidal anti-inflammatory drug is defined as an amount that provides reduced antipyretic activity in the absence of the zwitterionic phospholipid. Such amounts of the drug sub-therapeutically effective amounts thereof. This activity or lack of activity is observed in the absence of zwitterionic phospholipid, while the same or about the same amount of the NSAID does demonstrate pharmacological activity in the presence of zwitterionic phospholipid. In this regard, the phenomenon is observed that the combination of low amounts of nonsteroidal anti-inflammatory drugs with phospholipid have potent pharmacological activity, while doses of the drug alone (i.e., without zwitterionic phospholipid) do not.

The present method employs compositions that may include any variety of those drugs generally classified as nonsteroidal anti-inflammatory drugs. By way of example, these drugs include ibuprofen, piroxicam, salicylate, aspirin, naproxen, indomethacin, diclofenac, or any mixture thereof. In particular embodiments, the nonsteroidal anti-inflammatory drug is salicylate. By way of example and not limitation, NSAID's useful in the practice of the invention, include those noted in Table 1.

TABLE 1

Nonsteroidal Anti-Inflammatory Drugs To Be Used in Combination with Zwitterionic Phospholipids

Propionic acids	Fenoprofen calcium	Nalfon®
	Flurbiprofen	Ansaid®

	Suprofen	
	Benoxaprofen	
	Ibuprofen (prescription)	Motrin®
	Ibuprofen (200 mg. over the counter)	Nuprin, Motrin 1B®
	Ketoprofen	Orduis, Oruvall®
	Naproxen	Naprosyn®
	Naproxen sodium	Aleve, Anaprox, Aflaxen®
	Oxaprozin	Daypro®
Acetic acids	Diclofenac sodium	Voltaren®
	Diclofenac potassium	Cataflam®
	Etodolac	Lodine®
	Indomethacin	Indocin®
	Ketorolac tromethamine (intramuscular)	Acular, Toradol®
	Ketorolac (oral)	Toradol®
Ketones	Nabumetone	Relafen®
	Sulindac	Clinoril®
	Tolmetin sodium	Tolectin®
Fenamates	Meclofenamate sodium	Meclomen®
	Mefenamic acid	Ponstel®
Oxicams	Piroxicam	Dolibid®
Salicylic acid	Diflunisal	Feldene®
	Aspirin	
Pyrazolin acid	Oxyphenbutazone	Tandearil®
	Phenylbutazone	Butazolidin®

NSAIDs such as benoxaprofen, ketoprofen, oxaprozin, etodolac, ketorolac tromethamine, ketorolac and nabumetone, together with zwitterionic phospholipid, comprise still other particular embodiments of the invention, again both with and without neutral lipid.

In particular embodiments, the zwitterionic phospholipid in the compositions is dipalmitoyl phosphatidylcholine, phosphatidyl choline, or a mixture thereof.

The pharmacological, particularly anti-pyretic, activity of NSAIDs, is shown to be enhanced several-fold over preparations with similar doses without zwitterionic phospholipid. Surprisingly, the present inventors have found that combination of the afore-described nonsteroidal anti-inflammatory drugs with zwitterionic phospholipid dramatically enhances the anti-pyretic activity and potency of the drug, even at sub-therapeutically active doses, and in some cases from about 2-fold to about 6-fold relative to non-phospholipid containing preparations. Hence, the methods are expected to be particularly efficacious in reducing fever in a mammal having reduced tolerance for NSAID's.

Amounts ranging between one-tenth and one-half that typically necessary to elicit a fever-reducing response in a mammal may thus be realized employing the present inventive methods and compositions. In this regard, it is expected that amounts of between 2 mg/kg to about 300 mg/kg will provide fever-reducing therapeutic activity. Of course, the amount/dose used will depend in specific cases on the particular pharmacological characteristics of the NSAID or combination of NSAIDs included. Further defined ranges of the drug expected to provide the anti-pyretic activity herein disclosed range from between about 10 to about 150 mg/kg or about 20 or 50 mg/kg to about 150 mg/kg.

The present inventors have also observed the claimed compositions are useful for enhancing the platelet retarding activity of a non-steroidal anti-inflammatory drug. This method for inhibiting platelet aggregation comprises providing a non-covalently associated combination of zwitterionic phospholipid and an amount of non-steroidal anti-inflammatory agent that provides reduced inhibition of platelet aggregation in the absence of zwitterionic phospholipid. In some embodiments, this composition is essentially free of anionic phospholipid and/or includes an amount of anionic phospholipid that is a biologically inert component of the preparation. The amounts of non-steroidal anti-inflammatory drug employed as part of the composition, again, is relatively low, and may be further described as an amount that generally provides reduced pharmacological activity

in the absence of zwitterionic phospholipid. Again, the zwitterionic phospholipid of choice is, in some embodiments, DPPC, PC, or a combination thereof.

The compositions of the afore-described methods may further include a neutral lipid, such as a triglyceride. For a partial listing of representative neutral lipids, such as the triglycerides, reference is specifically made to U.S. Patent Number 4,950,656 and 5,043,329. Both saturated and unsaturated triglycerides may be employed in the present compositions, and include such triglycerides as tripalmitin (saturated), triolein and trilinolein (unsaturated). However, these particular triglycerides are listed here for convenience only, and are merely representative of a variety of useful triglycerides, and is further not intended to be inclusive.

Turning now to another aspect of the invention, methods for enhancing the analgesic activity of a non-steroidal anti-inflammatory drug are provided. These methods again comprise providing a non-covalently associated composition comprising zwitterionic phospholipid and an amount of a non-steroidal anti-inflammatory drug that provides reduced pharmacological activity in the absence of zwitterionic phospholipid. In some embodiments, these compositions are essentially free of anionic phospholipid, or include amounts of anionic phospholipid that are biologically inert. In particular embodiments, the nonsteroidal anti-inflammatory drug is one or more of those listed in Table 1. In particular embodiments, the NSAID is aspirin, salicylate, a salt thereof, or a combination thereof. While any of a number of different zwitterionic phospholipids may be employed in the composition of the method, in some embodiments, the phospholipid is dipalmitoyl phosphatidyl choline, phosphatidyl choline, or a combination thereof. In these and other embodiments of the described method, an anionic phospholipid that may be excluded or included only in biologically inert amounts is phosphatidyl glycerol (PG).

In still another aspect, the invention provides methods of enhancing the anti-inflammatory activity of non-steroidal anti-inflammatory drugs. The method comprises proving a non-covalently associated combination of zwitterionic phospholipid with an amount of a non-steroidal anti-inflammatory drug. The amount of NSAID in the composition is again defined as an amount that provides reduced pharmacological activity in the absence of zwitterionic phospholipid. In some embodiments, the method employs compositions that are essentially free of anionic phospholipids, such as the DPPG or PG,

or includes amounts of anionic phospholipid that are biologically and pharmacologically inert.

In particular embodiments, the composition is further defined as comprising an equimolar amount of NSAID and zwitterionic phospholipid. The present inventors' studies demonstrate the described combination of ingredients provides an enhancement of the anti-inflammatory activity and potency of these drugs compared to drug preparations that do not include zwitterionic phospholipid. Also demonstrated is the reduction in anti-inflammatory activity observed where pharmacologically active (i.e., non-biologically insert amounts) amounts of anionic phospholipid, such as PG (PI), are included in the preparation. Hence, preparations that include pharmacologically active amounts of DPPG or PI would not necessarily provide for the same enhanced anti-inflammatory activity, or the afore-described enhanced pharmacological activity (i.e., antipyretic or enhanced reduction in platelet aggregation activity), described in the present methods. Pharmacologically active amounts of other negatively-charged phospholipids would also not be contemplated as particularly useful in view of these results.

Turning now to still a further aspect of the present invention, a method for enhancing the antipyretic potential of sub-therapeutically effective amounts of nonsteroidal anti-inflammatory drug is disclosed. In some embodiments, the method comprises again combining zwitterionic phospholipid with an amount of non-steroidal anti-inflammatory drug to provide a noncovalently associated composition thereof. In some embodiments, the composition is further defined as essentially free of biologically active amounts of anionic (or negatively charged) phospholipid.

As used in the description of the above method, a sub-therapeutically effective amount of NSAID is defined as an amount that provides reduced antipyretic activity in the absence of a zwitterionic phospholipid. The enhancement in activity of low amounts of NSAID illustrated in the various *in vivo* studies disclosed herein demonstrate that while doses of aspirin of about 9 mg/kg in combination with the zwitterionic phospholipid, DPPC, did provide for a fever reducing (anti-pyretic) pharmacologic response, treatment with this same dose of NSAID without phospholipid did not.

The present invention also discloses particular pharmaceutical preparations. These pharmaceutical preparations are further described as suitable for enteral or oral

administration, and comprise a non-covalently associated combination of zwitterionic phospholipid, non- steroidal anti-inflammatory drug , and a pharmaceutically acceptable carrier. These compositions are formulated to provide a non-covalently linked composition that is further described as being essentially free of biologically active (pharmacologically active) amounts of anionic phospholipid, and in some embodiments, essentially free of DPPG.

In some applications, the non-steroidal anti-inflammatory drug is one or more of those listed in Table 1, such as naproxen, indomethacin, diclophenac, salicylate, aspirin, or any mixture thereof. In particular embodiments, the non-steroidal anti-inflammatory drug of choice is salicylate. While any variety of zwitterionic phospholipids may be employed alone or in combination with the described drugs, some of the representative phospholipids include phosphatidyl choline, dipalmitoyl phosphatidylcholine, phosphatidyl serine, other zwitterionic phospholipids, or mixtures thereof. The compositions may further include a neutral lipid, such as a triglyceride. Representative triglycerides are described in U.S. Patent 4,950,656, which reference is specifically incorporated herein by reference for this purpose. In particular embodiments, the pharmaceutical preparation is defined as comprising an equimolar amount of zwitterionic phospholipid and NSAID.

In yet another aspect, the present invention provides a method of maintaining and/or enhancing the therapeutic activity of a non-steroidal anti-inflammatory drug in the presence of a drug that reduces or inhibits gastric secretion. In one embodiment, the method comprises administering a combination of a non-steroidal anti-inflammatory agent and a zwitterionic phospholipid to an animal having received an anti-secretory agent. By way of example, drugs that reduce or inhibit gastric acid secretion in an animal include omeprazole.

The present invention also provides methods for maintaining or enhancing therapeutic activity of a non-steroidal anti-inflammatory agent in the presence of an H2 receptor blocker. In some embodiments, the method comprises administering the anti-inflammatory agent in association with a zwitterionic phospholipid to the animal having received an H2 receptor blocker, wherein the pH of the gastric secretions in the animal are maintained at fasting levels. As used in the description of the present invention, fasting

levels are defined as a pH of about a pH 1 to about pH 4. By way of example, H2 receptor blockers include Zantac, Tagamet, nizetidine, as well as those agents listed at Table 7.

The present invention further provides a method for maintaining and/or enhancing the bio-availability of a non-steroidal anti-inflammatory drug in an animal. In some embodiments, the method comprises administering a combination of a non-steroidal anti-inflammatory agent and a zwitterionic phospholipid to an animal having received an anti-secretory agent, wherein the pharmacological activity of the NSAID is greater than the pharmacological activity of the non-steroidal anti-inflammatory agent administered in the absence of a zwitterionic phospholipid. activity in an animal receiving an anti-secretory agent.

The present invention further provides a pharmaceutical preparation comprising a non-covalently associated combination of a non-steroidal anti-inflammatory agent, zwitterionic phospholipid, and an agent that reduces or inhibits gastric acid secretion. Such agents include, by way of example, antisecretory agents. By way of example, such anti-secretory agents include omeprazole. Such agents include both proton pump inhibitors (e.g. lansoprazole), and H2 receptor antagonists.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, anti-oxidant, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions and methods described herein is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

As used in the description of the present invention, the term "sub-therapeutically effective amount", particularly as it is used to described the amount of NSAID employed, is defined as an amount of the NSAID that provides reduced pharmacological (i.e., anti-pyretic) activity in the absence of non-covalent association with a zwitterionic phospholipid.

It is understood that as used in the present disclosure and appended claims, the terms "a" and "an," as in "an element" or "a molecule" are intended to include one or more

items or elements, and in no way limit the description or claimed element to one element or item.

The following abbreviations are employed in the description of the invention:

PI = phosphatidyl inositol

PC = phosphatidylcholine

PG = phosphatidylglycerol

L-NAME = N-nitro-L-arginine Methyl Ester

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1A. Photograph of test tubes containing 30 nM of the sodium salts of one of the five NSAIDs tested in chloroform in the absence and presence of 30 nM DPPC (naproxen, indomethacin, diclofenac, salicylate, aspirin).

FIG. 1B. The concentration of each NSAID in solution increases (quantified by UV absorption) in proportion to the molar equivalents of DPPC dissolved in chloroform (Naproxen = -▲-; Aspirin = -•-; Salicylate = -□-, Diclofenac = -△-; Indomethacin = -○-).

FIG. 2A. The DPPC-induced reduction in ASA's solubility in water was maximal when both reactants were present in equimolar concentration over a 60 min. incubation period at 25°C.

FIG. 2B. Time course at 25°C of the reduction in ASA's solubility in water induced by the presence of equimolar concentrations of DPPC. In contrast, the concentration of ASA in water was not significantly changed over time by the addition of an equimolar concentration of the anionic phospholipid, DPPG as a lipidic suspension (ASA only = -•-; DPPG/ASA = -□-; DPPC/ASA = -○-).

FIG. 3. The passive diffusion of ASA from water into cyclohexane is markedly accelerated by the presence of zwitterionic (DPPC) but not anionic (DPPG) phospholipids in the aqueous solution (ASA/DPPC = -○-; ASA/DPPG = -□-; DPPC = -•-; ASA = -x-).

FIG. 4A. The injurious potential of salicylate and ASA to induce gastric lesions (in rats subsequently challenged with 0.6N HCl), is remarkably decreased when the NSAIDs are intragastrically administered in association with DPPC (without DPPC = clear bar; with DPPC = hatched bar).

FIG. 4B. The injurious potential of non-salicylate NSAIDs to induce GI bleeding (in rats pre- and post-treated with L-NAME to increase the animal's susceptibility to the NSAID) is remarkably decreased when the drugs are intragastrically administered in association with DPPC (without DPPC = clear bar; with DPPC = hatched bar).

FIG. 4C. The anti-pyretic activity of the NSAID (aspirin - dose 90 mg/kg) is not diminished, and is augmented if administered as a lipidic suspension. Asterisk (*) represents a statistically significant difference between the values of rats treated with the NSAID alone (-DPPC) and those treated with the NSAID/DPPC complex (+ DPPC) (Saline = -•-; ASA = -□-; ASA/DPPC = -○-).

FIG. 5. Anti-inflammatory action of ASA and Phospholipon G™ (Phospholipid "G") as determined by the implanted string assay as described. Dosage is 90 mg/kg ASA. Each bar represents data for n=5 rats.

FIG. 6. Antipyretic activity of ASA\DPPC complex at a subthreshold ASA dosage (9.0 mg/kg) (Saline = -□-; ASA (9.0 mg/kg) = -▲-; ASA/DPPC (9 mg/kg) = -○-).

FIG. 7. Antipyretic activity of aspirin (ASA) alone when administered at doses which range from 2.5-90.0 mg/kg. In this and all subsequently figures the test agents were intragastrically administered 18 hrs after the rats were subcutaneously injected with 2g/kg Brewer's Yeast to induce a 0.5-1.0°C increase in body temperature. It can be appreciated that doses of aspirin of <10 mg/kg failed to reduce the fever during the 4 hour study period (Saline = -◇-; ASA, 2.5 mg/kg = -□-; ASA, 5 mg/kg = -○-; ASA, 10 mg/kg = -△-; ASA, 20 mg/kg = -■-; ASA, 45 mg/kg = -•-; ASA, 90 mg/kg = -▲-).

FIG. 8. In contrast to the above pattern aspirin (ASA) when complexed with an equimolar concentration of dipalmitoylphosphatidylcholine (DPPC) effectively reduced fever at a dose of 9.0 mg/kg, whereas the anionic phospholipid, DPPG, failed to augment aspirin anti-pyretic activity at this same sub-threshold dose. This figure also demonstrates that anionic phospholipid is essentially biologically inert in terms of enhancing the anti-pyretic action

of aspirin (ASA, 9.0 mg/kg = -•-; ASA/DPPC, 9.0 mg/kg = -□-; ASA/DPPG, 9.0 mg/kg = -○-; n=5/grp; * = p <0.05 vs ASA; † = p <0.05 vs ASA/DPPG).

FIG. 9. ASA at a subthreshold dose of 4.5 mg/kg, which had no antipyretic activity alone, effectively reduced fever when complexed with an equimolar concentration of DPPC (H_2O = -□-; H_2O/DPPC (4.5 mg/kg) = -▲-; ASA (4.5 mg/kg) = -⊖-; ASA/DPPC (4.5 mg/kg) =--○--).

FIG. 10. Compilation of all the data at subthreshold doses (2.5-9.0 mg/kg) of ASA. ASA alone (open symbols) data demonstrates the enhancement of antipyretic activity when ASA was complexed to DPPC (closed symbols) (Saline = -*-; ASA, 9mM = -□-; ASA, 4.5 mM = -○-; ASA, 2.5 mM = --△--; ASA/DPPC, 9mM = -■-; ASA/DPPC, 4.5mM = -•-; ASA/DPPC, 2.25 mM = --▲--).

FIG. 11. Dose-response analysis of the antipyretic activity of ASA alone and the ASA/DPPC (equimolar ratio) complex 1 hr (FIG. 11) (ASA = -•-; ASA/DDPC = -□-) after intragastric administration. The potency of the ASA, as reflected by the ED_{50} is increased ~ 10 fold when it is administered with the zwitterionic phospholipid.

FIG. 12. Dose response analysis of the anti-pyretic activity of ASA alone and the ASA/DPPC (equimolar ratio) complex 2 hours after intragastric administration.

FIG. 13. Effect of varying the ASA:DPPC ratio from 1:1 on antipyretic activity at 1 hr post intragastric administration. It can be appreciated that the ability of the zwitterionic phospholipid to enhance the antipyretic activity of ASA was lost when the molar concentration of DPPC was increased (from unity) by a factor of 4 or decreased by a factor of 10 (ASA =9.01 mg/kg).

FIG. 14. Effect of varying the ASA:DPPC ratio from 1:1 on the antipyretic activity of the complex 2 hours post intragastric administration (Legends same as in FIG. 13) (ASA = 9.01 mg/kg).

FIG. 15. At subthreshold doses the antipyretic efficacy of ASA could be clearly enhanced (0-120 min. post-administration), if the NSAID was administered as a microemulsion containing DPPC and tripalmitin (TP). In all cases the molar ratio of ASA:DPPC was maintained at 1:1, whereas the TP was administered in excess (weight ratio of DPPC:TP = 1:4) (n = 5/grp; * = p>0.05 vs ASA/DPPC; ASA/DPPC 1 mg/kg = -•-; ASA/DPPC/TP,1 mg/kg = --○--; ASA/DPPC, 9 mg/kg = --▲--; ASA/DPPC/TP, 9 mg/kg = --△--).

FIG. 16. It can be appreciated that the addition of the neutral lipid provided a further enhancement of antipyretic activity, even over that of ASA/DPPC, as can best be seen with the 1 mg/kg subthreshold ASA dose (n = 10/grp; * = p<0.05 vs ASA/DPPC; ASA, 1 mg/kg = -◆-; ASA/DPPC = -○-; ASA/DPPC/TP = --•--).

FIG. 17. The ability of DPPC to promote the movement of aspirin from water into a lipidic cyclohexane phase (as a membrane model) is greatly accelerated by the presence of the neutral lipid, tripalmitin (TP) to form a microemulsion. This may provide an explanation why the presence of neutral lipids further promote the therapeutic potency of the NSAID/DPPC complex (DPPC + TP (1:4), 2.5mM + ASA, 10mM (sonicated) = -○-; DPPC, 2.5mM + ASA, 10mM (sonicated) = -□-; TP, 2.5 mM + ASA, 10 mM = -■-).

FIG. 18. The ability of DPPC to enhance the anti-pyretic activity of ASA was seen with indomethacin (Saline = -•-; indomethacin (10 mg/kg) = -□-; indomethacin (10 mg/kg)/DPPC = -○-).

FIG. 19. Demonstrates the ability of DPPC to enhance the anti-pyretic activity of naproxen (25 mg/kg dose) (Saline = -•-; naproxen (25 mg/kg) = -□-; naproxen (25 mg/kg)/DPPC =-○-).

FIG. 20. Demonstrates the ability of DPPC to enhance the anti-pyretic activity of diclofenac, 10 mg/kg (Saline = -□-; diclofenac (10 mg/kg) = -□-; diclofenac (10 mg/kg)/DPPC = -○-; n = 5/grp; * = p<0.05 vs. DICLO).

FIG. 21. Demonstrates the ability of DPPC to enhance the anti-pyretic activity of salicylic acid (SA) (70 mg/kg dose) (Saline = - -; SA (70 mg/kg) = -○-; SA (70 mg/kg)/DPPC = -•-; n = 5/grp, * = p<0.05 vs. SA/DPPC).

FIG. 22. Antipyretic activity of ASA (18 mg/kg) and ASA/DPPC complex with IP injection of omeprazole (150 mg/kg) two hours prior to NSAID dosage. ASA (saline) = - • -; ASA/DPPC (Sal) = - ○ -; ASA (omeprazole) = --▲--; ASA/DPPC (omep) = --△--; n = 5/grp; * = p<0.05 vs. ASA (omep); † = p<.05 vs. ASA (Sal).

FIG. 23. Antipyretic activity of ASA (18 mg/kg) and ASA/DPPC complex with IP injection of Ranitidine (2 mg/kg) l hour prior to NSAID dosage. ASA (saline) = - • -; ASA (DPPC) = - ○ -; ASA (Ranitidine) = --▲--; ASA/DPPC (Ranitidine) = --△--; n = 5/grp; * = p<0.05 vs. ASA (rani); † = p<0.05 vs. ASA (Sal).

FIG. 24. Demonstrates that aspirin's ability to acutely induce gastric lesions was reduced to a variable degree in rats pretreated with an antisecretory agent (see open bars), with omeprazole and cimetidine being most protective. In contrast, antisecretory drugs consistently protected against aspirin-induced gastric lesions if the NSAID was chemically associated with DPPC (see stippled bars).

Pretreatment of rats with any one of the antisecretory agents, prior to the intragastric administration of phospholipid-complexed aspirin, afforded the animals full protection against NSAID-induced gastric lesion in this ulcer model.

FIG. 25. Indicates that the combination therapy of antisecretory agents with indomethacin/DPPC proved superior to either the acid-inhibitor or the phospholipid alone in the prevention of acute NSAID-induced GI bleeding over an 18 hour period.

FIG. 26A-26C. Indicates that the antipyretic activity of aspirin, administered at a dose of 18 mg/kg, was clearly attenuated if the rats were pretreated with antisecretory doses of omeprazole (FIG. 26A), ranitidine (FIG. 26B), or cimetidine © (FIG. 26C). However, the antisecretory agents' ability to attenuate aspirin's antipyretic activity was completely reversed if the NSAID was preassociated with DPPC, to increase the drug's lipophilic characteristics.

FIG. 27. Demonstrates that the proton pump inhibitor, omeprazole had an effect of reducing the antipyretic activity of indomethacin, administered at a dose of 4 mg/kg, which once again could be overcome if the NSAID was administered as a complex with DPPC.

FIG. 28A-28B. Demonstrate that the analgesic activity of both aspirin and indomethacin was similarly attenuated if rats were pretreated with an antisecretory agent. Under control conditions the "pain pressure threshold" of the inflamed paw increased approximately two-fold, from 40-60 mm Hg to 90- 100 mm Hg, 2 hours after rats were administered the ED_{50-80} dose of either NSAID alone. This analgesic action of both aspirin and indomethacin was clearly reduced or abolished altogether if the rats were pretreated with one of the potent antisecretory agents (compare open-bars). Furthermore, the analgesic activity of both aspirin and indomethacin could be restored in rats pretreated with an inhibitor of gastric acid secretion, if the NSAIDs were administered in the lipid-associated state (compare stippled- to open-bars for each antisecretory agent). An analgesic activity pattern very similar to that described above were observed 26 hours after rats received three consecutive

treatments of aspirin or indomethacin ± DPPC (at 0, 6, and 24 hours) preceded 1 hour earlier with an injection of an antisecretory agent. Once again the NSAID-induced analgesia was significantly reduced by pretreatment with one of the three antisecretory agents which could be overcome if the NSAIDs were chemically associated with DPPC. It is also important to note that at both time-points in the absence of one of the above antisecretory agents, both the antipyretic and analgesic activities of aspirin and indomethacin were at least as good and most cases enhanced when the drug was complexed to DPPC over that observed with the NSAID alone (compare stippled to open-bars of rats treated with saline).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

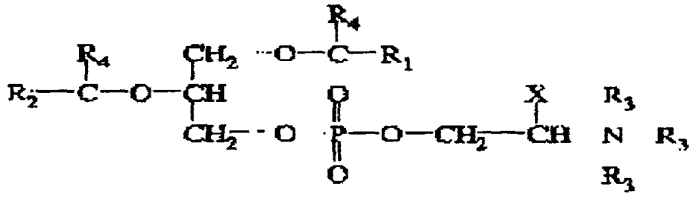
The present invention arises from the discovery that aspirin and other NSAIDs chemically associate with zwitterionic phospholipids, such as dipalmitoyl phosphatidylcholine (DPPC). Important embodiments of the invention include methods of enhancing the various therapeutic activities of NSAID's, such as antipyretic, anti-inflammatory, and analgesic pharmacological activities. Surprisingly, these responses are observed without evidence of gastrointestinal side effects as demonstrated in acute and chronic animal models of NSAID injury in the present disclosure.

The data disclosed herein indicates that NSAIDs have the capacity to chemically associate with zwitterionic phospholipids in both organic and aqueous solvent systems, and in doing so, both classes of molecules undergo profound changes in their physical and chemical properties. Complex formation in aqueous solvent systems is shown to occur more efficiently at pH values at or slightly below the pKa of the NSAID. Therefore, without being bound by any theory, it is contemplated that the intermolecular bonding is not covalent, but is instead both hydrophobic and electrostatic, with the latter association being between the negatively charged carboxyl group of the NSAID and the positively charged nitrogen of the phospholipid. This possible interaction has been supported by computer assisted molecular modeling programs (Quanta and CHARMm), which also indicate that the NSAID/phospholipid complex has a lower molecular free energy (greater thermodynamic stability) than either reactant alone.

According to the present invention, it would be expected that orally administered NSAIDs would chemically associate with the intrinsic zwitterionic phospholipids that coat the luminal aspects of the mucus gel layer of the upper GI tract. A description of luminal aspects of the mucus gel layer is described in Goddard *et al.*, (1990) and Kao et al. (1990). While not intending to be limited to any particular mechanism of action, this intermolecular association is thought to be the basis for the attenuation in surface activity and/or the loss of stability of the interfacial extracellular phospholipid layer, and to culminate in an NSAID induced decrease in mucosal hydrophobicity and barrier properties.

Description of the Lipid Compounds

The phospholipids of the present invention are characterized generally by the formula:



wherein R_1 and R_2 are saturated or unsaturated substitutions ranging from 8 to 32 carbon atoms; R_3 is H or CH_3, and X is H or COOH; and R_4 is = 0 or H_2.

As will be appreciated by those of skill in the art, the foregoing chemical structure defines a zwitterionic phospholipid structure and embraces a wide range of phospholipids, including but not limited to phosphatidyl chorines, phosphatidyl ethanolamines, phosphatidyl serines and various other zwitterionic phospholipids.

Other phospholipids that may be employed in the composition include: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin, and other ceramides, and mixtures thereof.

Description of the NSAID's

A few of the non-steroidal anti-inflammatory agents that may be employed in the methods and compositions disclosed herein include by way of example: pyrazolones,

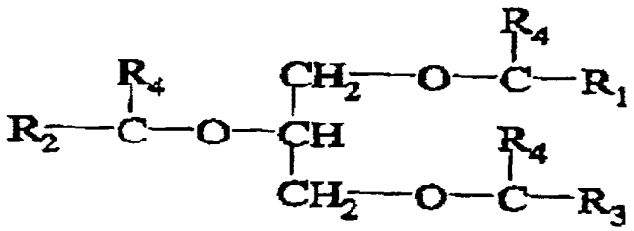
phenylbutazone (4-butyl-1,2-diphenylpyrazolidine-3,5-dion), and oxyphenbutazone (4-butyl-2-(4-hydroxyphenyl)-1-p-phenylpyrazolidine-3,5-dion), salicylic acid derivatives such as salicylic acid salicylic acid amide, acetyl-salicylic acid, benorilate (4-acetamidophenyl-o-acetylsalicylate), and diflunisal (5-(2,4-difluorophenyl)-salicylic acid); Indoles, especially indometacine and its analogs such as indometacine (1-(p-chlorobenzyl)-5-methoxy-2-methylindole acetic acid), glucametacine (1-(p-chlorobenzoyl-5-methoxy-2-methylindole-3-yl acetic acid glucose amide), acemetacine (1-(p-chlorobenzoyl)-5-methoxy-3-methylindole-3-acetic acid-glycolic acid-ester), and sulindac (5-fluor-2-methyl-1-p-(methylsulphenyl)-benzylidene-indene-3-acetic acid); Phenyl acetic acid or phenyl propionic acid derivatives such as ibuprofen (2-(4-isobutylphenyl)-propinic acid); naproxen (2-(6-methoxy-2-naphthyl)-propinic acid), alclofenac (4-allyloxy-3-chlorophenyl-acetic acid), ketoprofen (2-(3-benzylphenyl)benzoic acid), diclofenac (2-(2,6-dichlorophenylamino)-phenylacetic acid), fenoprofen (2-(3-phenyloxyphenyl)-acetic acid), tolmetin (1-methyl-5-(p-toluyl)-pyrrole-2-yl-acetic acid), flurbiprofen (2-2-fluorobiphenyl-4-ye-proprionic acid), and suprofen (p-2-thenoyl-hydratropic acid) phenyl-propionic acid); Anthranilic acids and their nitrogen analogs such as flufenamino acid (N-(m-trifluoromethylphenyl)-anthranilic acid), mefenamino acid (N-(2,3-dimethylphenyl)-anthranilic acid), and niflumin acid (2-(3-trifluoromethylaminolino)-nicotinic acid).

Phospholipid compounds found to be particularly useful in the practice of the present invention are dilinoleoyl phosphatidylcholine (DLL-PC), dipalmitoyl phosphatidylcholine (DPPC) and egg phosphatidylcholine (Egg-PC or PC_e). In DPPC, a saturated phospholipid, the saturated aliphatic substitution R_1 and R_2 are CH_3--$(CH_2)_{14}$, R_3 is CH_3 and X is H. In DLL-PC, an unsaturated phospholipid, R_1 and R_2 are CH_3--$(CH_2)_4$--CH=CH--CH_2--CH=CH--$(CH_2)_7$, R_3 is CH_3 and X is H. In Egg PC, which is a mixture of unsaturated phospholipids, R_1 primarily contains a saturated aliphatic substitution (e.g., palmitic or stearic acid), and R_2 is primarily an unsaturated aliphatic substitution (e.g., oleic or arachidonic acid).

Description of the Neutral Lipids

Neutral lipids form another component of some embodiments of the compositions described herein. This class of lipids include the triglycerides.

The triglycerides useful in the practice of the present invention are generally characterized by the formula:



wherein R_1, R_2 and R_3 are each saturated or unsaturated substitutions ranging from 4 to 32 carbon atoms; and R_4 is either ==0 or H_2.

As will be appreciated, this structure embraces a wide range of triglycerides, both saturated and unsaturated, and include, for example, triglycerides such as tripalmitin (saturated), triolein and trilinolein (both unsaturated). A further listing of saturated and unsaturated fatty acids that can be esterified or ether-linked to the triglyceride in question is provided in U.S. 5,032,585, which is specifically incorporated herein by reference.

In a particular anticipated pharmaceutical preparation, the preparation will be provided in a pill form suitable for human ingestion, and contain about 2 to about 300 mg per kg aspirin or salicylate, together with an equimolar amount of PC, DPPC, or a combination thereof, or any other zwitterionic phospholipid. In the described methods, the compositions include the NSAID and the zwitterionic phospholipid in molar ratios ranging from about 1:0.1 to about 1:20, and preferably from about 1:0.5 to about 1:2. In a most preferred embodiments, the ingredients are included in a molar ratio of about 1:1.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit aid scope of the invention.

EXAMPLE 1
GI LEGIONARY AND BLEEDING - EFFECT OF NSAID AND ZWITTERIONIC PHOSPHOLIPID COMPOSITIONS

The present example demonstrates the utility of the present invention for providing compositions that reduce the GI-related side-effects commonly associated with NSAIDS.

In order to retard the ability of NSAIDs to interact with the extracellular phospholipid lining of the mucus gel layer, several NSAIDs were preassociated with zwitterionic phospholipids and the effect was determined in various rat ulcer models. The present example shows that ability of the NSAIDs to induce acute and/or chronic GI lesions and bleeding was remarkably decreased when the drugs were administered as a complex with DPPC or related phospholipids. Surprisingly, the anti-pyretic and anti-inflammatory activity of aspirin appeared to be consistently enhanced when associated with zwitterionic phospholipids. This is in contrast to previous side effects of other formulations that reportedly suffer from reduced therapeutic efficacy or onset (Alpsten *et al.*, 1982; Mojaverian *et al.*, 1987).

Ulcer Models

Gastric lesions were acutely induced in rats in accordance with the following techniques. For the salicylate-based NSAIDs, fasted male Sprague Dawley rats (150-200g) were intragastrically injected with saline (control), ASA or salicylate, or the drugs preassociated with an equimolar concentration of DPPC (all solutions adjusted to a pH of 3.1). Ten minutes later, the rats were intragastrically challenged with 1 ml of 0.6 N HCl. Gastric lesions were macroscopically scored 60 minutes later in accordance with a previously outlined method (Lichtenberger *et al.*, 1983, incorporated herein by reference).

In order to investigate the effects of non-salicylate NSAIDs to induce GI bleeding, fasted rats were subcutaneously injected with N-nitro-L-arginine Methyl Ester (L-NAME), 1 hr. before and 1 and 6 hrs. after intragastrically receiving 1 ml of the NSAIDs; indomethacin, diclofenac, and naproxen administered alone and in association with an equimolar concentration of DPPC. The Nitric Oxide synthesis inhibitor, L-NAME, is administered before and after the NSAID to increase the rat's sensitivity to the drug in accordance with the method of Chen et. al., *Gastroenterology* **104**: A53, 1993). Eighteen

hours after receiving the NSAID, the distal half of the intestine was excised and flushed with 10 ml of saline. The hemoglobin (Hb) concentration of the intestinal perfusate was measured as an estimate of GI bleeding in accordance with a previously described method (Lichtenberger *et al.*, 1983).

Rats were treated with ASA over a two week period to investigate the chronic effects of NSAID exposure (in the presence and absence of phospholipids) on hematocrit, gastric mucosal hydrophobicity, and granuloma formation. In order to ensure that the rat had an empty stomach prior to receiving the daily intragastric dose of ASA, they were placed on a reverse lighting schedule (9AM/lights off; 5PM/lights on), and were only provided access to chow during the day (dark period). The test solutions (saline, ASA and ASA/Phospholipon 90G complex) were intragastrically administered between 8AM - 9AM daily during the two week study period. In these chronic exposure experiments, ASA was complexed with an equimolar concentration of Phospholipon 90G (purified soya lecithin, prepared and obtained from Nattermann GmbH of Cologne, Germany) instead of DPPC. At the completion of the study period, blood was collected into a capillary tube for the determination of hematocrit and the stomach was excised for contact angle analysis.

One of a number of NSAIDs was intragastrically administered to rats alone or preassociated with an equimolar concentration of DPPC or Phospholipon 90 G (purified soya lecithin, prepared by Nattermann GmbH of Cologne Germany) in several models of acute and chronic injury of the upper GI tract. Two contrasting animal models were employed to determine the ability of DPPC to protect against acute NSAID injury to the GI tract. For salicylate-based NSAIDs, which primarily induce stomach injury, gastric lesions were scored in fasted rats who were initially treated with aspirin or salicylate alone or complexed with DPPC and challenged 10 minutes later with a supra-physiological dose of HCl. For non-salicylate NSAIDs (indomethacin, diclofenac and naproxen) which predominantly induce injury to the mid-distal regions of the small intestine, the quantity of intraluminal blood was assessed in the distal half of the small intestine of rats who were pre- and post-treated with the Nitric Oxide synthetase inhibitor, L-NAME (N-nitro-L-arginine methyl ester).

The results, shown in FIG. 4A and FIG. 4B, respectively, clearly demonstrate (in rats sensitized to the drugs) that the injurious potential of both salicylate-based and non-

salicylate NSAIDs to induce acute GI lesions and bleeding is significantly reduced, by >85%, if the NSAID is preassociated with the zwitterionic phospholipid prior to administration. Similarly, daily administration of aspirin to rats over a 2 week period resulted in a significant fall in both hematocrit and gastric mucosal hydrophobicity, which was prevented in rats that received the aspirin/Phospholipon G complex (Table 2).

Table 2. Effect of DPPC on Aspirin's Chronic GI Side Effects

Group	Gastric Mucosal Hydrohobicity (degrees, Contact Angle)[b]	Hematocrit
Saline (Control	52.2 ± 2.7(6)	51.7 ± 0.4 (10)
Aspirin (90 mg/kg)	15.8 ± 6.6[c](6)	47.8 ± 0.2[c](10)
Aspirin + Phospholipon G	55.3 ± 5.8[c,d](6)	53.1 ± 0.4[c,d](10)

[b] Gastric mucosal hydrophobicity was measured by contact angle analysis as described in Hills *et al.*, 1983; Goddard *et al.*, 1987; Goddard *et al.*, 1990.

[c] $p<0.05$ in comparison to saline-treated control values.

[d] $p<0.05$ in comparison to values of aspirin-treated rats.

(n) number of rats/group

EXAMPLE 2 - SOLUBILITY STUDIES; COMPLEX FORMATION BETWEEN NSAIDS and ZWITTERIONIC PHOSPHOLIPIDS

To demonstrate the effect of DPPC on the solubility of the sodium salts of the five NSAIDs (naproxen, indomethacin, diclofenac, salicylate and aspirin) in chloroform, each NSAID was added to chloroform at a 30 nM final concentration. DPPC was dissolved in the chloroform that was contained in half the tubes at a final concentration which ranged between 5 - 40 nM, prior to the addition of the NSAID salt. DPPC, as well as PC and other zwitterionic phospholipids useful in the practice of the invention, may also be dissolved in other organic solvents, such as ethanol, in the practice of the present invention. The tubes were gently mixed at 25°C for 16 hrs, after which they were photographed and/or centrifuged (2,000g for 15 min) and the supernatant collected to determine the concentration of NSAID in solution. The latter was assessed by measuring the NSAID's

UV absorbance at 290 nm, and comparing these readings to the appropriate standard curves for each NSAID. It should be noted that for each of the five NSAIDs a linear relationship existed between the drug's concentration (nM) in water and the UV absorbance reading. The equations for the regression lines for each of the NSAIDs were as follows: aspirin, y = 2.603x -0.004, r= 0.999; salicylate, y = 8.780x + 0.123, r = 0.999; indomethacin, y = 18.325x + 0.156, r= 0.999; diclofenac, y = 21.523x + 0.008, r = 0.999; and naproxen, y = 3.732x + 0.005, r =0.999. In all cases the readings for the unknowns fell within the linear portion of the standard curves. Further, the presence of DPPC in the solvent did not interfere with these analyses, as it failed to contribute to the W absorbance reading in the absence of the NSAID.

To assess the effect of DPPC on the solubility of the sodium salt of ASA in water, ASA was dissolved in water at a final concentration of 30 nM (pH adjusted to 6.0), and its intrinsic fluorescence (290 nm/excitation; 406 nm/emission) was monitored. DPPC was present as a lipidic suspension in half the tubes at a final concentration which ranged between 15 - 60 nM. The tubes were gently mixed at 25°C for the desired incubation period, after which they were centrifuged (2,000g for 15 min) and the supernatant collected to determine the concentration of ASA in solution. Once again a linear relationship (y = 14.02 + 0.353, r = 0.999) was found between the fluorescent reading and the concentration of aspirin in solution.

It can be appreciated from FIG. 1A and FIG. 1B that the sodium salts of these NSAIDs are insoluble in chloroform unless an equimolar or greater concentration of DPPC is added to the organic solvent, at which point complete solvation takes place. Conversely the sodium salts of the NSAIDs are readily soluble in either saline or water, and are removed from solution as a complex within minutes after an equimolar concentration of DPPC is added as a lipidic suspension. The solubility of aspirin in saline can be followed either by monitoring its intrinsic fluorescence or radioactivity (employing ^{14}C-labeled aspirin). FIG. 2A and FIG. 2B demonstrate that the injection of DPPC into an aqueous solution results in the precipitation of the NSAID, presumably as a complex with the phospholipid. The rapid change in solubility of the NSAID in both the organic and aqueous solvent systems does not occur if DPPC is substituted by the anionic phospholipid, dipalmitoylphosphatidylglycerol (DPPG).

EXAMPLE 3 - GRANULOMA FORMATION AND ENHANCED ANTI-INFLAMMATORY ACTIVITY

The present example demonstrates the utility of the present methods for enhancing the anti-inflammatory activity of ASA accomplished when ASA, or other non-steroidal anti-inflammatory drug complexed with Phospholipon 90G. A foreign-body granuloma model widely used by those of skill in the art to assess anti-inflammatory action was employed.

The inventors used the rat model of foreign-body granuloma formation. This model is recognized by those of skill in the art as a representative model for granuloma formation and anti-inflammatory activity, as described in Ucelay *et al.*, (1988); and Castro *et al.*, (1980). These references are specifically incorporated herein for the purpose of providing details associated with the use of this model.

Methods

The above model has been successfully employed in both rats and invertebrates to quantify this most basic component of a tissue's response to injury (Ucelay *et al.*, 1988; Castro *et al.*, 1980; Clatworthy *et al.*, 1994, each incorporated herein by reference). Sterile tared cotton string was surgically implanted (bilaterally) under the abdominal skin of ether anesthetized rats on day 1 of the study period, and then randomly placing rats in a group to be daily treated with saline, aspirin or the NSAID complexed with Phospholipon 90G over a two-week period.

At the completion of the study period the string with the adherent granuloma tissue was surgically dissected from the euthanized rats and dried in a vacuum for several days at room temperature, until a baseline dry weight was obtained. The difference between this value and the initial dry weight of the string prior to implantation divided by the latter value provided an estimate of the weight of granuloma tissue. This technique proved to be very reproducible and accurate, as determined both by the close agreement between the changes in weight of the two pieces of string that were implanted contralaterally in each animal (< 12.5% difference in values between the right and left string); and the low variance (< 8%) in values of granuloma formation within a group of animals.

Improvement in the anti-inflammatory efficacy of ASA was observed over a 2 week study period. This effect is seen when the NSAID was administered as a complex with Phospholipon G at concentrations below the maximally effective dose for this particular drug (see Table 3, FIG. 5).

Table 3. Effect of Aspirin ± DPPC Formulations on Foreign-Body Granuloma Formation.

Group	± DPPC	n	Granuloma Formation[a]
Saline (Control	-	5	3.20 ± 0.10
ASA (90 mg/kg)	-	5	1.90 ± 0.10[b]
	+	5	1.4 ± 0.10[b,c]
ASA (140 mg/kg)	+	6	1.00 ± 0.15[b]

[a] The values represent the dry weight of the string with adherent granuloma tissue -dry weight of string/dry weight of the string.

[b] $p<0.05$ in comparison to saline-treated control values.

[c] $p<0.05$ in comparison to values of rats treated with NSAIDs alone.

EXAMPLE 4 - CONTACT ANGLE ANALYSIS

The present example is provided to describe the model which was used to examine the surface tension reducing action of the compositions of a combination of non-steroidal anti-inflammatory agent and zwitterionic phospholipid.

Contact angle analysis was performed with the use of a goniometer on excised gastric mucosal tissue, that was lightly blotted and dried, as previously outlined (Hills *et al.*, 1983; Goddard *et al.*, 1987; Goddard *et al.*, 1990, each incorporated herein by reference). Briefly, this was accomplished by applying a droplet of water (~5μl) to the tissue surface, and employing the telescopic eyepiece of the goniometer to measure the maximal angle that is dissected at the triple point, where the solid/liquid/ and air interface meet.

EXAMPLE 5 - ANTIPYRETIC ACTIVITY

An established rat fever model was used in the present example to demonstrate the utility of the invention for enhancing the anti-pyretic activity of a NSAID by combining these class of agents with a phospholipid.

The rat fever model involves the injection of rats with Brewer's yeast (2g/kg, s.c.) to induce an increase in fever of 0.5 -1.5°C. These models are described in Adams *et al.* (1968) and Ucelay *et al.* (1988), which references are specifically incorporated herein for this purpose. The animals were intragastrically treated (instilled) with either saline, 90 mg/kg ASA, or 90 mg/kg ASA preassociated with an equimolar concentration of DPPC. Similar antipyretic analyses were performed with the sodium salts of the following NSAIDs: diclofenac (10 mg/kg), indomethacin (10 mg/kg) and naproxen (30 mg/kg), alone and complexed with an equimolar concentration of DPPC. All test solutions were titrated to a pH of 4.5 prior to intragastric administration. Rectal temperatures were monitored in conscious, restrained rats at all indicated times. This technique provided a very reliable and reproducible estimate of the antipyretic activity of NSAID formulations as indicated by the fact the variance within a group was low, with the standard errors < 5% of the mean values, and the fact that the difference in mean temperature values for a given group varied < 2 % between separate experiments.

EXAMPLE 6 - PHARMACEUTICAL COMPOSITIONS

The complexes described in the present example are preferably for oral administration, for example, with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds (i.e., NSAID and Zwitterionic phospholipid and/or neutral lipid) may be incorporated with excipients or carriers and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain enough active NSAID compound to achieve an effective plasma level of the active drug. Aspirin, for example, would be provided in doses of from about 10 mgm, or about 20 mg, or 32.5 mg or even up to 60 mg, or 300 mgs/kg would be contained in each tablet or dose, where employed for

administration to animals having a weight of about 60 kg - 70 kg. The dose will vary depending on the NSAID or combinations of NSAIDs used. The amount of the NSAID in particular preparations is more generally described as an amount of NSAID or combination of NSAIDs effective to provide a pharmacologically active plasma concentration of the drug when used in combination with zwitterionic phospholipid. These amounts are determinable by one of ordinary skill in the pharmaceutical arts given the data disclosed herein, and general pharmaceutical references such as Remingtons Pharmaceutical Sciences.

The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose, aspartame or saccharin may be added, or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both.

A syrup of elixir may contain the active compounds sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor, again formulated for oral administration. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

The present compositions may also be formulated as injectable formulations, or as formulations suitable for enteral administration, according to those techniques known to those of ordinary skill in the medicinal arts.

EXAMPLE 7
LIPID-PERMEABILITY OF NSAID/PHOSPHOLIPID COMPLEX

The present example illustrates the utility of the invention for providing compositions and methods for enhancing the big-absorption and big-availability of

NSAID's. The diffusion of aspirin (alone and complexed with phospholipid) from water into cyclohexane was used as a model to estimate the membrane permeability of the drug.

Permeability Analysis

The sodium salt of ASA (also salicylate) was dissolved in 5 ml of water at a final concentration of 100mM (pH adjusted to 6.0) and gently stirred at 25°C. An equal volume of cyclohexane was layered over the aqueous solution and the entry of the NSAID into the organic phase was monitored fluorometrically over time. In order to determine the effect of phospholipid association on the lipid permeability of the NSAID, ASA (or salicylate) at the above concentration was sonicated in the presence of 0.5 mM phospholipid (DPPC or DPPG) in water (adjusted to a pH of 7), and its rate of diffusion into the cyclohexane phase was measured fluorometrically. This was accomplished by removing 1 ml of the top phase chloroform solution by pipette, injecting it into the cuvette to obtain the fluorescence reading, and returning the sample to the incubation vessel to assure that the volume did not change. This entire process could be completed in < 30 seconds.

In these studies, the concentration of NSAID:phospholipid in water was adjusted to a molar ratio of 200:1, creating a large driving force to promote NSAID flux into the hydrocarbon phase, and minimizing the turbidity encountered with high phospholipid concentrations.

Results

Under neutral conditions, the passive diffusion of aspirin across an aqueous - hydrocarbon interface, as assessed fluorometrically, was negligible unless it was chemically associated with the zwitterionic phospholipid, DPPC (See Figure 3). Furthermore, this increase in the flux rate into the organic phase, was simply not a consequence of liposomal encapsulation since the NSAID failed to enter cyclohexane if the anionic phospholipid, DPPG was substituted for DPPC. These studies also revealed that DPPC promoted the flux of sodium-salicylate from the aqueous to the organic phase in a similar manner.

EXAMPLE 8
ANTI-INFLAMMATORY AND ANTI-PYRETIC ACTIVITY OF NSAID/PHOSPHOLIPID COMPLEX

This example demonstrates the utility of the present invention for enhancing the fever-reducing potential of the NSAID (20 mg/kg dose) when chemically associated with a zwitterionic phospholipid. The ability of NSAIDs (administered alone and complexed with DPPC) to reduce fever in rats was determined. Fever was induced 18 hrs prior to drug treatment by the subcutaneous administration of Brewer's yeast (Adams *et al.*, 1968; Ucelay *et al.*, 1988).

The results (See FIG. 4C) indicate that the anti-pyretic activity of the ASA/DPPC complex was significantly greater than that of the NSAID alone, at all time periods examined.

The enhancement in anti-pyretic activity of the NSAID/DPPC complex compared to the NSAID alone over the first three hours after intragastric administration, was observed to a lesser degree with sodium salts of the following drugs; diclofenac (-0.29°C), indomethacin (-0.28°C), and naproxen (-0.30°C).

EXAMPLE 9
ENHANCEMENT OF ANTIPYRETIC ACTIVITY OF ASA/DPPC COMPLEX AT SUBTHRESHOLD ASA DOSAGE

The antipyretic activity of the ASA/DPPC complex was determined in rats as in Example 8 (supra), this time at much lower doses, more than 2 times lower than in Example 8. In the present example, a dosage of 9.0 mg/kg ASA was administered either alone or complexed with DPPC. The data from this study is summarized in FIG. 6.

As can be seen in FIG. 6, ASA alone does not have significant antipyretic activity at this dosage level, however ASA complexed with DPPC does have significant antipyretic activity over a five hour period. The dosage level in the present example is a 10-fold reduction of the standard dosage of 90 mg/Icg, as used in Example 8, and reported in FIG. 4C.

Based on the recommended human dosages of 90 mg/kg for juvenile rheumatoid arthritis, or 325 to 650 mg for antipyretic or analgesic treatment in adults (See pages 1110-1111, Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Company,

Easton, Pennsylvania, 1990, incorporated herein by reference), it is expected that ASA complexed with DPPC, or other zwitterionic phospholipid, at a 10-fold lower dosage, i.e. approximately 9 mg/kg for juvenile rheumatoid arthritis or 32.5 to 65 mg for antipyretic activity in adults, would be as effective as the normal dosage of ASA alone to provide fever-reducing activity.

EXAMPLE 10
ANALGESIC EFFECT OF COMPLEXED-NSAIDS

It is contemplated that, in light of the demonstrated enhancement of the effects of NSAIDs (anti-inflammatory and antipyretic) when complexed with a zwitterionic phospholipid, or even further administered in combination (i.e., in a mixture) with a neutral lipid (triglyceride) that the analgesic effects of these drugs will also be enhanced when complexed with the phospholipids. Two established pain tests, using rats, may be employed to demonstrate enhancement of this effect. Tail retraction occurs in response to a noxious stimulus that generates pain. Tail retraction response may be observed and timed in rats given a placebo in order to determine a base line reaction time. If after receiving either DPPC-complexed or uncomplexed aspirin the time latency between pain induction (with a laser heat source) and tail retraction increases, this is a direct reflection of the analgesia. Retraction times will be compared between placebo and aspirin-treated rats. The test will be conducted with varying dosages and varying times after the administration of the placebo, aspirin, and/or other NSAIDs, or complex to determine if dosage or duration have some effect on the strength of the analgesia.

A model for analgesic effect in rats involves animals injected with formalin. The rats are injected under the dorsal surface of the right hind paw with a 0.05 ml volume of 15% formalin and saline solution (Helmstetter and Fanselow, 1987). The treated rats are then given the aspirin, and/or other NSAIDs, aspirin/DPPC or PC complex, or placebo at various times before and after injection with the irritating solution. The rats are then placed in a cage and their behavioral responses to the painful stimulus are be observed (employing a video camera system) and graded as follows: (1) freezing - an absence of all activity other than respiration; (2) paw lifting - the rat holds its treated paw close to its body; (3) paw licking - the rat either licks the treated paw or has some other type of mouth contact with

it; and (4) general activity - this involves any other type of general movement. The behavioral response of the rat is indicative of its sensitivity. The formalin test, along with the tail flick test, comprises two distinct and separate tests to evaluate pain sensitivity and analgesia effectiveness.

EXAMPLE 11
EFFECTS OF LOW DOSE ASPIRIN (9 MG/KG) ALONE AND AS A COMPLEX WITH PHOSPHOLIPID/NEUTRAL LIPIDS ON FOREIGN-BODY GRANULOMA FORMATION IN RATS[A]

The present example is provided to demonstrate the utility of the present invention for reducing inflammation. The string granuloma model described herein was again used to demonstrate the activity of the presently disclosed methods for treating this condition.

Sterile tared string was surgically implanted. Five days later rats were intragastrically administered twice a day with either saline, ASA alone, or ASA lipid mixtures. Rats were sacrificed after each rat received 5 doses of the test compounds and the string and granuloma excised and weighed.

*p<0.05 vs. ASA alone. ASA = Aspirin;
DPPC = Dipalmitoylphosphatidylcholine,
TP = Tripalmitin; TO = Triolein

TABLE 4

Saline	ASA	ASA DPPC/TP	ASA PhospholiponG/TO
4.08(8)	3.89(8)	3.44(8)	3.49(8)*
±25	±0.06	±0.24	±0.17

Table 4. As demonstrated in the data provided in Table 4, a phospholipid (DPPC)/neutral lipid (TO) microemulsion appeared to modestly enhance the anti-inflammatory activity of ASA when the NSAID was administered at a subthreshold dose (9 mg/kg) for this activity.

EXAMPLE 12
EFFECT OF DPPC ON ASPIRIN'S INHIBITORY EFFECT ON PLATELET AGGREGATION

The present example demonstrates the ability of Non-Steroidal anti-inflammatory Drugs (NSAIDs) to inhibit cellular activation resulting in either platelet aggregation or the synthesis and release of inflammatory mediators. The activity is shown to be enhanced if the NSAIDs are administered as a complex with zwitterionic phospholipids alone or together with neutral lipids.

TABLE 5

High Ristocetin (1.5 mg/ml) Test		
	% Platelet Aggregation	
ASA Conc	-DPPC	+DPPC
0.00	93%	100%
0.01mM	100%	100%
0.1 mM	83%	87%
1.0 mM	95%	35%
1.0 mM[a]	100%	84%
Low Ristocetin (0.75) mg/ml Test		
ASA Conc	-DPPC	+DPPC
0.00	75%	95%
0.01mM	65%	12%
0.1mM	26^%	20%
1.0mM	80%	6%
1.0mM[a]	39%	15%

Abbreviations:ASA= aspirin

DPPC = dipalmitoylphosphatidylcholine

DPPC was added at a conc. equimolar to the aspirin.

In the absence of aspirin, DPPC was added at a final concentration of 1 mM.

[a] Second study.

The advantage of this invention is it will allow the NSAIDs to be administered at a lower than normal dose, due to their enhanced efficacy and potency-thus increasing their effectiveness and minimizing their side-effects on the GI tract and other organ systems.

A number of embodiments of the invention would include the combination of NSAIDs with zwitterionic phospholipids alone and together with neutral lipids. These combinations would both increase the efficacy and potency of NSAIDs to inhibit the activation of: 1) platelets; 2) neutrophils; 3) monocytes/macrophages; 4) lymphocytes; 5) Pans and 6) other bone-marrow derived cell types.

Considering the interest in the pharmaceutical industry in the role of NSAIDs in the prevention of cardiovascular disease and tissue/joint inflammation, the presently described pharmaceutical preparations would provide alternative clinical management protocols with a improved bioavailability at lower doses of the sometimes irritative NSAID regimen.

This list would include at minimum the 20-40 pharmaceutical companies presently marketing an NSAID.

EXAMPLE 13
ANTI-SECRETORY AGENTS TOGETHER WITH NSAID AND LIPIDS

The present example demonstrates the utility of the present invention for compositions that include an anti-secretory drug, such as Tagamet, or other histamine type 2 receptor antagonist, or Omeprazole (Prilosec™, or other proton-pump inhibitor or H^+/K^+ATPase inhibitors), either before or along with an NSAID complexed with phospholipid and/or neutral lipid (such as a triglyceride). These embodiments of the invention are further described in Example 14.

While not intending to be limited to any particular mechanism of action, by including a phospholipid and/or neutral lipid, the poor absorption of NSAID's that sometimes results with an anti-secretory agent administered therewith, may to some degree be prevented or lessened. Hence, the bioavailability and therapeutic action of the NSAID when administered together with an anti-secretory agent may be maintained, and in some cases enhanced.

The present example also demonstrates that the therapeutic (antipyretic) activity of aspirin, and other NSAIDs, is attenuated if animals are pre-treated with an agent that

inhibits gastric acid secretion. Omeprazole (sold under the name Prilosec™ is in the class of "proton pump inhibitors", also called "H^+/K^+ ATPase inhibitors" that act by irreversibly binding to and inhibiting H+/K+ ATPase of the parietal cell, the rate limiting enzyme in gastric HCl secretion. Ranitidine (sole under the name Zantac™ is in the class of "H_2 receptor antagonists" that prevents histamine from binding to its type-2 receptor on the parietal cell to inhibit gastric acid secretion. It can be appreciated from figures 22 and 23 that the blocking effect of these two classes of antisecretory drugs on the therapeutic actions of the NSAID, however, is overcome if the NSAID is complexed with a zwitterionic phospholipid. As demonstrated in Figures 23 and 23, aspirin at a dose of 20 mg/kg failed to reduce fever in rats if the NSAID was administered in conjunction with either the proton pump inhibitor (Prilosec™ or a histamine blocker, Ranitidine (Zantac™). This block in therapeutic activity due to inhibition of gastric acid secretin was overcome if the NSAID was administered as a complex with phospholipid, DPPC.

EXAMPLE 14
THERAPEUTIC REGIMENS

The present example is provided to demonstrate various therapeutic combination regimens for treating fever, inflammation and pain. These regimens include the administration of NSAIDs together with phospholipid and/or neutral lipid.

Agents that include phospholipid, such as lecithin - tablets and the like, may be used as part of the regimens disclosed herein for the enhancement of NSAID activity.

Therapeutic Regimen

For use as an improved regimen (e.g., anti-pyretic, platelet aggregation, analgesic), the present invention contemplates an initial administration of the phospholipid, such as in a tablet or phospholipid-containing agent, such as lecithin tablets, that contain phospholipid (e.g., phosphatidyl choline).

Either at the same time or following the administration of a phospholipid or phospholipid containing agent, the patient would then be given an NSAID. The NSAID may also be administered in combination with the phospholipid as a single composition,

or alternatively as a combination with both a phospholipid and a neutral lipid, such as a triglyceride (e.g., TO).

The present inventors also propose regimens that include the administration of an NSAID and phospholipid and/or neutral lipid, either before or at the same time as an anti-secretory drug, such as Tagamet™ and Prilosec™. This is because antisecretory drugs are observed by the present inventor to reduce the anti-pyretic action of NSAIDs. It is expected that the inclusion of phospholipid and/or neutral lipid will improve the observed reduced absorption of NSAID's observed when NSAIDs are administered with an antisecretory agent alone. Typically, this reduced absorption required that a higher dose of the NSAID be administered to the patient in order to provide the desired therapeutic effect.

Combinations of NSAIDs with zwitterionic phospholipids alone or in combination with neutral lipids will promote the ability of this family of drugs to influence certain target cells, such as neutrophils, platelets, eosinophils, macrophages, and others. In doing so, said phospholipids will increase the efficacy and potency of the NSAIDs to inhibit the cellular cyclo-oxygenase and the formation of arachidonic acid-derived products and other agents involved in cellular aggregation, adhesion, and the synthesis and release of inflammatory mediators and/or cytokines.

EXAMPLE 15
GI SIDE-EFFECTS OF NSAIDS ARE REDUCED BY ANTISECRETORY AGENTS

Rodent model systems were employed in the present study to investigate the effects of combination therapy on the GI toxicity and therapeutic activity of aspirin and indomethacin. A comparison was then made of these results to phospholipid-associated NSAIDs.

Methods

Fasted rats were pretreated with either saline or an antisecretory dose of omeprazole, ranitidine or cimetidine and 1-2 hours later were intragastrically administered saline, aspirin or indomethacin. In ulcer models, aspirin-treated rats were post-challenged with intragastric HCl and gastric lesions measured, whereas indomethacin treated rats were pre- and post-challenged with L-NAME, and GI bleeding assessed. For antipyretic and

analgesic activity rectal body temperature in febrile rats was measured, and the rat's pain sensitivity to pressure applied to an inflamed limb respectively.

Results

NSAID-induced GI ulceration and bleeding were reduced in rats pretreated with antisecretory agents and abolished in rats administered phospholipid-associated NSAIDs in combination with inhibitors of acid secretion. The antipyretic and analgesic activity of both NSAIDs were attenuated in rats pretreated with an antisecretory agent. This pH-dependent block in therapeutic activity was overcome if the NSAID is preassociated with a phospholipid to enhance the drug's lipophilic characteristics. The present example demonstrates that the combination therapy of antisecretory agents and NSAIDs, chemically associated with phospholipids, has distinct advantages with regards to both low GI toxicity and restored therapeutic activity.

EXAMPLE 16
PROTON PUMP INHIBITORS AND H2 RECEPTOR ANTAGONISTS, EFFECTS ON ACUTE GI TOXICITY, ANTIPYRETIC, AND ANALGESIC ACTIVITY OF NON-STEROIDAL ANTI-INFLAMMATORY AGENTS

In the present study, rat model systems were also employed to investigate the effects of both proton pump inhibitors and H2 receptor antagonists on the acute GI toxicity, antipyretic and analgesic activity of aspirin and indomethacin. Parallel studies were performed with these NSAIDs coupled to phospholipids to demonstrate this as an effective strategy to increase the bioavailability of the NSAID in the presence of an anti-secretory agent, while providing enhanced protection against the drugs' injurious actions.

Materials and Methods

Animals

Male Sprague Dawley rats weighing 150-175 g were purchased from Harlan Sprague Dawley Inc (Indianapolis, IN) and housed in our institution's Animal Care Center 5-10 days before study, during which time they had *ad libitum* access to water and Harlan Teklab F-6 Rodent Diet (Madison, WN). All animal protocols employed exceeded NIH guidelines in the treatment and welfare of laboratory animals.

Gastric pH

Fasted rats were injected intraperitoneally with saline (control) or various doses of omeprazole (Astra Hässle AB, Molndal, Sweden), cimetidine or ranitidine (Sigma Chemical Co., St. Louis, MO). One hour later, the rats were ether anesthetized and after a midline incision, the stomachs were exteriorized, and 2 ml of H_2O was injected into the gastric lumen and collected for pH analysis.

Ulcer Models and Chemical Association of NSAIDs with DPPC

Rats were fasted overnight and then injected i.p. with an acid-inhibitory dose of omeprazole (400, μmoles/kg or 140 mg/kg) ranitidine (5 mg/kg) or cimetidine (50 mg/kg). One hour later, the rats were intragastrically administered an ED_{50-80} dose of aspirin (ASA, 20 mg/kg) and challenged 10 min later with an intragastric dose of 0.6 N HCl as outlined previously. In this and all subsequent studies, the NSAIDs were administered either alone or chemically associated with an equimolar concentration of dipalmitoylphosphatidylcholine (DPPC).

The NSAID/DPPC complex was prepared, by initially dissolving the required amount of DPPC in chloroform, followed by exhaustive overnight evaporation of the organic solvent under vacuum. The NSAID salt, which was dissolved in water and subsequently titrated to the desired pH, was added to the tube containing the lipid film, followed by 15 min of sonication in a bath type sonicator (Laboratory Supplies Co. Inc., Hicksville, N.Y.). Sixty min after administration of ASA±DPPC, the rats were euthanized by CO_2 asphyxiation and the stomachs removed, and gastric lesions scored in accordance to a previously described method as described in Lichtenberger *et al.* (1995) (Natural Medicine, 1:154-158), which reference in specifically incorporated herein by referenced for this purpose.

The second animal model was based on a method described in Lichtenberger *et al.* (1995), in which fasted rats were administered N^G-nitro-L-arginine methyl ester (L-NAME, 20 mg/kg, i.p.) 1 hour before and 3 and 6 hours after the animals intragastrically received indomethacin± DPPC, at a dose of 10 mg/kg or saline (control). In this study the antisecretory agents were administered (i.p.) 90 min prior to indomethacin (or saline) treatment. Eighteen hours after the administration of the NSAID (or saline), the rats were

euthanized (CO_2 asphyxiation) and the distal half of the intestine was flushed with 10 ml of saline which in turn was collected for hemoglobin analysis to provide an estimate of GI bleeding as outlined in Lichtenberger *et al.* (1995).

Antipyretic Activity

The antipyretic activity of NSAIDs was evaluated and compared in conscious rats who were made febrile by the subcutaneous injection of 2 g/kg Brewers yeast and fasted thereafter, in accordance to the method described by Adams *et al.*, (1968) J. Pharm. Pharmac. 20:305-312 (incorporated herein by reference). Eighteen hours later, the fasted febrile rats (body temperature increased by 0.5-1.0.°C) were injected with an antisecretory agent or an equivalent volume of vehicle injected by the same route. One hour later, the rats intragastrically received 1 ml of an approximate ED_{50-80} dose of either aspirin (18 mg/kg) or indomethacin (18 mg/kg) alone or the drugs preassociated with DPPC (adjusted to a pH of 7.0). Rectal body temperature was monitored over the subsequent 4 hour period.

Analgesic Activity

The ability of NSAIDs to reduce the hyperalgesia (i.e., increased sensitivity to pain) associated with an inflamed hindpaw (induced 4 days previously by the injection of 0.05 ml complete Freund's Adjuvant) was assessed. A modified version of the Randall-Selitto test was used to assess pain sensitivity of each hindpaw. (Lichtenberger *et al.* (1995) (Randall *et al.* (1957) Arch. Int. Pharmacolog. 111:409-419.) Rats were restrained in Plexiglass tubes and external pressure was applied sequentially to the uninflamed and inflamed hindpaws (0-250g at a rate of 16 g/sec with an Analgesymeter (Life Sciences Inst., Woodland Hills, CA). The "pain pressure threshold" was defined as the pressure at which an animal exhibits paw withdrawal. In these studies, rats were fasted overnight and pretreated with an antisecretory agent or saline, 1 hour before receiving an ED_{50-80} dose of the NSAID alone (10 mg/kg aspirin, 4 mg/kg indomethacin), or the NSAID preassociated with DPPC as described above. Two hours later, pain pressure thresholds were assessed on the both hindpaws. The dosing regimen described above was repeated at 6 and 24 hours, and the sensitivity to externally applied pressure was measured 2 hours after the animals received the last NSAID dosage.

The results were analyzed with the analysis of variance (ANOVA) procedure. If the ANOVA procedure yielded a significant interaction between variables, post *ad hoc* comparisons were made with Fisher's LSD test ($p<0.05$). Data are expressed as mean ± standard error of the mean, with $p \leq 0.05$ being considered statistically significant.

Results

Table 6 reveals that in the conscious rats, gastric acid secretion was maximally inhibited by the injection of omeprazole at a dose of 400 μmoles/kg, with gastric pH not being significant from neutrality. The H_2 - receptor blockers, ranitidine and cimetidine, at doses of 5 and 50 mg/kg respectively, were somewhat less efficacious in inhibiting gastric acid secretion in the rat, raising the gastric pH to values >6.0 in 1-2 hours.

Table 6. Ability of Antisecretory Drugs to Neutralize Gastric Juice pH in Rats[a]

Groups	Dose	Gastric Juice pH
Saline	0	3.45±0.05
Omeprazole[b]	40 μmoles/kg	4.15±0.60
	400 μmoles/kg	7.21±0.15*
Ranitidine[c]	5 mg/kg	6.03±0.27
	20 mg/kg	6.30±0.24*
Cimetidine[c]	20 mg/kg	3.58 ±0.46
	50 mg/kg	6.13 ±0.32*

[a]Fasted rats were injected i.p. with omeprazole, ranitidine, cimetidine or saline. 2 hours later under other anesthesia a laparotomy was performed. Clamps were then placed at the esophageal and duodenal ends and 2 ml. of water was injected through the gastric wall into the lumen. After mixing, he gastric fluid was collected for pH analysis.

[b]Omeprazole was suspended in 1 part 10 mg% polyethylene glycol in 5 parts 19mM $NaHCO_3$.

[c]Ranitidine and cimetidine were solubilized in distilled H_2O.

*=$p<0.05$ vs. The gastric juice pH of control rats injected with saline.

Proton pump inhibitors and H2 - receptor antagonists are presently being recommended for both the prevention and treatment of gastroduodenal ulcers associated with NSAID usage based upon encouraging clinical findings.[4-8] Pretreatment of rats with antisecretory doses of omeprazole, ranitidine and cimetidine were moderately effective in reducing GI injury and bleeding induced by acute exposure to aspirin or indomethacin. The GI toxicity of the above potent antisecretory agents were used in combination with phospholipid-associated NSAIDs. The results presented above clearly indicate that this strategy proved the most effective in eliminating the acute GI side-effects of both aspirin and indomethacin.

The results presented here based upon rodent model systems, indicate that the powerful anti-secretory agents - omeprazole, ranitidine and cimetidine appear to attenuate the therapeutic activity of anionic NSAIDs to reduce fever, pain and/or inflammation. This apparent reduction in bioavailability was best observed when the antisecretory agents were administered at a dose to increase gastric juice pH > 6.0 and the NSAIDs were administered at an ED_{50-80} dose to induce anti-pyretic, anti-inflammatory/analgesic activity. These findings thereby establish that although combination therapy of antisecretory agents with NSAIDs appears to effectively reduce the GI toxicity of NSAIDs, due to neutralization of gastric acidity, it may be contra-indicated in clinical situations where relief from pain, inflammation and fever is needed.

The mechanism by which omeprazole, ranitidine and cimetidine blocks the therapeutic activity of both aspirin and indomethacin most likely relates to their common ability to neutralize gastric acidity and block the acid-dependent conversion of the NSAIDs into their membrane-permeable lipidic state. Orally administered NSAIDs are absorbed across the upper GI tract primarily in their undissociated lipidic state (McCormack, K & Brune, K).[28] The present inventors' view of the data presented here thus propose that pharmacological neutralization of the gastric juice to pH > pKa of the NSAID would limit the absorption of the drugs across the gastroduodenal mucosa. This block in the gastric absorption of the drugs would result in the movement of the NSAIDs into, and possibly their absorption from, lower regions of the small intestine where they could be exposed to metabolism by both pancreatic and/or brush border enzymes.

The data presented in this invention demonstrated that the antipyretic, and anti-inflammatory activity of the complex was superior to that of the NSAID alone. This apparent enhancement in therapeutic activity may be attributable to an increase in the lipid permeability/solubility of the phospholipid-associated NSAID. It was predicted that ionic binding between the DPPC and the NSAID may shield the NSAID from undergoing pH dependent changes in charge, and that unlike the free-NSAID, the complex would remain lipophilic even as the intragastric pH approached neutrality. Evidence reported here appears to support this assertion, as the block in the NSAIDs' antipyretic and anti-inflammatory/analgesic activities observed with the three potent antisecretory test agents could be overcome if the NSAIDs were administered to rats as a preformed complex with DPPC. It was also of interest to note that even in the absence of the antisecretory agents, both NSAIDs when administered as a lipidic complex appeared to have equivalent or greater therapeutic efficacy to that of the NSAID alone in reducing fever and pain, confirming the present findings on the ability of DPPC to enhance the therapeutic activity of NSAIDs.

Table 7. Inhibitors of Gastric Acid Secretion
H2-Receptor Antagonists

Chemical Name	Brand Name
Cimetidine	Tagamet®
Ranitidine	Zantac®
Famotidine	Pepsid®
Nizatidin	Axid®
Proton-Pump Inhibitors	
Omeprazole	Prilosec®
Lansoprazole	Prevacid®

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred

embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically, pharmacologically, and/or physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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WHAT IS CLAIMED IS:

1. A method for maintaining or enhancing therapeutic activity of a non-steroidal anti-inflammatory drug in the presence of an H2 receptor blocker in an amount comprising:

administering an anti-inflammatory agent in association with a zwitterionic phospholipid,

wherein the pH is increased above fasting levels in the animal.

2. The method of claim 1 wherein the H2 receptor blocker is ranitidine or cimetidine.

3. The method of claim 1 wherein fasting levels are about pH 1 to about pH 4.

4. A method for maintaining and/or enhancing the big-availability of a non-steroidal anti-inflammatory agent in the presence of an anti-secretory agent, comprising:

administering a combination of a non-steroidal anti-inflammatory agent and a zwitterionic phospholipid to an animal having received an anti-secretory agent.

wherein the pharmacological activity of the non-steroidal anti-inflammatory agent is greater than the pharmacological activity of the non-steroidal anti-inflammatory agent in the absence of a zwitterionic phospholipid to an animal receiving an anti-secretory agent.

5. The method of claim 4 wherein the composition is further defined as comprising an equimolar amount of zwitterionic phospholipid and non-steroidal anti-inflammatory drug.

6. The method of claim 1or 4 wherein the non-steroidal anti-inflammatory drug is salicylate, naproxen, indomethacin, diclofenac, aspirin, or a mixture thereof.

7. The method of claim 1 or 4 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidyl choline.

8. The method of claim 1 or 4 wherein the composition further comprises a neutral lipid.

9. The method of claim 8 wherein the neutral lipid is a triglyceride.

10. A pharmaceutical preparation comprising a non-covalently associated combination of nonsteroidal anti-inflammatory agent, zwitterionic phospholipid, and anti-secretory agent.

11. The pharmaceutical preparation of claim 10 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidyl choline and the neutral lipid is tripalmitin.

12. The pharmaceutical preparation of claim 10 wherein the antisecretory agent is omeprazole.

FIG. 1 A

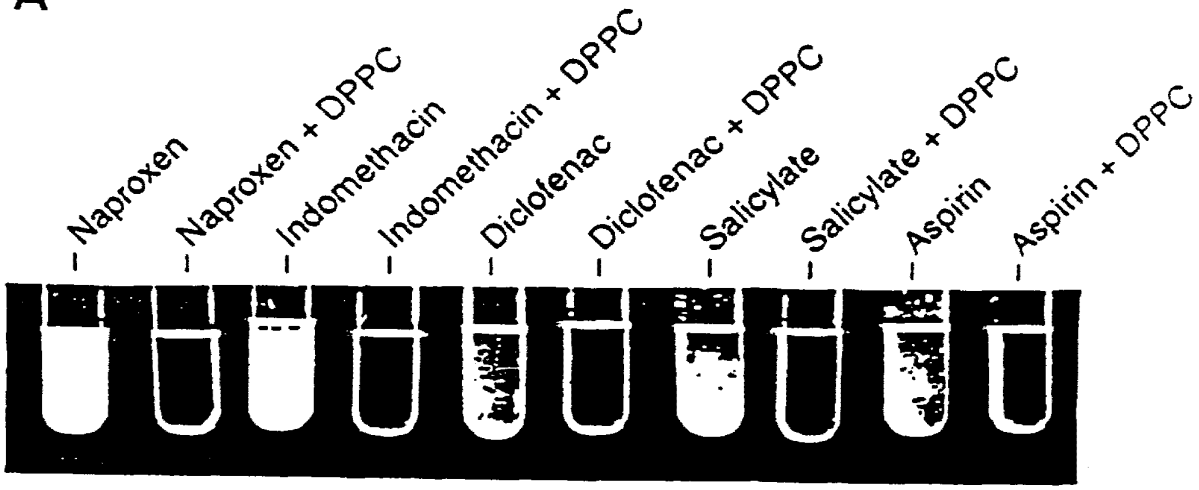

Naproxen
Naproxen + DPPC
Indomethacin
Indomethacin + DPPC
Diclofenac
Diclofenac + DPPC
Salicylate
Salicylate + DPPC
Aspirin
Aspirin + DPPC

FIG. 1 B.

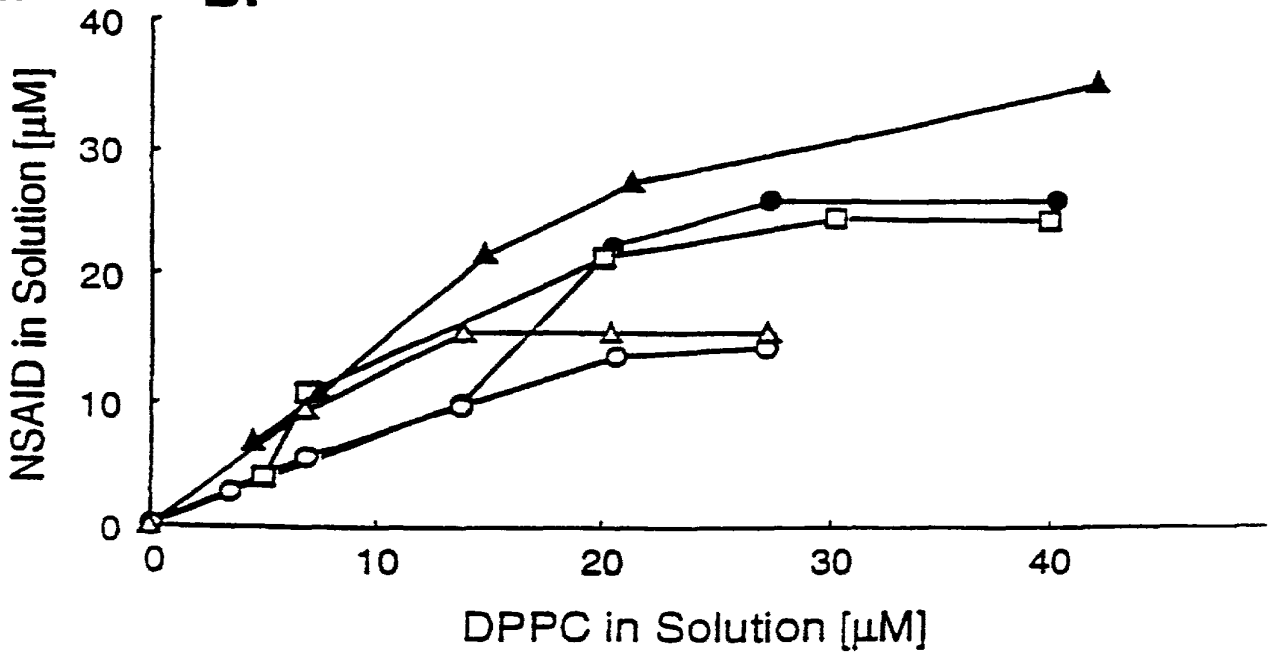

NSAID in Solution [µM]
DPPC in Solution [µM]
0
10
20
30
40

FIG. 2A

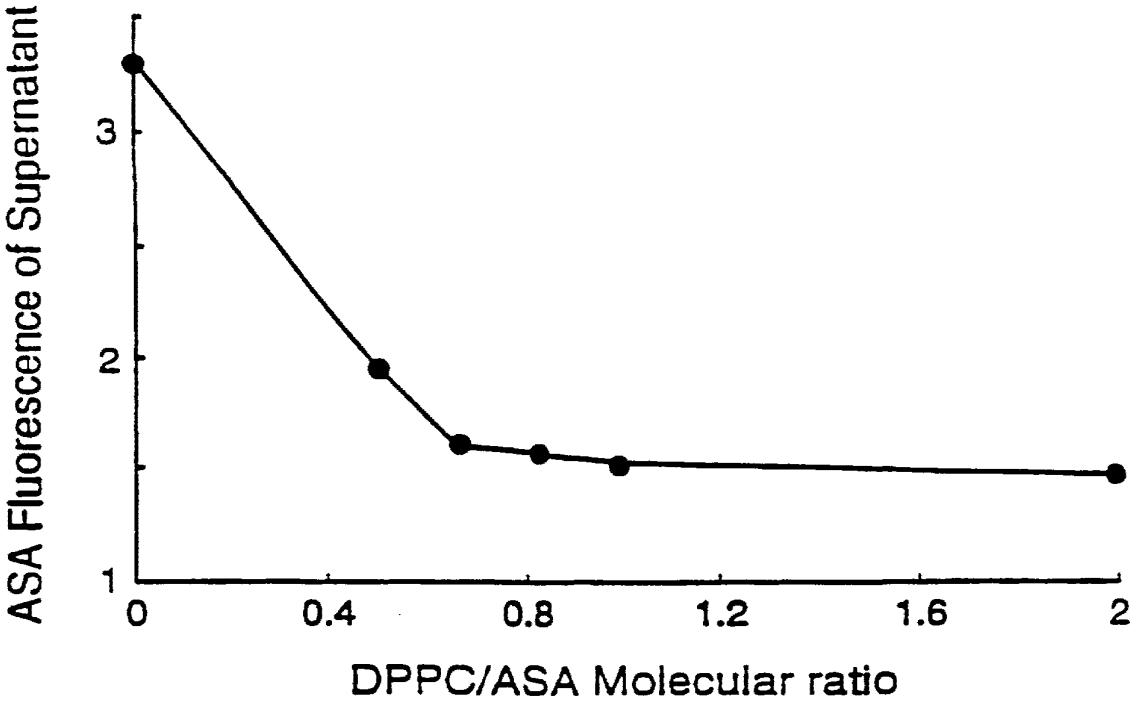
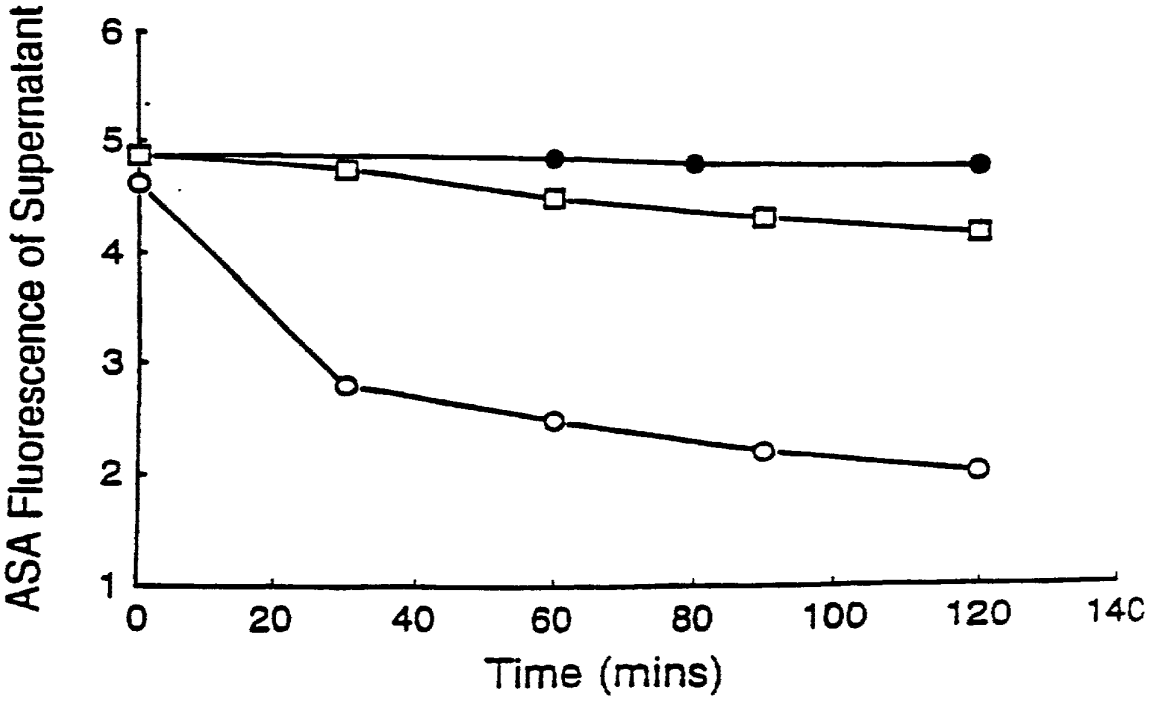

ASA Fluorescence of Supernatant
3
2
1
0
0.4
0.8
1.2
1.6
2
DPPC/ASA Molecular ratio

FIG. 2B


ASA Fluorescence of Supernatant
6
5
4
3
2
1
0
20
40
60
80
100
120
140
Time (mins)

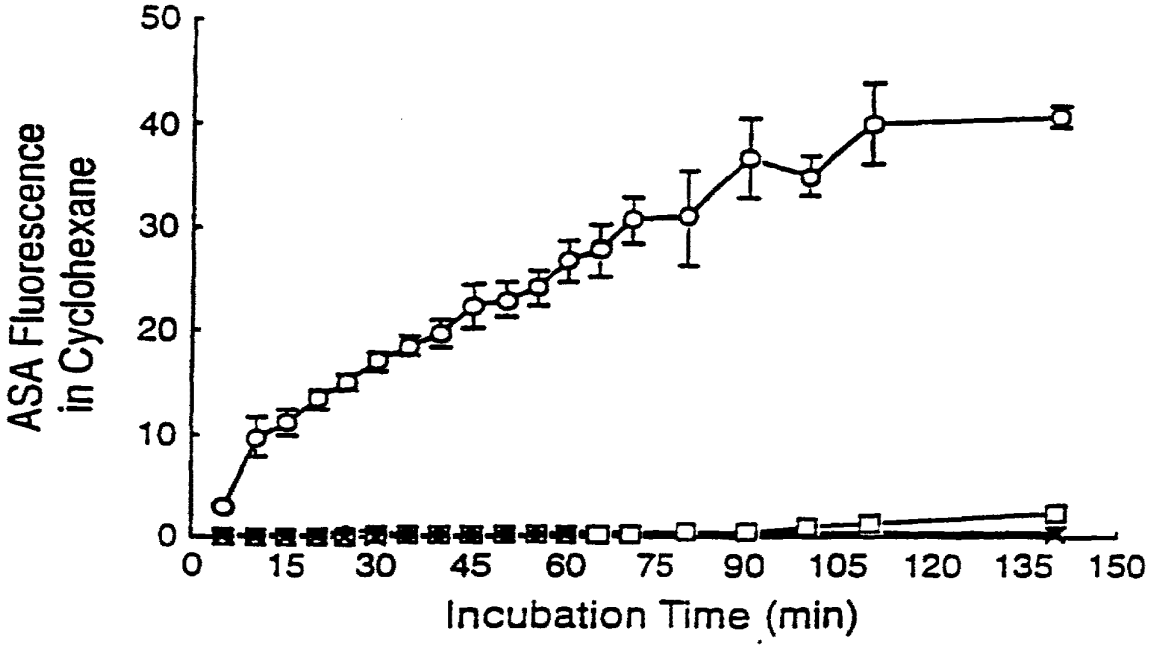

ASA Fluorescence in Cyclohexane
50
40
30
20
10
0
0
15
30
45
60
75
90
105
120
135
150
Incubation Time (min)

FIG. 3

FIG. 4A

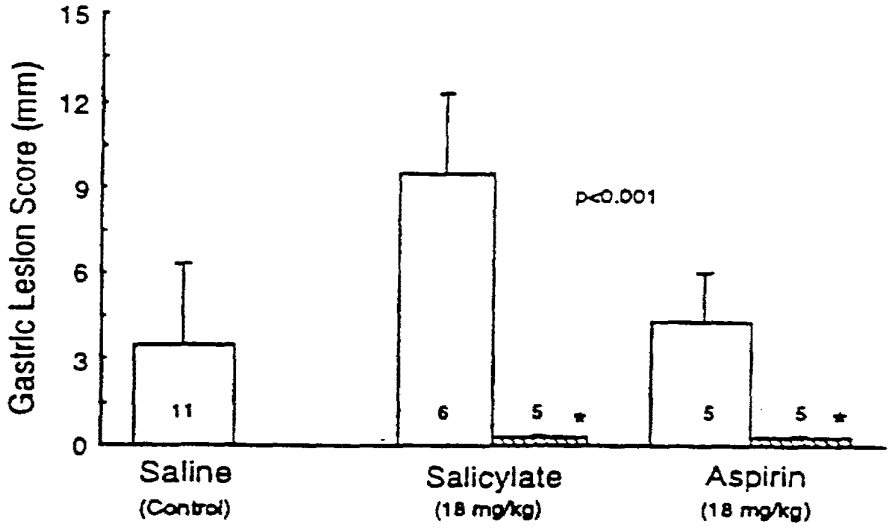


FIG. 4B

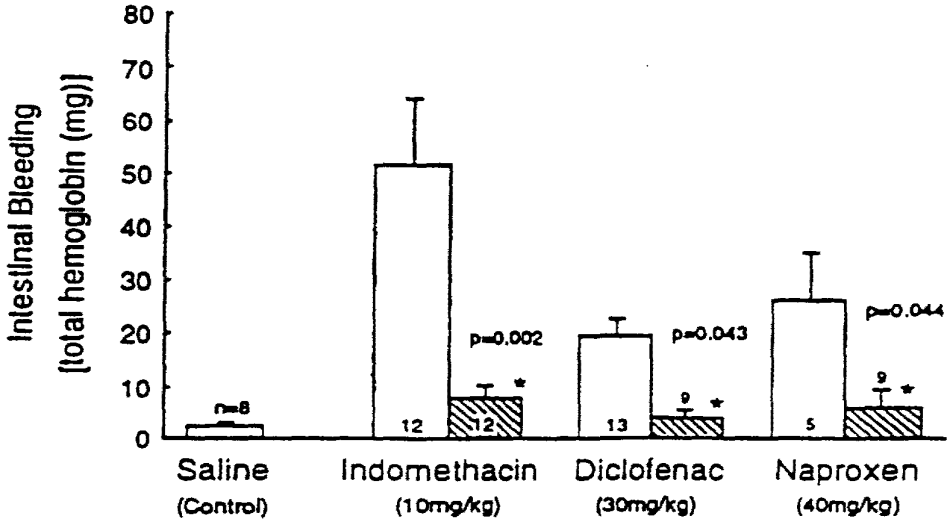
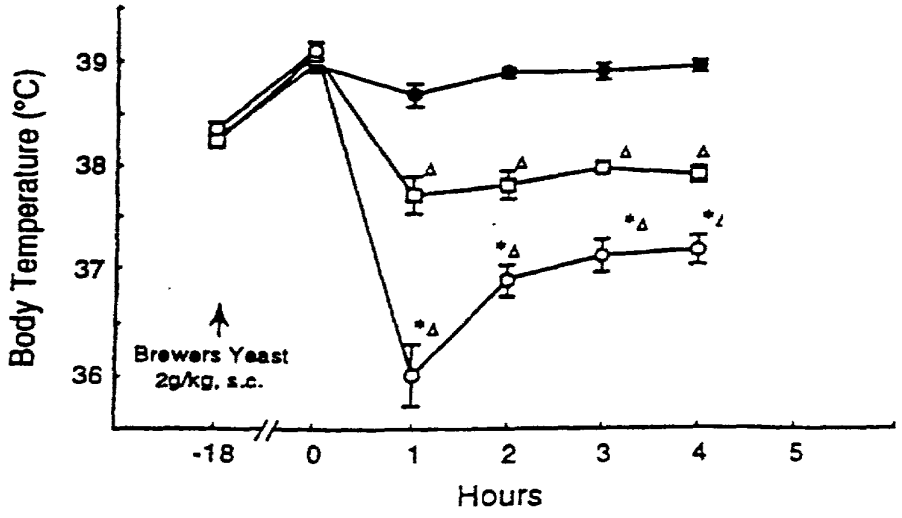


FIG. 4C



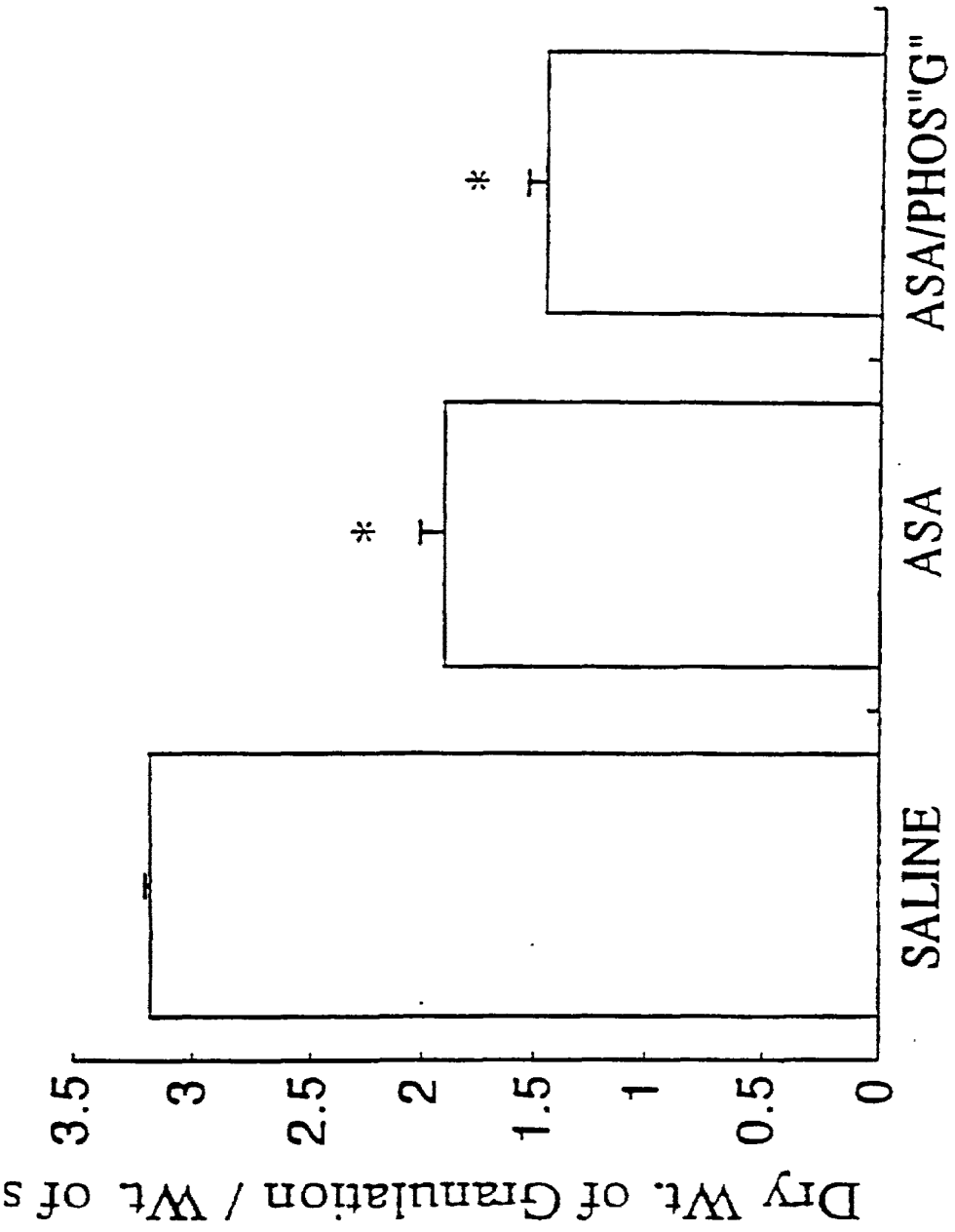

Anti-inflammatory Activity of ASA and PHOSPHOLIPID "G"
Dry Wt. of Granulation / Wt. of string
3.5
3
2.5
2
1.5
1
0.5
0
*
*
SALINE
ASA
ASA/PHOS"G"
GROUP

FIG. 5

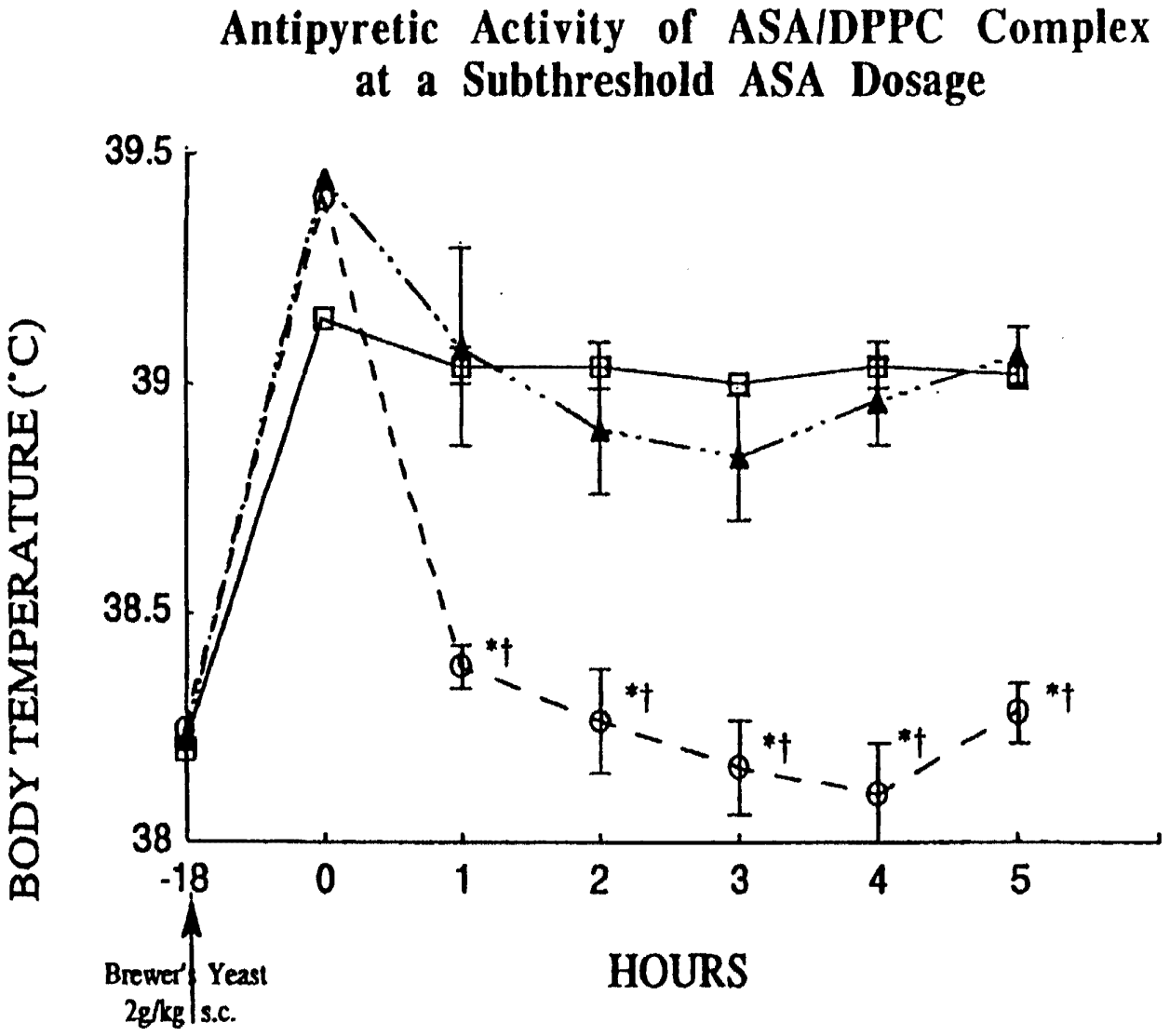

Antipyretic Activity of ASA/DPPC Complex
at a Subthreshold ASA Dosage
BODY TEMPERATURE (°C)
39.5
39
38.5
38
-18
0
1
2
3
4
5
HOURS
*†
Brewer's Yeast
2g/kg s.c.

FIG. 6

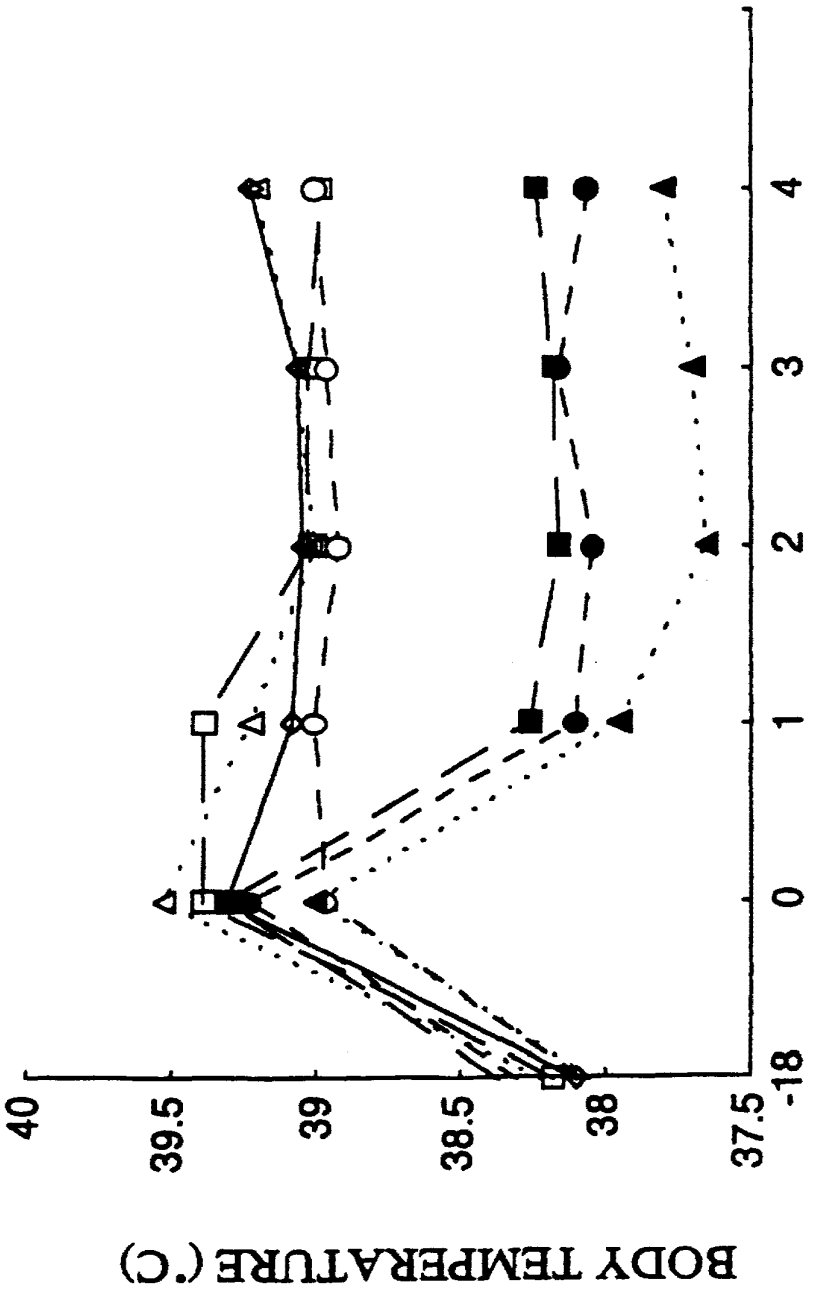


FIG. 7

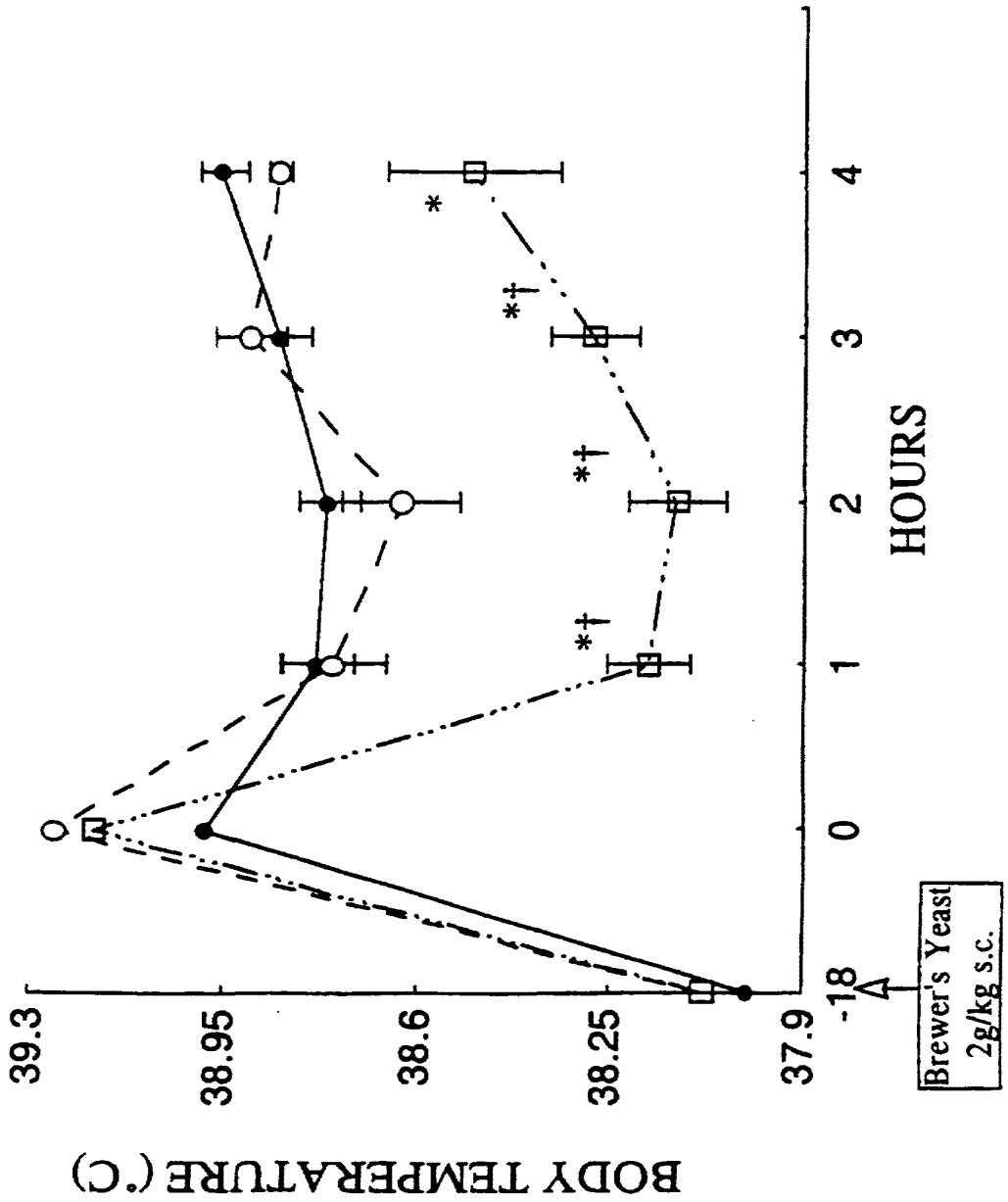

BODY TEMPERATURE (°C)
39.3
38.95
38.6
38.25
37.9
-18
0
1
2
3
4
HOURS
Brewer's Yeast
2g/kg s.c.
*
*†
*†
*†

FIG. 8

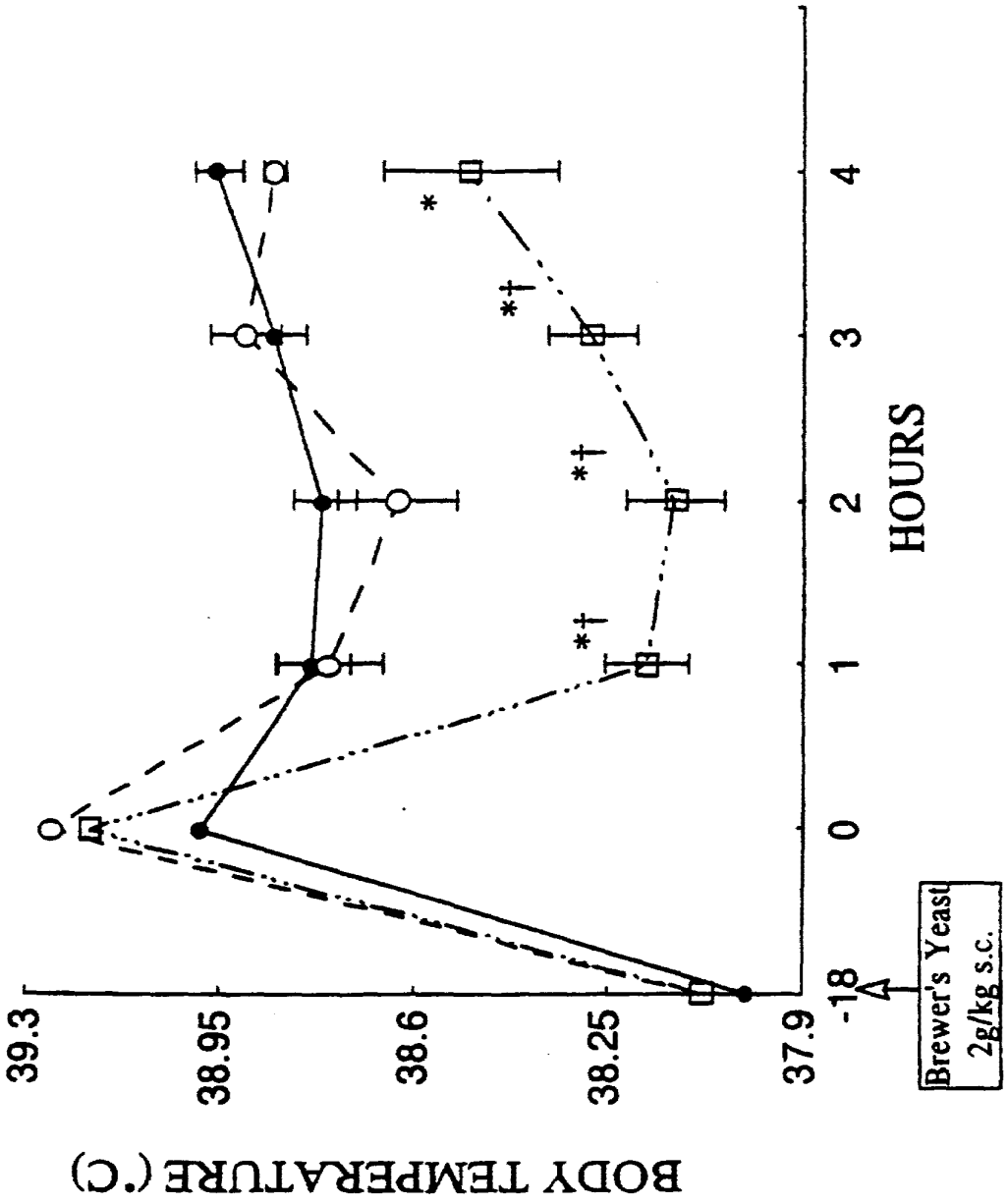

BODY TEMPERATURE (°C)
39.3
38.95
38.6
38.25
37.9
-18
0
1
2
3
4
HOURS
Brewer's Yeast
2g/kg s.c.
*
*†
*†
*†

FIG. 8

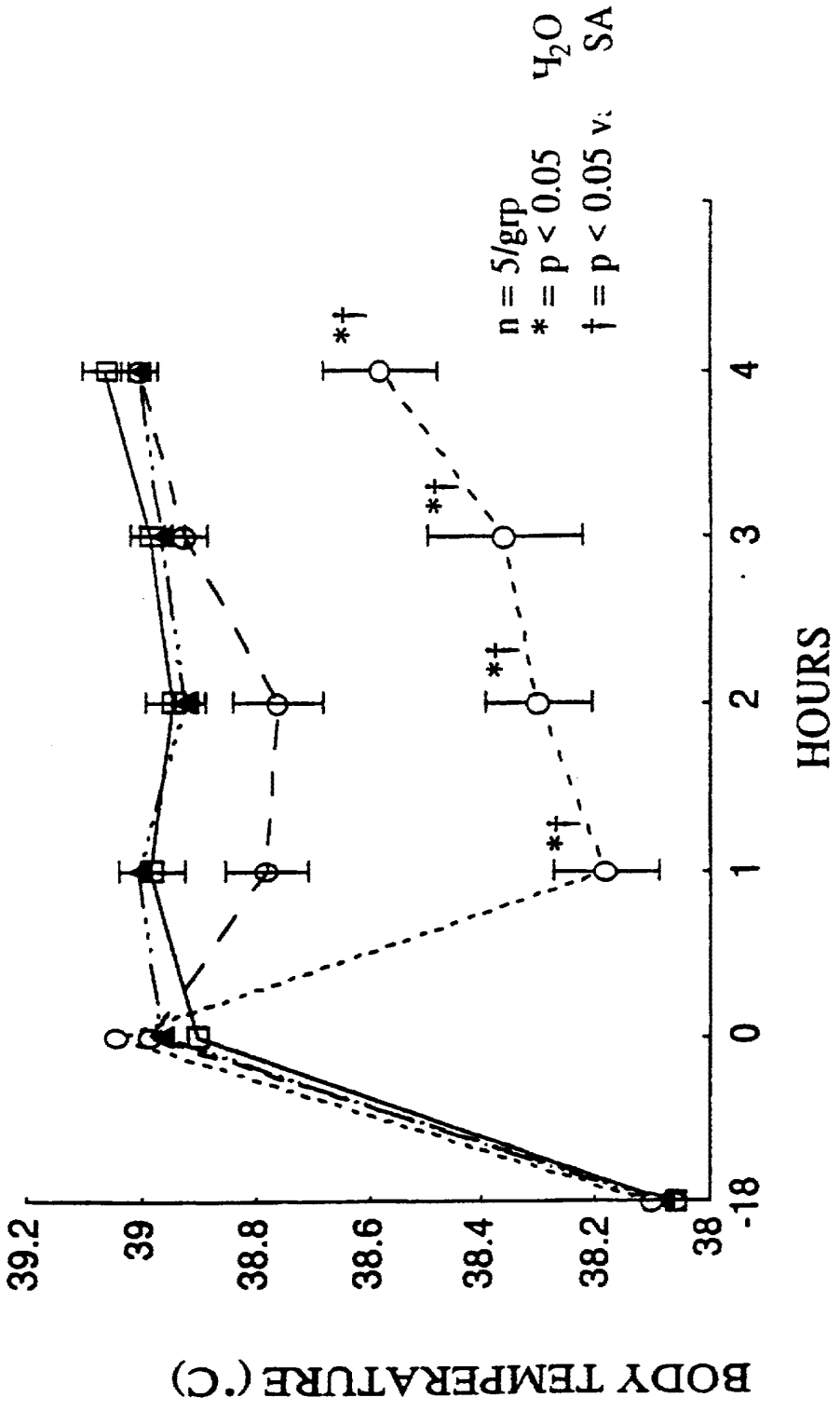

BODY TEMPERATURE (°C)
39.2
39
38.8
38.6
38.4
38.2
38
-18
0
1
2
3
4
HOURS
n = 5/grp
* = p < 0.05
† = p < 0.05 v
H_2O
SA

FIG. 9

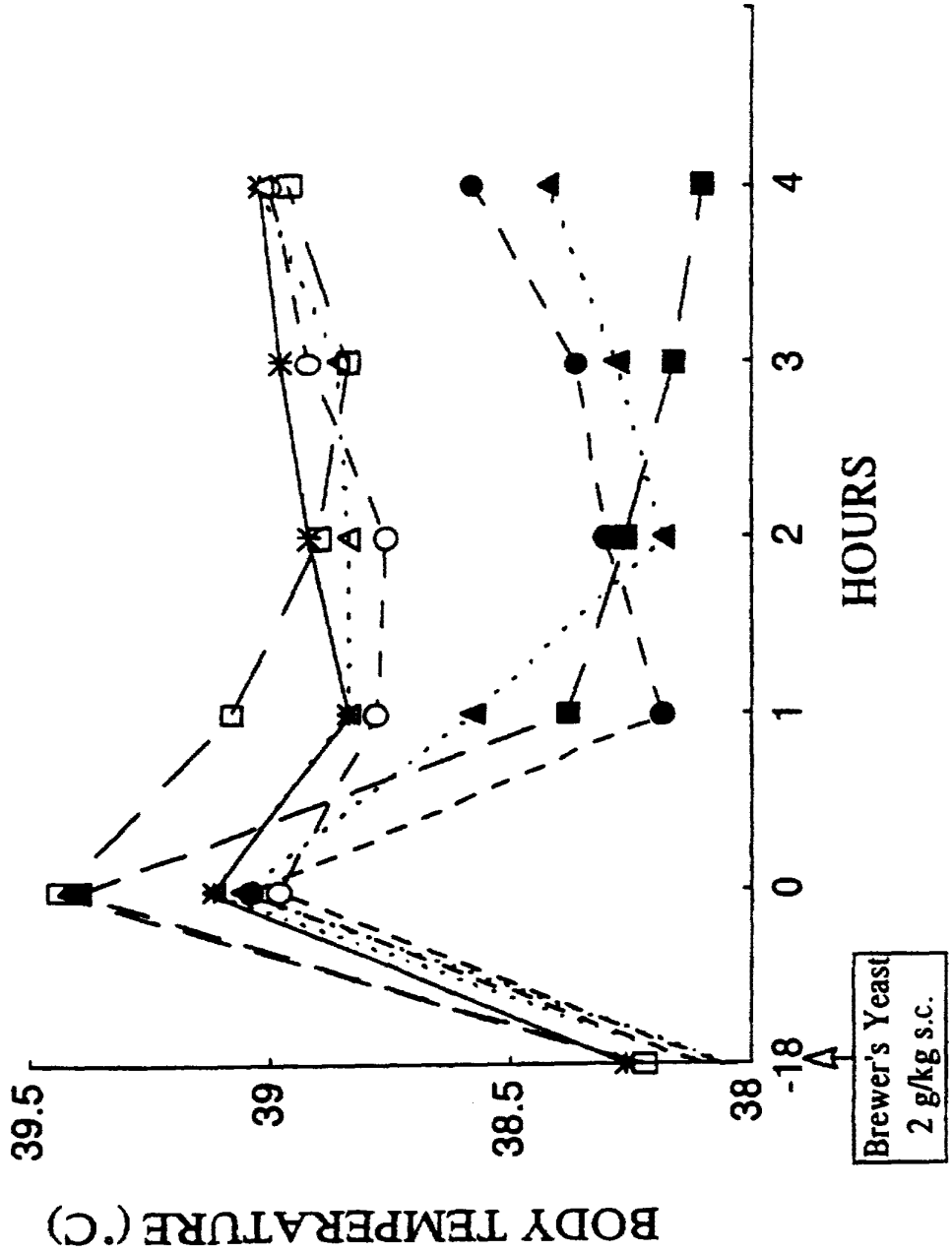

BODY TEMPERATURE (°C)
39.5
39
38.5
38
-18
0
1
2
3
4
HOURS
Brewer's Yeast
2 g/kg s.c.

FIG. 10

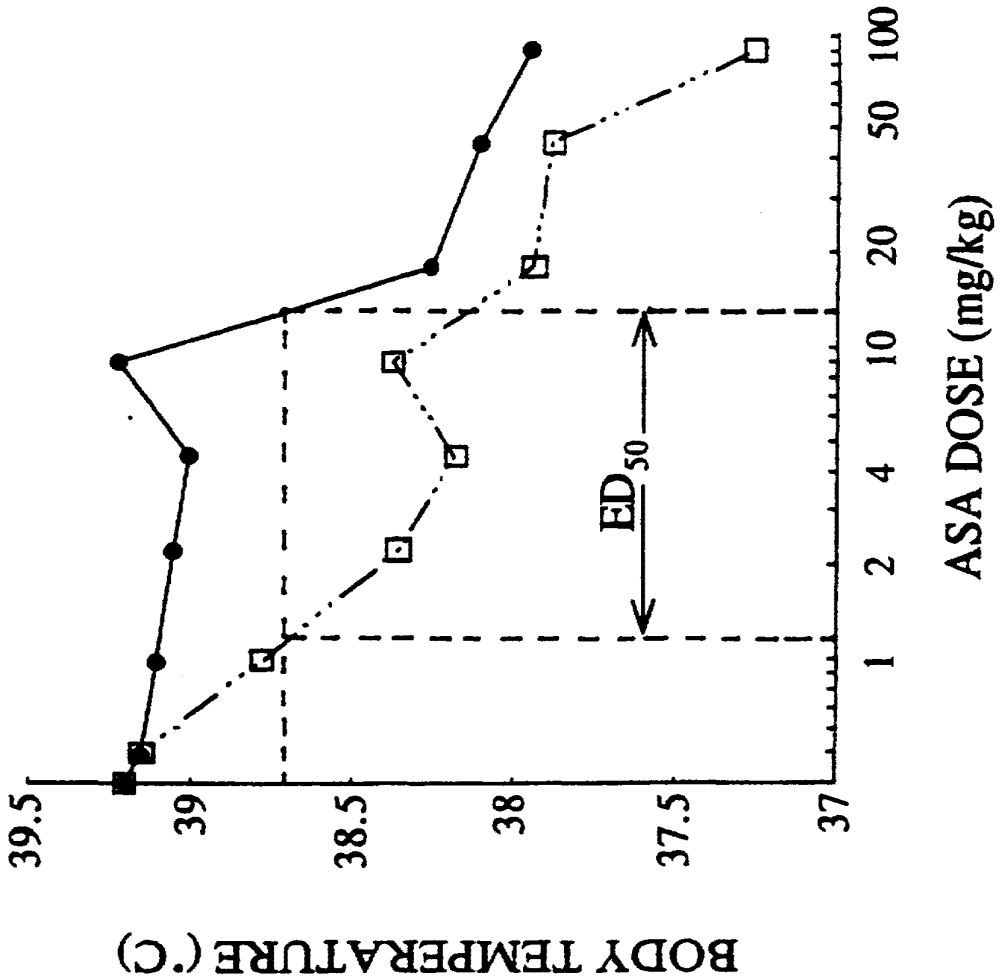

BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
ASA DOSE (mg/kg)
1
2
4
10
20
50
100
ED_{50}

FIG. 11

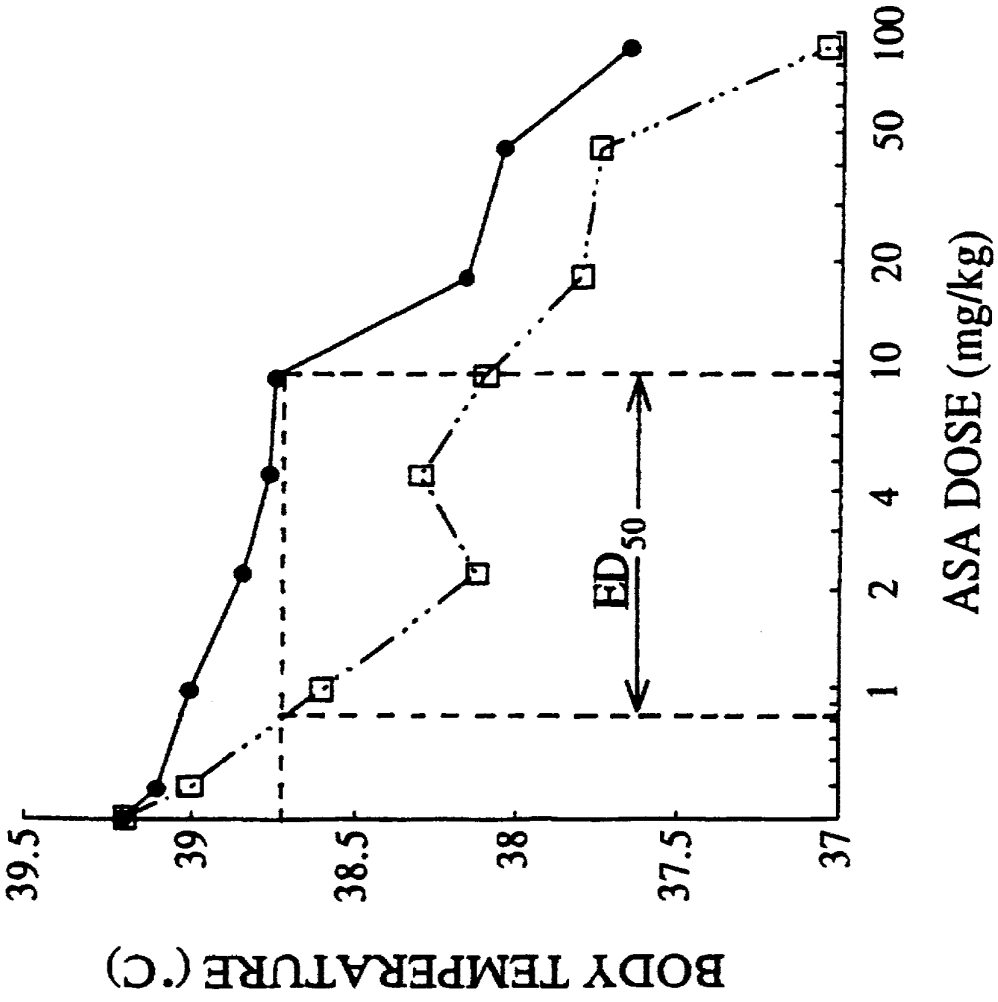

BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
ED50
ASA DOSE (mg/kg)
1
2
4
10
20
50
100

FIG. 12

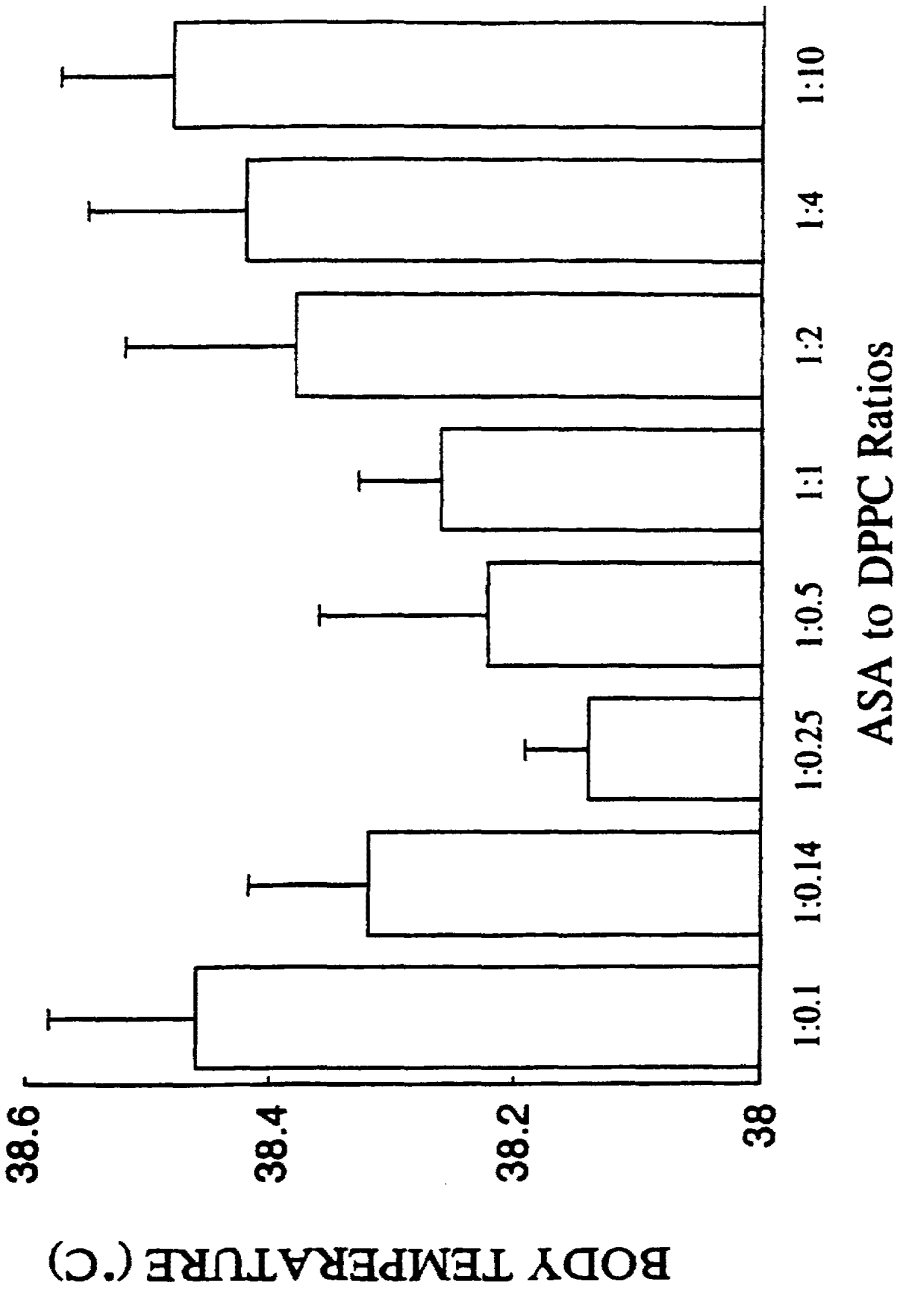

BODY TEMPERATURE (°C)
38.6
38.4
38.2
38
1:0.1
1:0.14
1:0.25
1:0.5
1:1
1:2
1:4
1:10
ASA to DPPC Ratios

FIG. 13

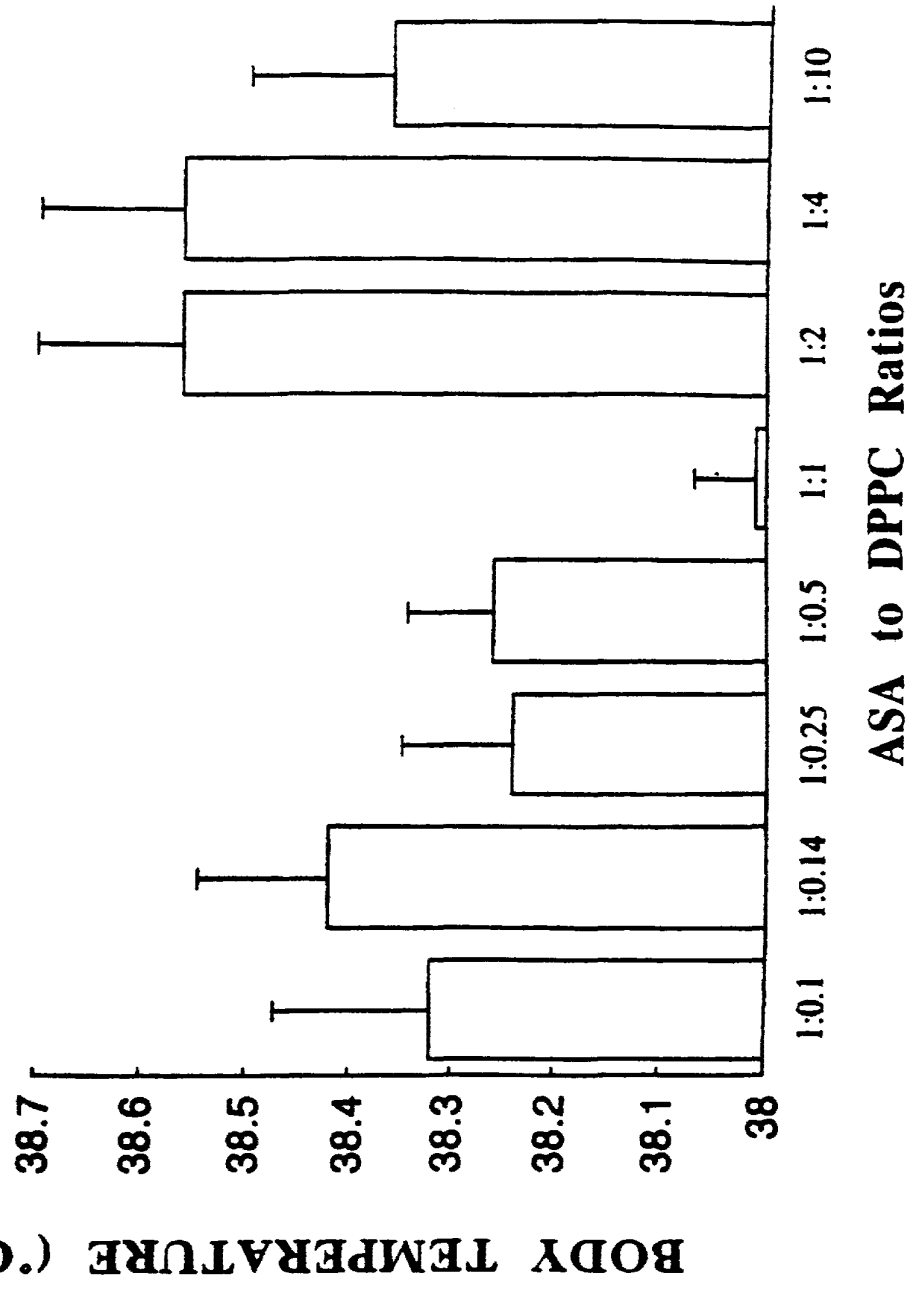

BODY TEMPERATURE (°C)
38.7
38.6
38.5
38.4
38.3
38.2
38.1
38
1:0.1
1:0.14
1:0.25
1:0.5
1:1
1:2
1:4
1:10
ASA to DPPC Ratios

FIG. 14

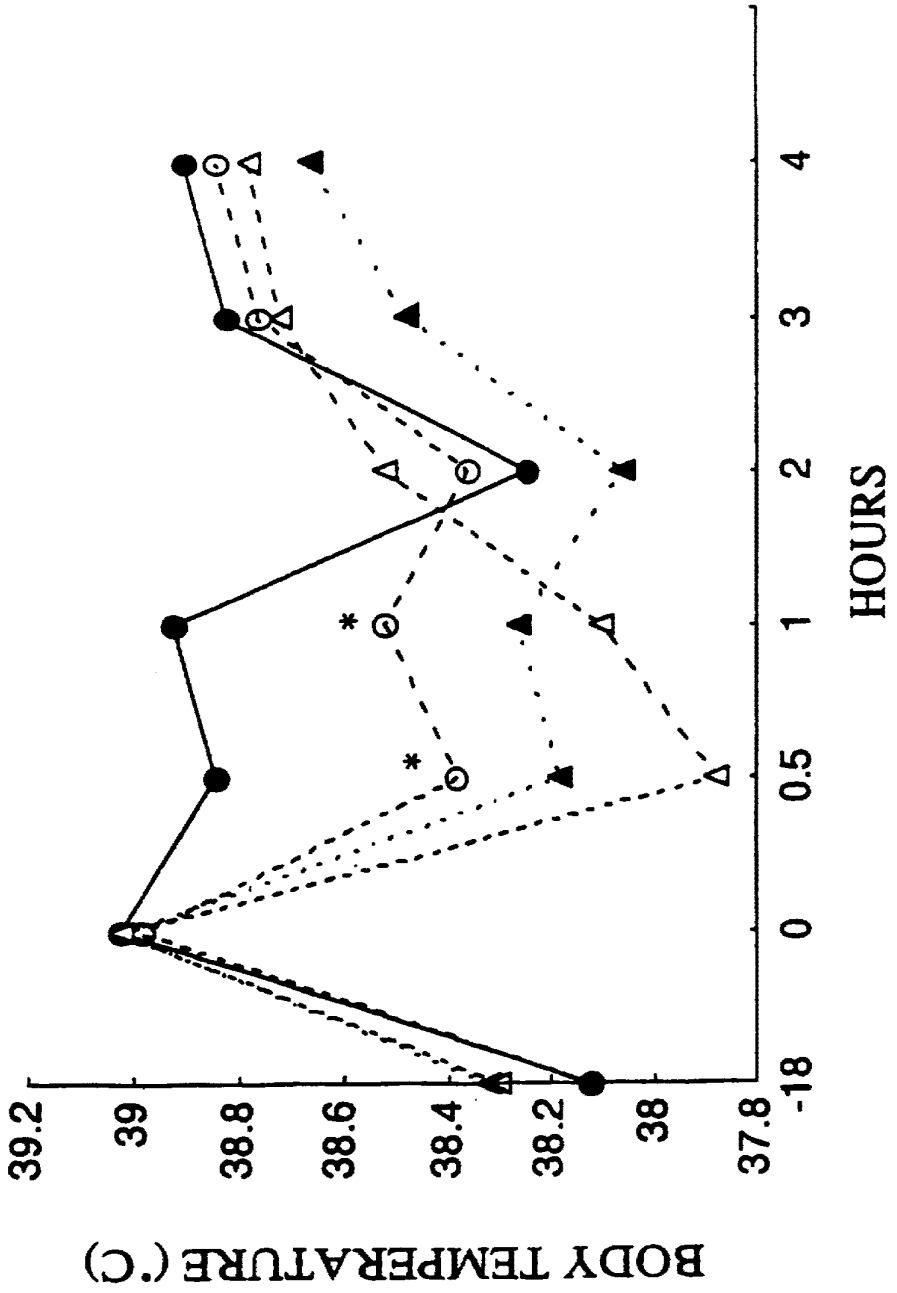

BODY TEMPERATURE (°C)
39.2
39
38.8
38.6
38.4
38.2
38
37.8
-18
0
0.5
1
2
3
4
HOURS

FIG. 15

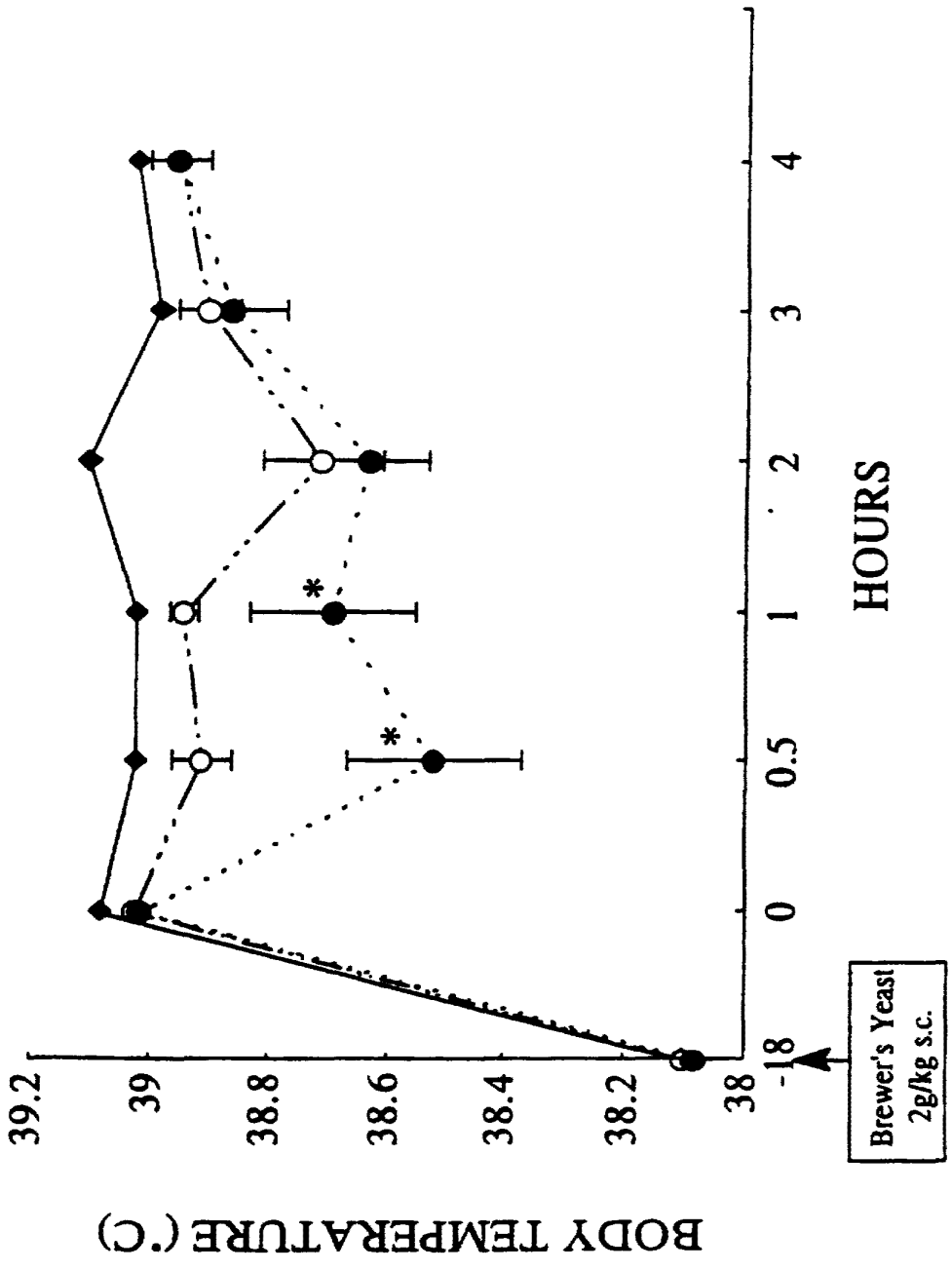

BODY TEMPERATURE (°C)
39.2
39
38.8
38.6
38.4
38.2
38
-18
0
0.5
1
2
3
4
HOURS
Brewer's Yeast
2g/kg s.c.
*
*

FIG. 16

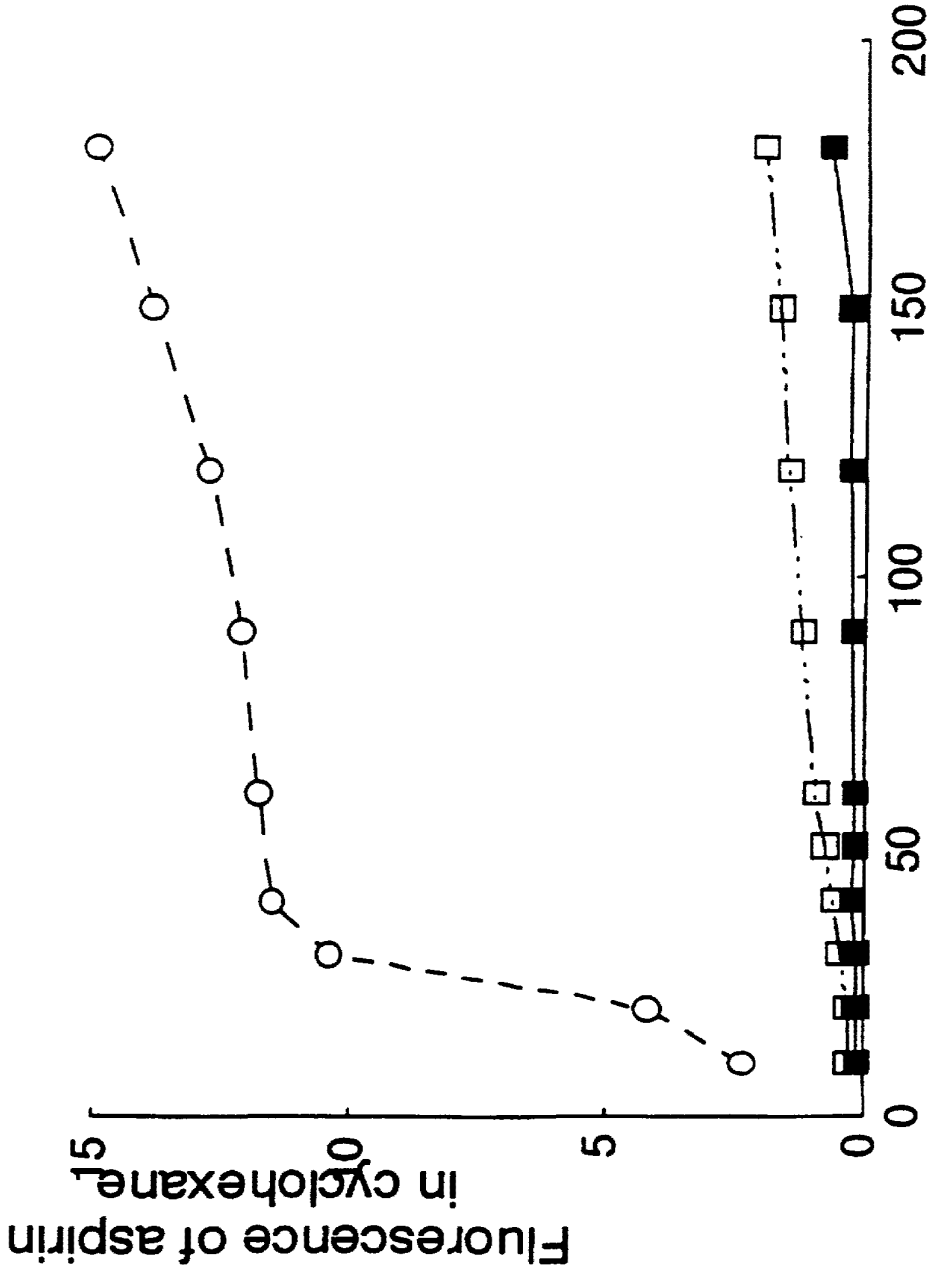

Fluorescence of aspirin
in cyclohexane
15
10
5
0
0
50
100
150
200
Time (Minute)

FIG. 17

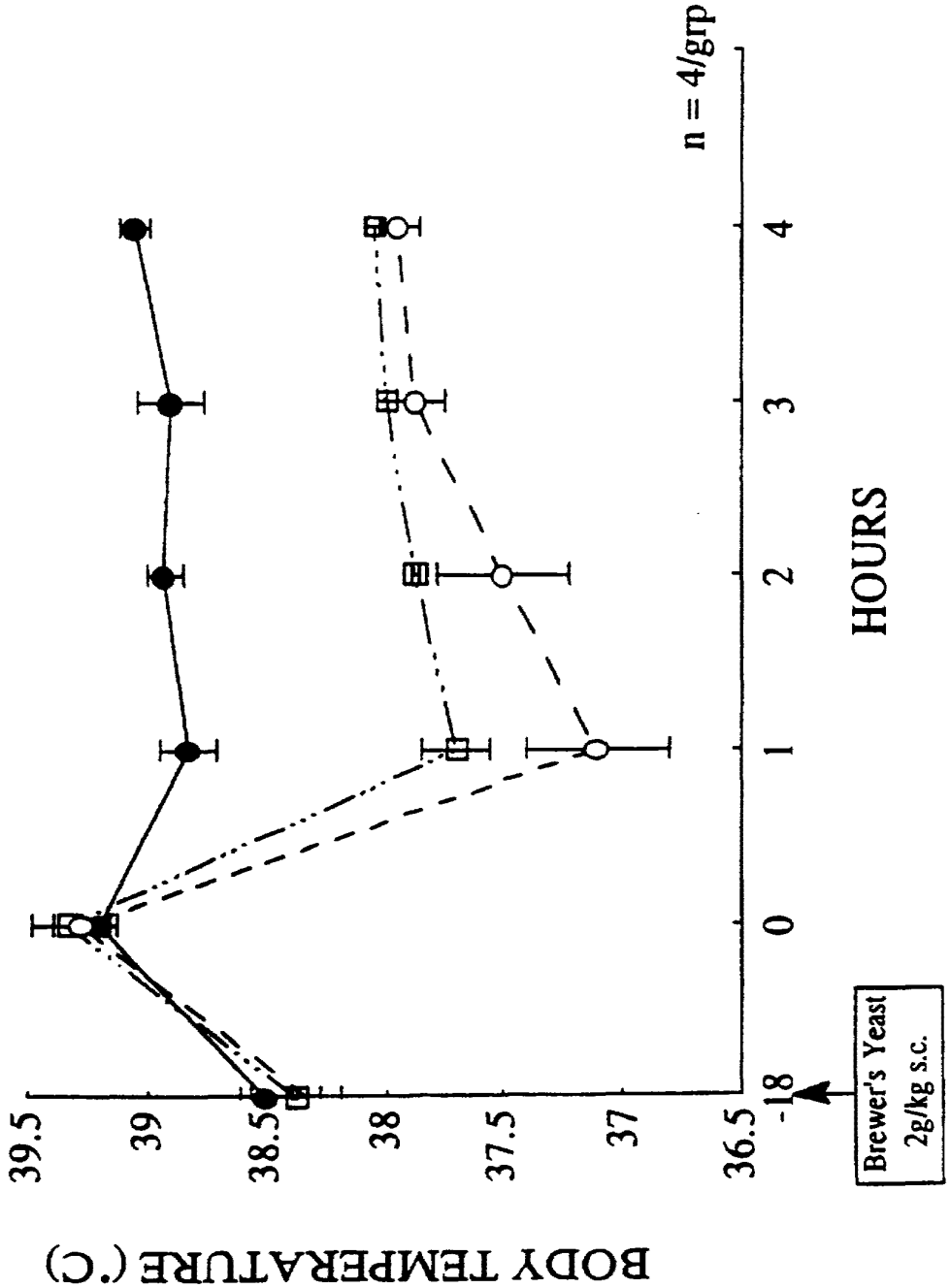

BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
HOURS
n = 4/grp
Brewer's Yeast
2g/kg s.c.

FIG. 18

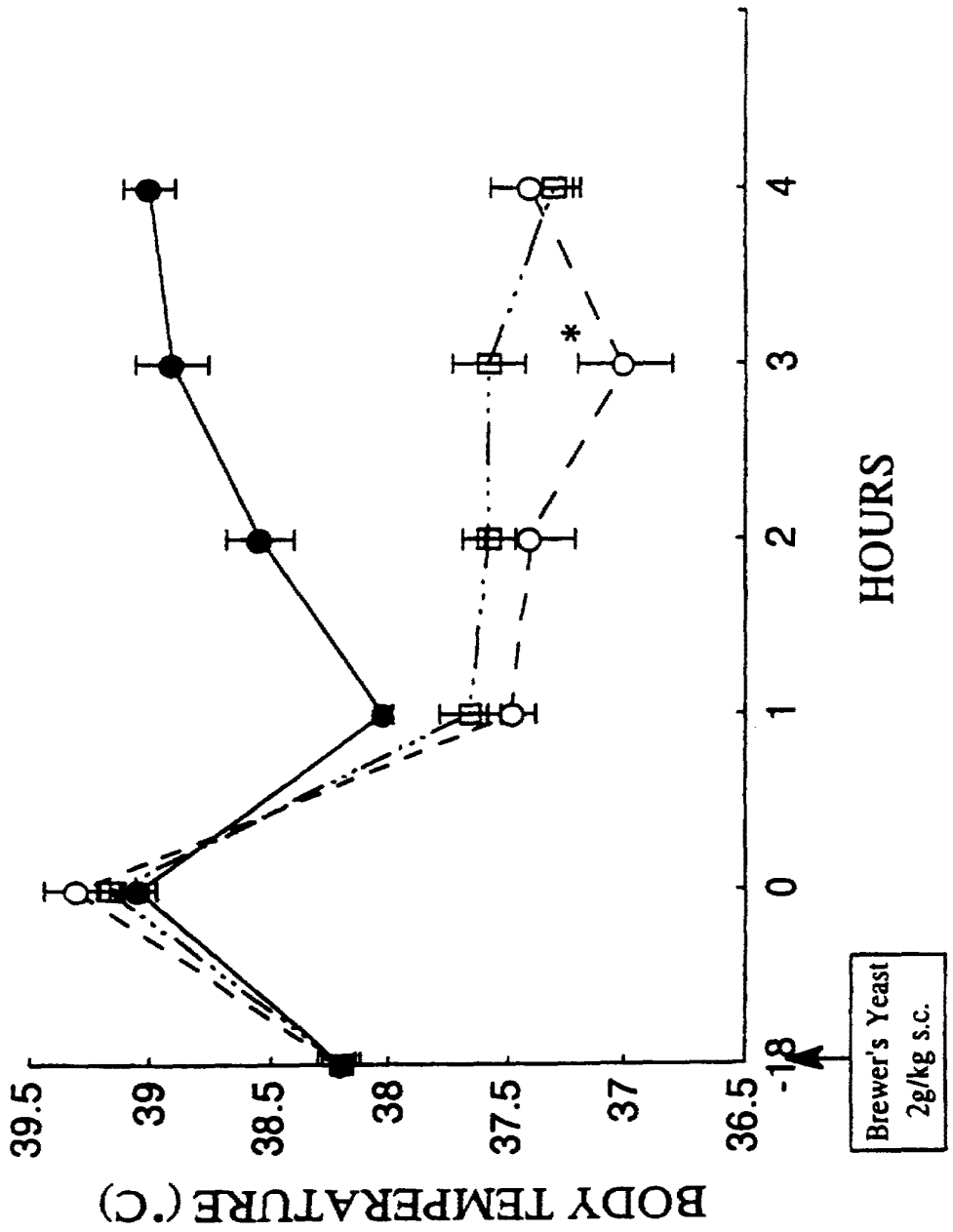

BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
HOURS
*
Brewer's Yeast
2g/kg s.c.

FIG. 19

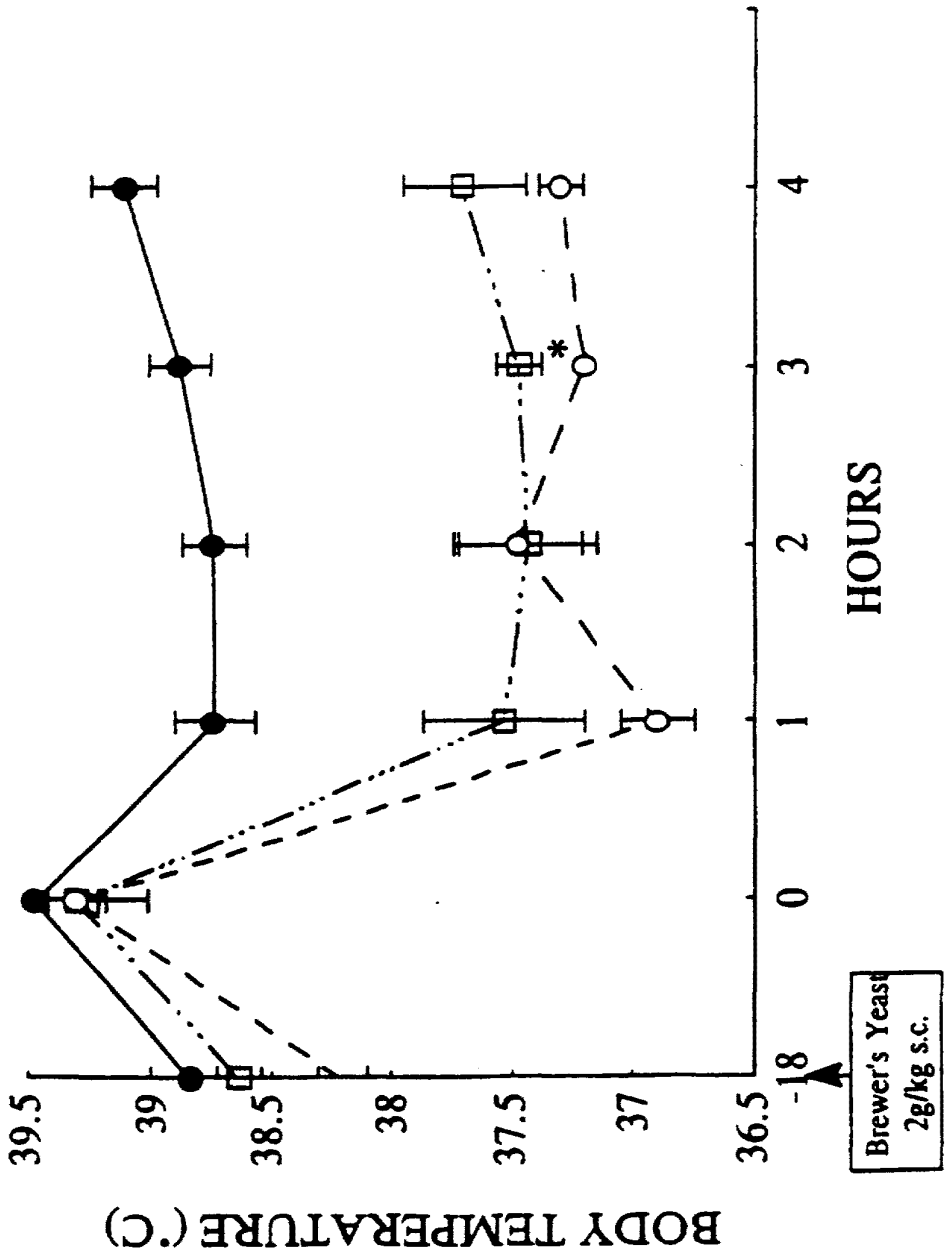

BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
HOURS
Brewer's Yeast
2g/kg s.c.
*

FIG. 20

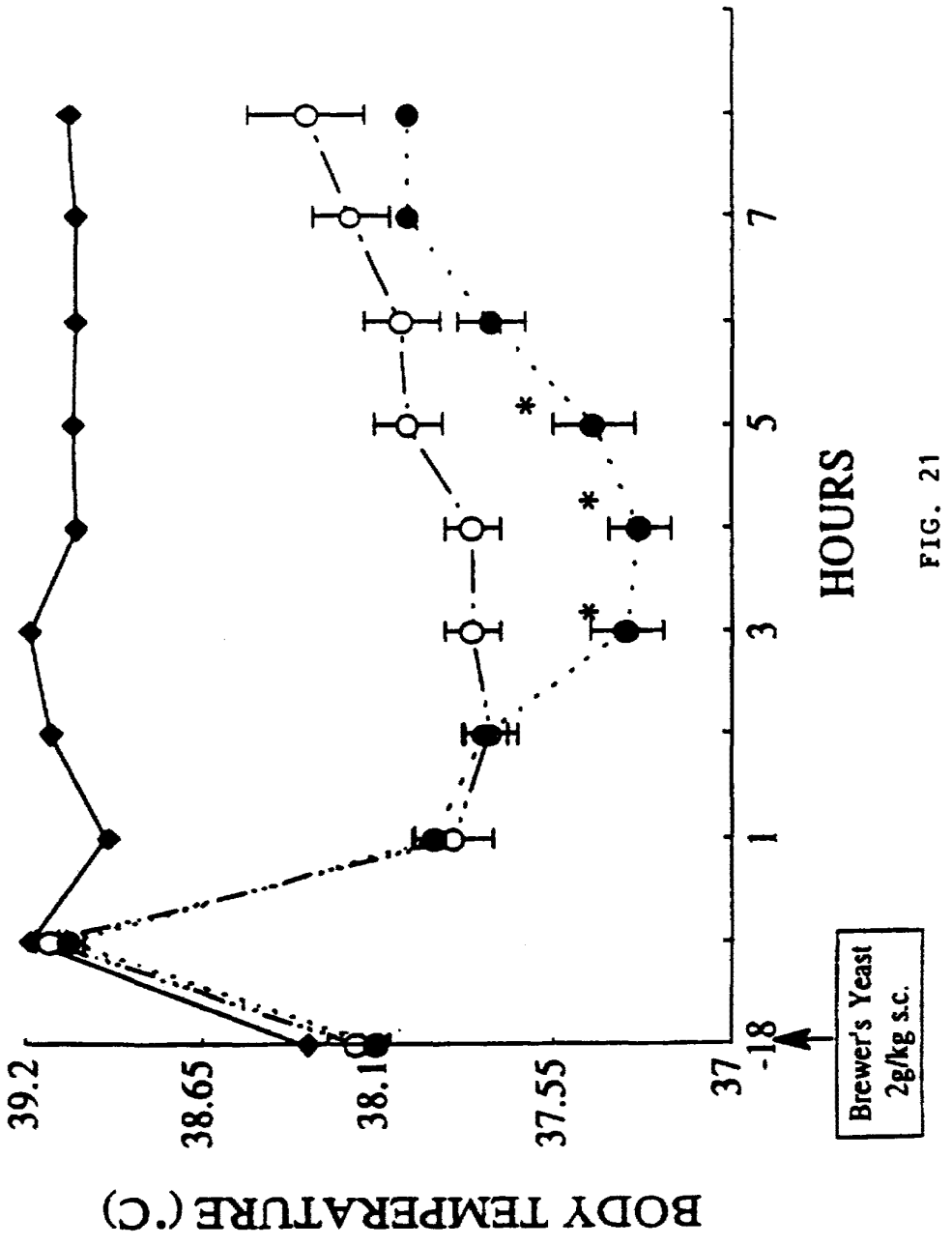

BODY TEMPERATURE (°C)
39.2
38.65
38.1
37.55
37
-1
1
3
5
7
HOURS
Brewer's Yeast
2g/kg s.c.

FIG. 21

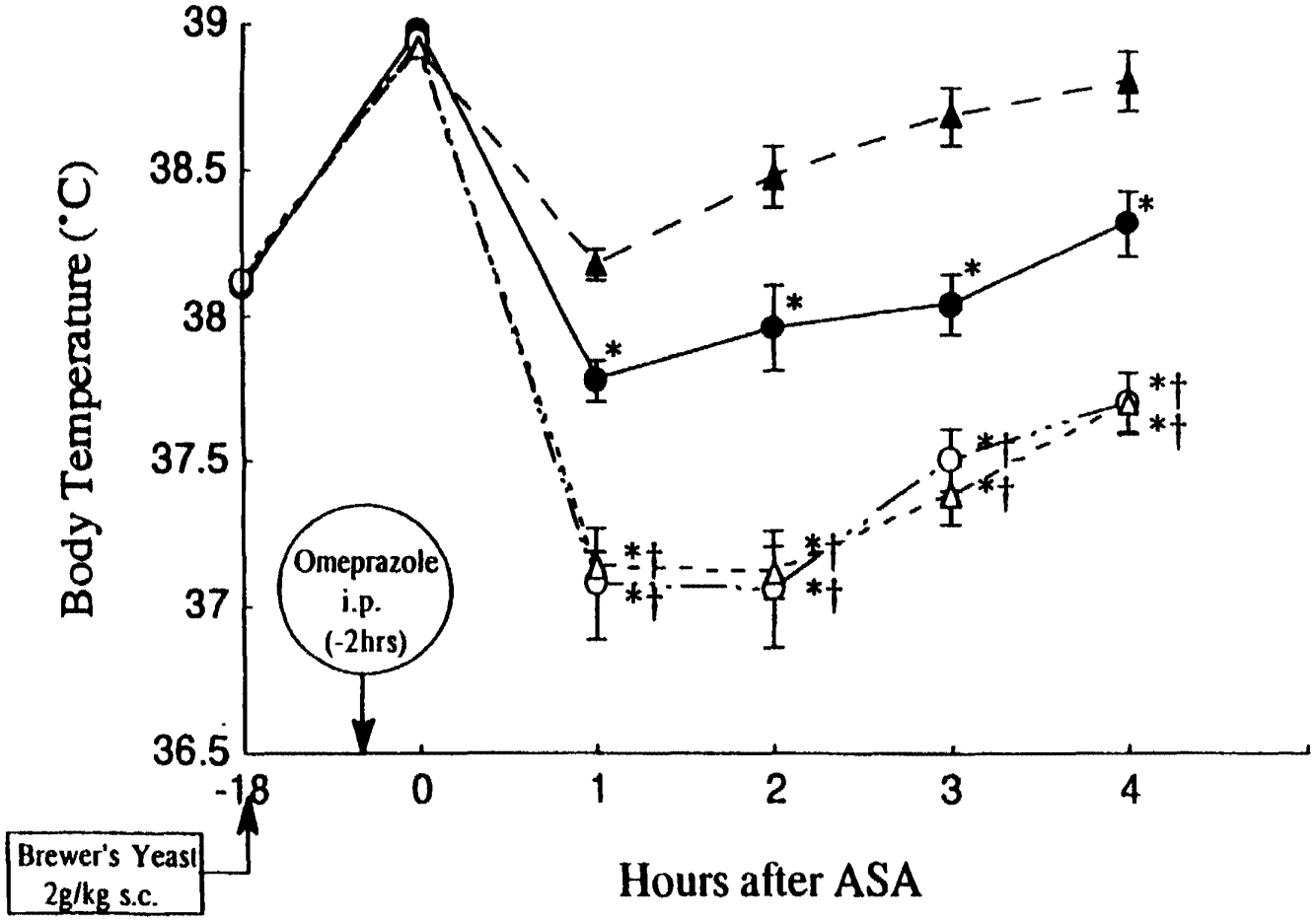

39
38.5
38
37.5
37
36.5
Body Temperature (°C)
-18
0
1
2
3
4
Hours after ASA
Omeprazole
i.p.
(-2hrs)
Brewer's Yeast
2g/kg s.c.

FIG. 22

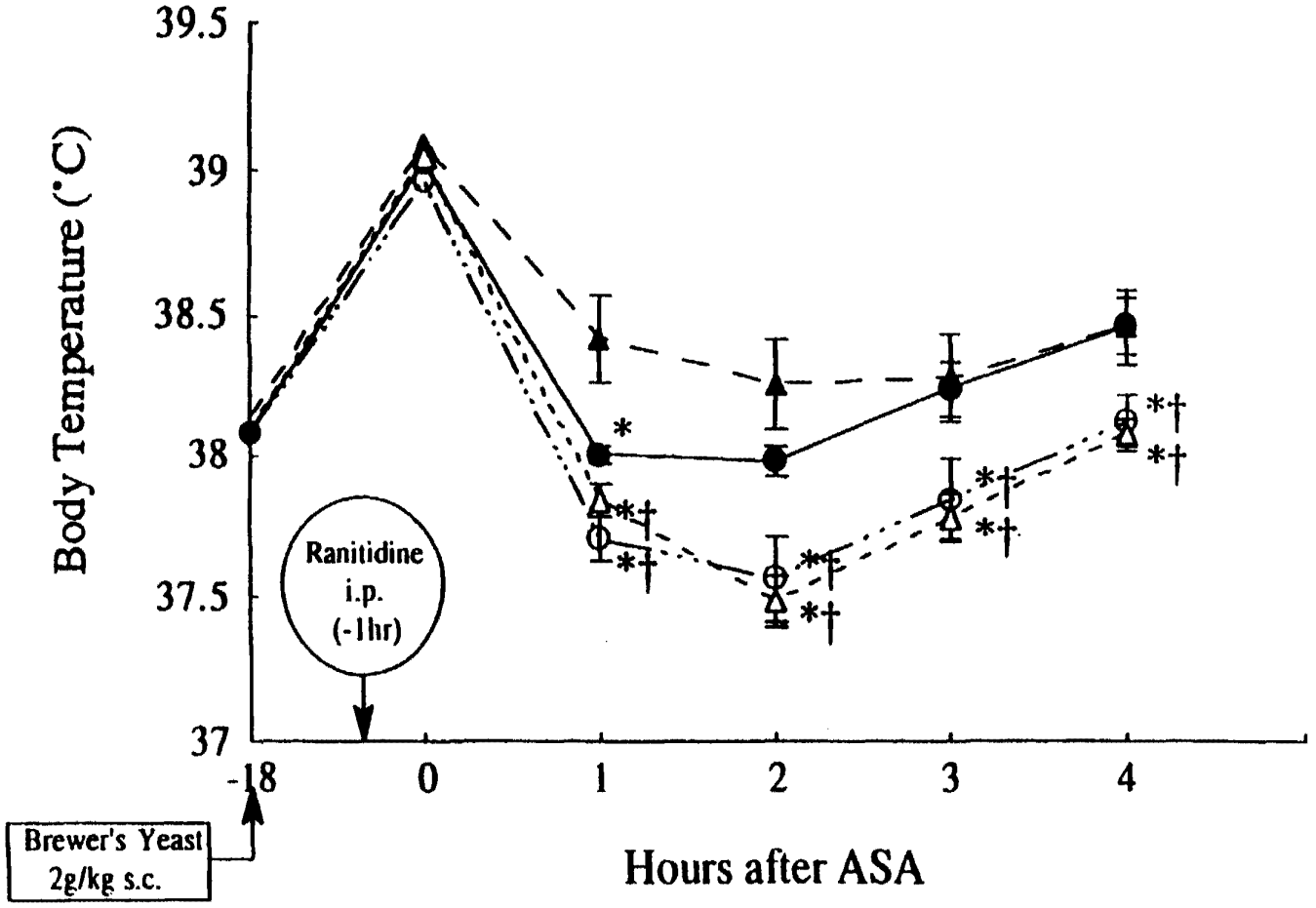

39.5
39
38.5
38
37.5
37
Body Temperature (°C)
-18
0
1
2
3
4
Hours after ASA
Ranitidine
i.p.
(-1hr)
Brewer's Yeast
2g/kg s.c.

FIG. 23

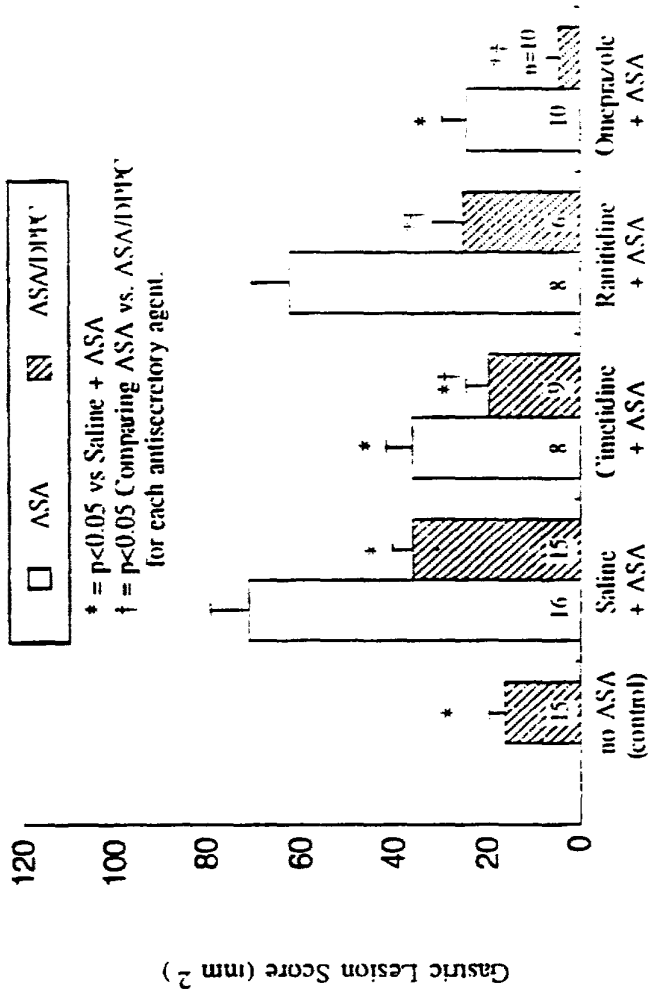

Gastric Lesion Score (mm ²)
120
100
80
60
40
20
0
ASA
ASA/DDTC
* = p<0.05 vs Saline + ASA
† = p<0.05 Comparing ASA vs. ASA/DDTC for each antisecretory agent.
16
15
8
9
8
10
n=10
no ASA (control)
Saline + ASA
Cimetidine + ASA
Ranitidine + ASA
Omeprazole + ASA

FIGURE 24

Indo
Indo/DPPC
* = p<0.05 vs. Saline + Indo
† = p<0.05 Comparing ASA vs. ASA/DPPC
for each antisecretory agent.
Intestinal Bleeding
(mg of Hb)
40
35
30
25
20
15
10
5
0
*
n=4
8
8
4
4
4
4
n=4
n=5
Saline
(control)
Saline
+ Indo
Cimetidine
+ Indo
Ranitidine
+ Indo
Omeprazole
+ Indo

FIGURE 25

Antipyretic Acitivity of ASA (18mg/kg) and ASA/DPPC complex with Rats Pretreated with Anitsecretory Agent.

FIGURE 26 A. Omeprazole

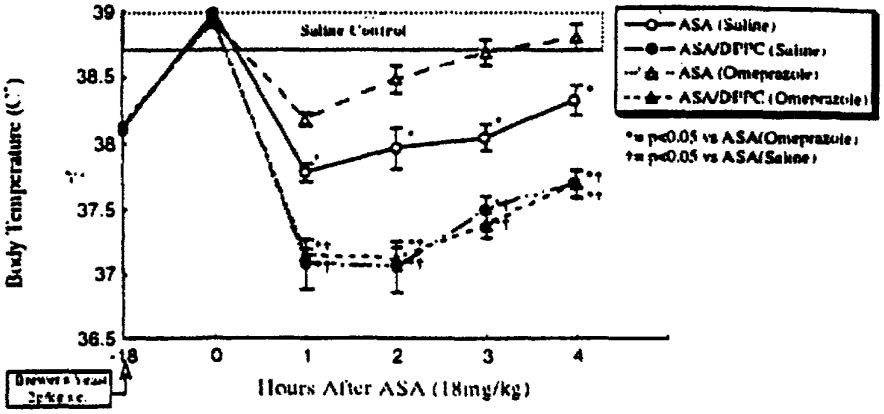


FIGURE 26 B. Ranitidine

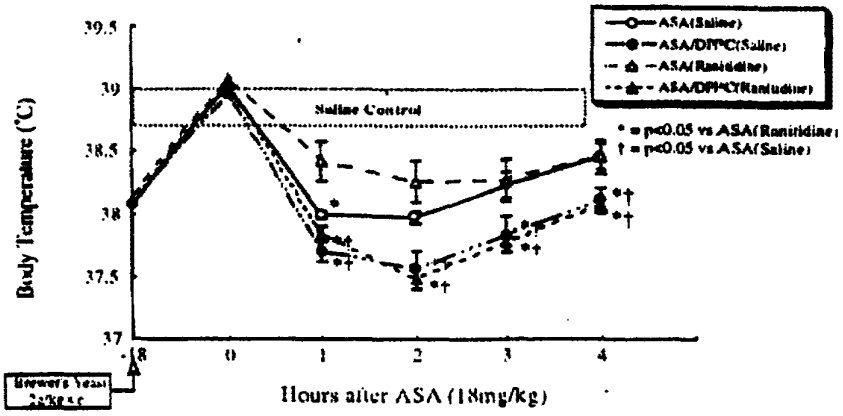
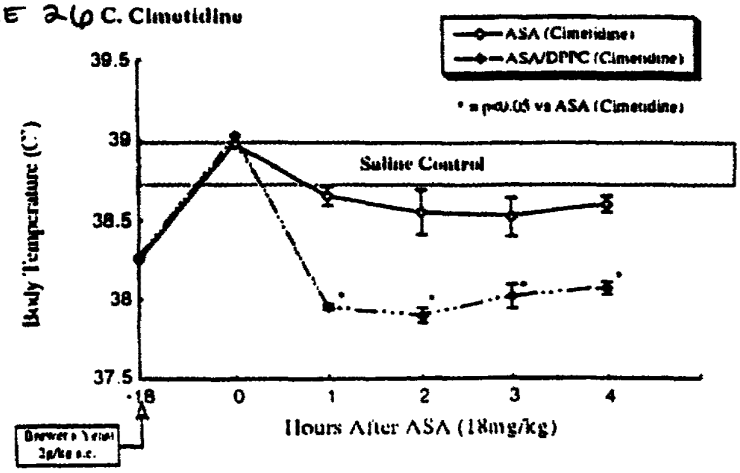


FIGURE 26 C. Cimetidine



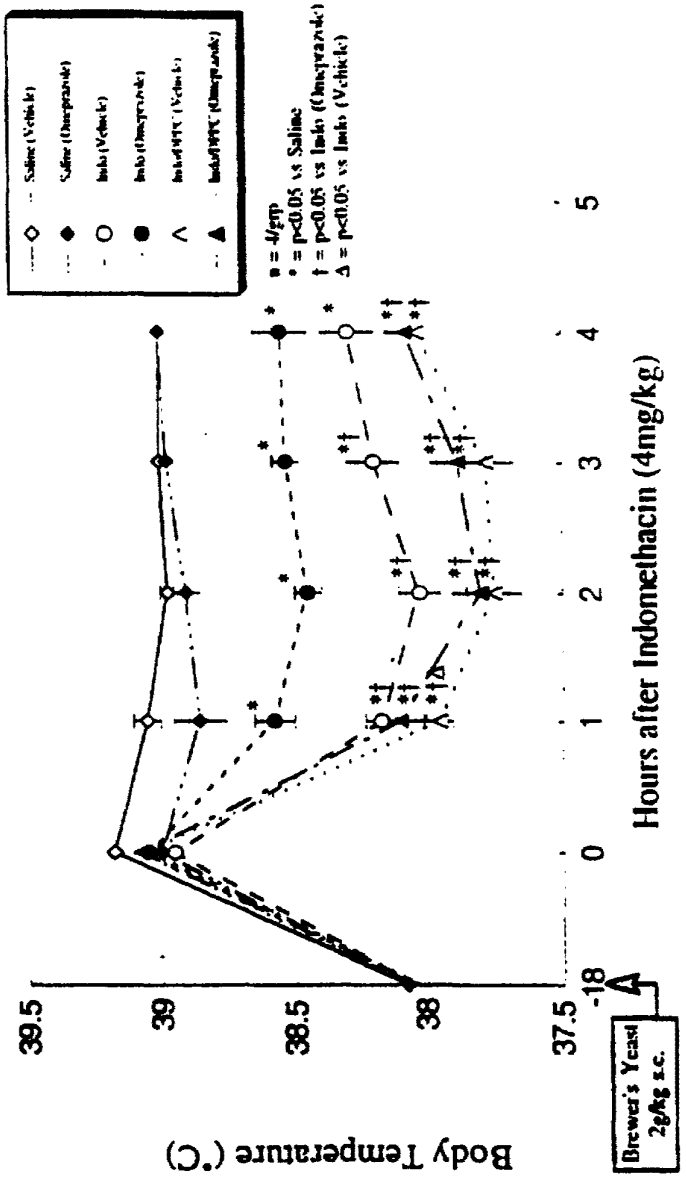

Body Temperature (°C)
39.5
39
38.5
38
37.5
-18
0
1
2
3
4
5
Hours after Indomethacin (4mg/kg)
Brewer's Yeast 2g/kg s.c.
n = 4/grp
* = p<0.05 vs Saline
† = p<0.05 vs Indo (Diazepam)
Δ = p<0.05 vs Indo (Vehicle)

FIGURE 27

FIG. 28 **A. Aspirin (ASA)**

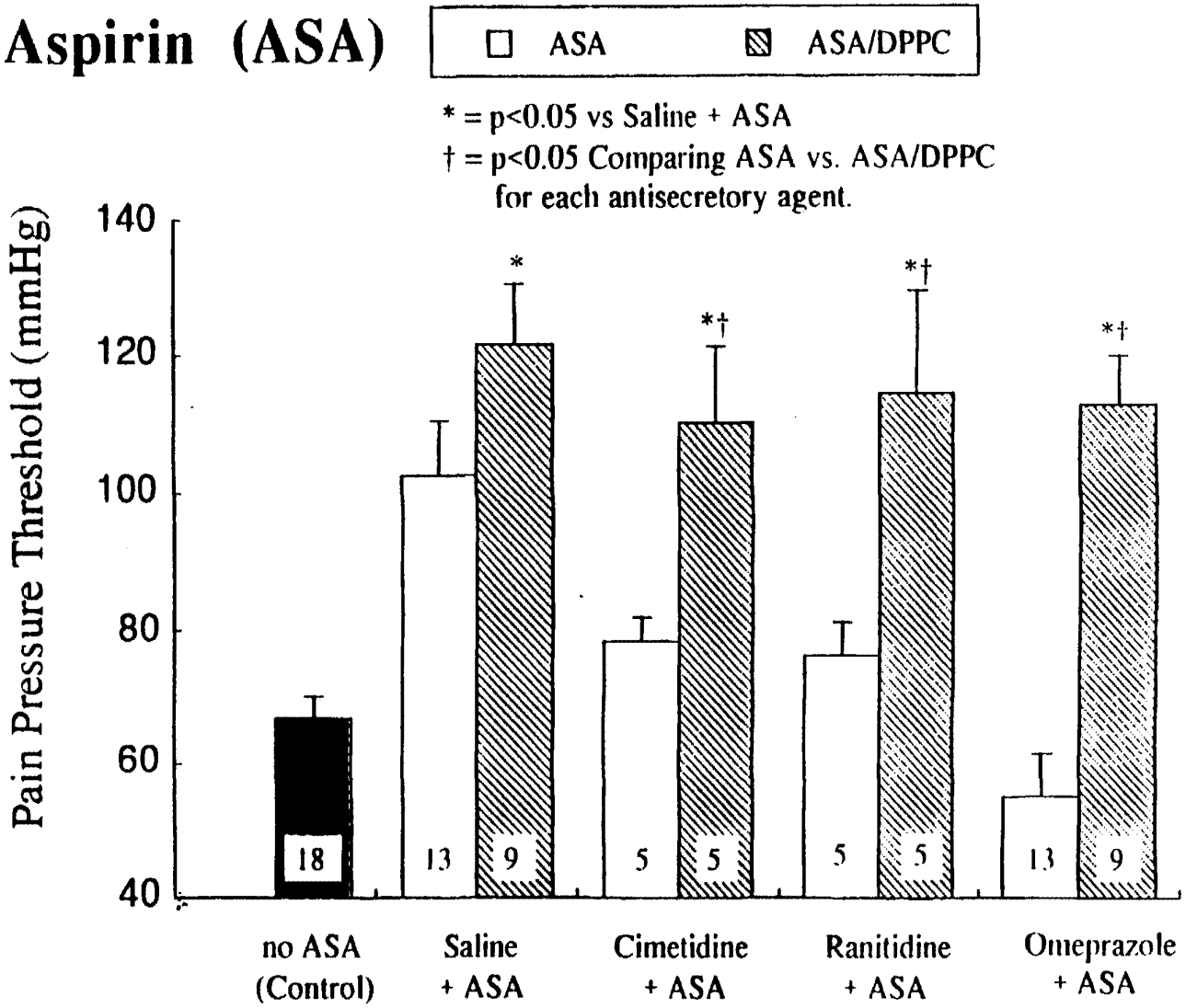
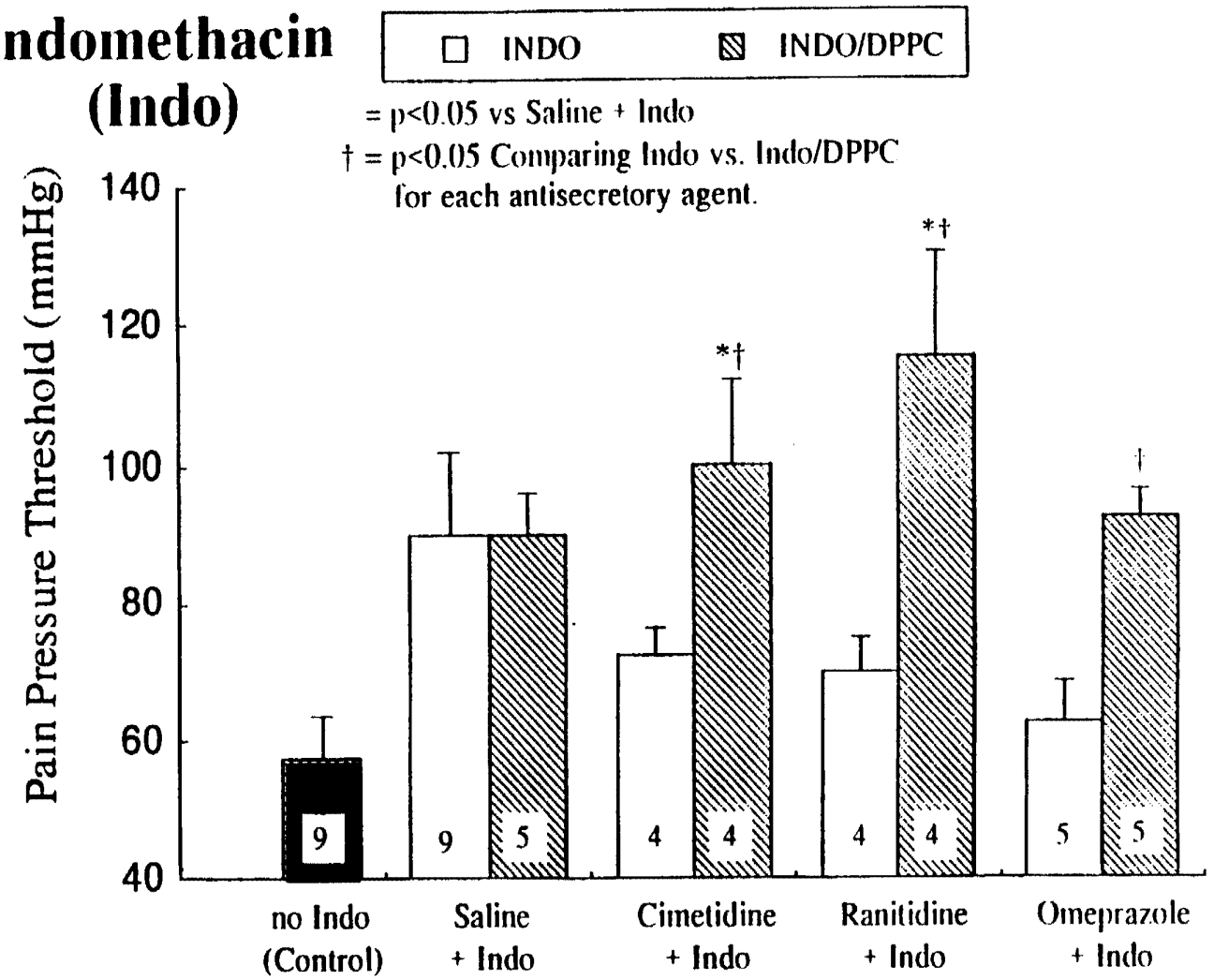


FIG 28 **B. Indomethacin (Indo)**



INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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US CL : 514/360

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/360

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,918,063 A (LICHTENBERGER) 04 April 1990, see entire document.	1-12
Y	Database Chemical Abstracts on STN, AN 1989:107568, Liversidge et al, 'Influence of indomethacin amphoteric gel on gastric ulcerogenicity and absorption of indomethacin in rats,' Pharm. Res. (1989), 6(1), pp. 44-8. See entire abstract.	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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(74) Agent: MAYFIELD, Denise, L.; Vinson & Elkins, L.L.P., 2300 First City Tower, 1001 Fanin Street, Houston, TX 77002–6760 (US).

(81) Designated States: CA, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) Title: METHODS OF ENHANCING THE THERAPEUTIC ACTIVITY OF NSAIDs AND COMPOSITIONS OF ZWITTERIONIC PHOSPHOLIPIDS USEFUL THEREIN

(57) Abstract

Disclosed are compositions comprising non–steroid anti–inflammatory drugs (NSAIDs) complexed with zwitterionic, neutral phospholipids, or both, having reduced gastrointestinal irritating effects and enhanced anti–pyretic, analgesic, and anti–inflammatory activity. Also disclosed are improved methods of using the complexes for treating fever, inflammation, and preventing platelet aggregation. In some embodiments, the anti–pyretic activity of sub–therapeutically used amounts of NSAIDs are enhanced to elicit anti–pyretic activity *in vivo* when associated (Noncovalently) with zwitterionic phospholipids, such as dipalmitoyl phosphatidyl choline. Methods and compositions useful for enhancing the therapeutic activity of non–steroidal anti–inflammatory agents in the presence of anti–secretory agents are also discussed.

*(Referred to in PCT Gazette No. 31/1998, Section II)

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METHODS OF ENHANCING THE THERAPEUTIC ACTIVITY OF NSAIDS AND COMPOSITIONS OF ZWITTERIONIC PHOSPHOLIPIDS USEFUL THEREIN

This application claims priority to U.S. Serial No. 08/719,134 filed September 24, 1996.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the fields of pharmacology and medicine and more particularly, it concerns the treatment and prevention of fever, pain and inflammation with non-steroidal anti-inflammatory drugs (NSAIDs) complexed with phospholipids, and in other embodiments in further combination with neutral lipids. The invention also provides methods for retarding platelet aggregation, and the application of these methods in treating cardiovascular and vascular diseases as it relates to platelet activity.

2. Description of the Related Art

The consumption of NSAIDs among the general populace is unparalleled by any other drug class due to their great efficacy in the treatment of pain, inflammation and fever (Rainsford, 1985). The widespread usage of these drugs is anticipated to increase even further due to their efficacy in the treatment of osteoarthritic and generalized aches and pain as the elderly increase as a percentage of the population (Alexander *et al.*, 1985; Jolobe and Montgomery, 1984), and as NSAIDs are employed in the treatment/prevention of stroke and cardiovascular disease.

The major concern with these developments relates to the tendency of NSAIDs to induce gastrointestinal (GI) mucosal lesions, perforations and bleeding resulting in significant morbidity and mortality, even in occasional NSAID users (Rainsford, 1989; Graham, 1989; Allison *et al.*, 1992). Strategies to reduce the gastroduodenal injurious effects of these drugs with enteric coatings, have had limited success due to the delayed therapeutic actions of these specially packaged NSAIDs (Alpsten *et al.*, 1982; Mojaverian *et al.*, 1987).

Although it is clear that the GI side-effects of NSAIDs are in part attributable to their ability to inhibit the biosynthesis of gastroprotective prostaglandins, a significant amount of evidence exists that NSAIDs act locally on the mucosa to induce GI ulcers and bleeding by a prostaglandin-independent mechanism (Rainsford, 1989; Whittle *et al.,* 1980; Whittle, 1981; Ligumsky *et al.,* 1982; McCormack and Brune, 1987).

The present inventor and others have obtained evidence that the mucosa of the stomach and other regions of the GI tract have hydrophobic, non-wettable properties, that protect the underlying epithelium from gastric acid and other luminal toxins (Hills *et al.,* 1983; Goddard *et al.,* 1987; Goddard *et al.,* 1990; Kao *et al.,* 1990). This biophysical characteristic, which can be quantified by contact angle analysis, appears to be attributable to the presence of an extracellular lining of surfactant-like phospholipid on the luminal aspects of the mucus gel layer (Goddard *et al.,* 1990; Kao *et al.,* 1990). Evidence has also come forth that these zwitterionic phospholipids are synthesized in surface mucus cells of the stomach, as well as those present in discrete submucosal glands of the GI tract, where they are stored in specific organelles and secreted by a prostaglandin-dependent pathway (Kao and Lichtenberger, 1991). It has also been reported that aspirin and other NSAIDs have the ability to rapidly transform the gastric mucosa from a non-wettable to a wettable state within minutes after luminal administration, thereby increasing the tissue's susceptibility to the corrosive actions of gastric acid (Hills *et al.,* 1983; Goddard *et al.,* 1987; Goddard *et al.,* 1990; Kao *et al.,* 1990).

One solution to this problem has been to formulate injectable solutions of NSAIDs and thus bypass the GI tract completely. The low water solubility of these drugs, however, has caused problems with this technique. Stable, injectable solutions of indoleacetic and indanacetic acid derivatives have been developed to address this problem, by complexing these NSAIDs with phosphatidylcholine and phosphatidylethanolamine derivatives (See US Patent 4,309,420).

US Patent 4,421,747 describes NSAIDs complexed with phospholipids for oral administration. These complexes were shown to retain their anti-inflammatory action and to have reduced ulcer formation in rats. However, no enhancement of therapeutic effects was reported with these preparations.

WO 91/16920 (Vical Inc.) relates to phospholipid prodrug derivatives of a salicylate or non-steroidal, anti-inflammatory drug. These preparations are made by combining salicylic acid or NSAID with a phospholipid in the presence of a coupling agent, thereby producing a covalently linked NSAID-phospholipid compound. These prodrugs are described as useful in reducing the toxicity of high dose, long term usage of NSAID preparations.

JP 3176425 (Nippon Shinyaku KK) relates to compositions including nonsteroidal, anti-inflammatory drugs together with neutral lipids and phospholipids in a fat and oil emulsion. Although the method of preparation is not described in the abstract, these compositions appear to be encapsulated in lipid, such as in a micelle. The combination of the drug with the neutral lipids and the phospholipids is described as not affecting the drug's pharmacological actions.

JP 63048228 (Toe Eiyo KK) relates to topically applied compositions that include non-steroidal anti-inflammatory drug together with phospholipid and a "disintegrator". The disintegrator is described as providing for a preparation with improved dispersability and increased absorptivity. JP 63048226 (Ono Pharmaceutical KK) relates to compositions that include a phospholipid base (such as phosphatidylcholine) and an anti-inflammatory agent (such as acetylsalicylic acid and indomethacin). KK JP 58150508 (Ono Pharmaceutical) relates to topical compositions that include a phospholipid base (such as phosphatidylcholine) and an anti-inflammatory agent (such as acetylsalicylic acid and indomethacin).

US Patent 4,369,182 (Nattermann & CIE), relates to inflammation-preventing pharmaceutical compositions for oral administration. The compositions are prepared and then lyophilized into powder form. The described compositions include natural and synthetic phospholipids (dipalmitoylphosphatidylcholine (DPPC)), in combination with nonsteroidal agents including salicylic acid, acetyl-salicylic acid, diflunisal, indomethacin, glucametacine, acemetacin, sulindac, ibuprofen, naproxen, tolmetin and other NSAID's. Also described are NSAID's in combination with phosphatidylcholine preparations, named phospholipons. US Patent 4,421,747 relates to methods of alleviating inflammation with compositions as described in the '182 patent.

Despite the extensive work in the area of NSAIDs, a need continues to exist in the art for preparations that include reduced amounts of this useful class of drug without loss of therapeutic efficacy. Methods and compositions that provide for similar or enhanced anti-pyretic, anti-inflammatory, anti-platelet and analgesic activity at lower doses than currently prescribed for pharmacological activity would also render this very valuable class of drugs available to those previously unable to tolerate standard and/or prolonged therapeutic regimens of NSAID.

SUMMARY OF THE INVENTION

The present invention seeks to overcome these and other drawbacks inherent in the prior art by providing compositions and methods capable of maintaining and/or improving the pharmacological activity of non-steroidal anti-inflammatory drugs by noncovalent association with zwitterionic phospholipids. In some embodiments, these preparations may further include neutral lipids, such as the triglycerides.

The present invention illustrates the ability of one or more zwitterionic phospholipids to enhance the fever-reducing potential of an NSAID. Pharmacological activity of low dose NSAID to reduce inflammation and pain may also be observed, and in some cases enhanced by chemically associating the NSAID with zwitterionic phospholipid, such as phosphatidyl choline (PC), dipalmitoylphosphatidylcholine (DPPC), and other disaturated phosphatidyl cholines, and the like. In some embodiments, the association of NSAID and zwitterionic phospholipid is of a non-covalent nature. In still other embodiments, the NSAID and zwitterionic phospholipid compositions may be further described as including more or less equimolar amounts of these ingredients. The compositions may also, of course, comprise a pharmaceutically acceptable carrier in any form, such as solid powder, gel or liquid form.

The present invention focuses techniques that are demonstrated in some cases to enhance the therapeutic activities of NSAIDs. This is accomplished without the disadvantage of hindering the pharmacological activity or therapeutic bioavailability of the drug rendering the preparations effective even at low doses.

Accordingly, the present invention provides in one aspect a method for enhancing the antipyretic activity of a nonsteroidal anti-inflammatory drug (NSAID). This method comprises providing a non-covalently associated combination of a zwitterionic phospholipid with an amount of a nonsteroidal anti-inflammatory drug that provides reduced anti-pyretic activity in the absence of the zwitterionic phospholipid. Some embodiments of the present preparations are further described as being essentially free of anionic phospholipid, or as including an amount of anionic phospholipid that is biologically inert and/or not an active component, of the preparation.

The term "essentially free" as used in the description of the present invention, is understood to mean compositions that include less than about 0.10% of anionic phospholipid, and in even further defined embodiments, less than 0.01% anionic phospholipid. As used in the description of the present invention, the term zwitterionic phospholipid embraces a wide range of phospholipids, including but not limited to phosphatidylcholine, phosphatidylserine, phosphalidylethanolamine, sphingomyelin and other ceramides, as well as various other zwitterionic phospholipids. In some embodiments, these compositions are essentially free of anionic phospholipid.

In other embodiments of the described method, the amount of nonsteroidal anti-inflammatory drug is defined as an amount that provides reduced antipyretic activity in the absence of the zwitterionic phospholipid. Such amounts of the drug sub-therapeutically effective amounts thereof. This activity or lack of activity is observed in the absence of zwitterionic phospholipid, while the same or about the same amount of the NSAID does demonstrate pharmacological activity in the presence of zwitterionic phospholipid. In this regard, the phenomenon is observed that the combination of low amounts of nonsteroidal anti-inflammatory drugs with phospholipid have potent pharmacological activity, while doses of the drug alone (i.e., without zwitterionic phospholipid) do not.

The present method employs compositions that may include any variety of those drugs generally classified as nonsteroidal anti-inflammatory drugs. By way of example, these drugs include ibuprofen, piroxicam, salicylate, aspirin, naproxen, indomethacin, diclofenac, or any mixture thereof. In particular embodiments, the nonsteroidal anti-inflammatory drug is salicylate. By way of example and not limitation, NSAID's useful in the practice of the invention, include those noted in Table 1.

TABLE 1

Nonsteroidal Anti-Inflammatory Drugs To Be Used in Combination with Zwitterionic Phospholipids

Propionic acids	Fenoprofen calcium	Nalfon®
	Flurbiprofen	Ansaid®
	Suprofen	
	Benoxaprofen	
	Ibuprofen (prescription)	Motrin®
	Ibuprofen (200 mg. over the counter)	Nuprin, Motrin 1B®
	Ketoprofen	Orduis, Oruvall®
	Naproxen	Naprosyn®
	Naproxen sodium	Aleve, Anaprox, Aflaxen®
	Oxaprozin	Daypro®
Acetic acids	Diclofenac sodium	Voltaren®
	Diclofenac potassium	Cataflam®
	Etodolac	Lodine®
	Indomethacin	Indocin®
	Ketorolac tromethamine (intramuscular)	Acular, Toradol®
	Ketorolac (oral)	Toradol®
Ketones	Nabumetone	Relafen®
	Sulindac	Clinoril®
	Tolmetin sodium	Tolectin®
Fenamates	Meclofenamate sodium	Meclomen®
	Mefenamic acid	Ponstel®
Oxicams	Piroxicam	Dolibid®
Salicylic acid	Diflunisal	Feldene®
	Aspirin	
Pyrazolin acid	Oxyphenbutazone	Tandearil®

	Phenylbutazone	Butazolidin®

NSAIDs such as benoxaprofen, ketoprofen, oxaprozin, etodolac, ketorolac tromethamine, ketorolac and nabumetone, together with zwitterionic phospholipid, comprise still other particular embodiments of the invention, again both with and without neutral lipid.

In particular embodiments, the zwitterionic phospholipid in the compositions is dipalmitoyl phosphatidylcholine, phosphatidyl choline, or a mixture thereof.

The pharmacological, particularly anti-pyretic, activity of NSAIDs, is shown to be enhanced several-fold over preparations with similar doses without zwitterionic phospholipid. Surprisingly, the present inventors have found that combination of the afore-described nonsteroidal anti-inflammatory drugs with zwitterionic phospholipid dramatically enhances the anti-pyretic activity and potency of the drug, even at sub-therapeutically active doses, and in some cases from about 2-fold to about 6-fold relative to non-phospholipid containing preparations. Hence, the methods are expected to be particularly efficacious in reducing fever in a mammal having reduced tolerance for NSAID's.

Amounts ranging between one-tenth and one-half that typically necessary to elicit a fever-reducing response in a mammal may thus be realized employing the present inventive methods and compositions. In this regard, it is expected that amounts of between 2 mg/kg to about 300 mg/kg will provide fever-reducing therapeutic activity. Of course, the amount/dose used will depend in specific cases on the particular pharmacological characteristics of the NSAID or combination of NSAIDs included. Further defined ranges of the drug expected to provide the anti-pyretic activity herein disclosed range from between about 10 to about 150 mg/kg or about 20 or 50 mg/kg to about 150 mg/kg.

The present inventors have also observed the claimed compositions are useful for enhancing the platelet retarding activity of a non-steroidal anti-inflammatory drug. This method for inhibiting platelet aggregation comprises providing a non-covalently associated combination of zwitterionic phospholipid and an amount of non-steroidal anti-inflammatory agent that provides reduced inhibition of platelet aggregation in the absence of zwitterionic phospholipid. In some embodiments, this composition is essentially free of anionic phospholipid and/or includes an amount of anionic phospholipid that is a biologically inert component of the preparation. The amounts of non-steroidal anti-

inflammatory drug employed as part of the composition, again, is relatively low, and may be further described as an amount that generally provides reduced pharmacological activity in the absence of zwitterionic phospholipid. Again, the zwitterionic phospholipid of choice is, in some embodiments, DPPC, PC, or a combination thereof.

The compositions of the afore-described methods may further include a neutral lipid, such as a triglyceride. For a partial listing of representative neutral lipids, such as the triglycerides, reference is specifically made to U.S. Patent Number 4,950,656 and 5,043,329. Both saturated and unsaturated triglycerides may be employed in the present compositions, and include such triglycerides as tripalmitin (saturated), triolein and trilinolein (unsaturated). However, these particular triglycerides are listed here for convenience only, and are merely representative of a variety of useful triglycerides, and is further not intended to be inclusive.

Turning now to another aspect of the invention, methods for enhancing the analgesic activity of a non-steroidal anti-inflammatory drug are provided. These methods again comprise providing a non-covalently associated composition comprising zwitterionic phospholipid and an amount of a non-steroidal anti-inflammatory drug that provides reduced pharmacological activity in the absence of zwitterionic phospholipid. In some embodiments, these compositions are essentially free of anionic phospholipid, or include amounts of anionic phospholipid that are biologically inert. In particular embodiments, the nonsteroidal anti-inflammatory drug is one or more of those listed in Table 1. In particular embodiments, the NSAID is aspirin, salicylate, a salt thereof, or a combination thereof. While any of a number of different zwitterionic phospholipids may be employed in the composition of the method, in some embodiments, the phospholipid is dipalmitoyl phosphatidyl choline, phosphatidyl choline, or a combination thereof. In these and other embodiments of the described method, an anionic phospholipid that may be excluded or included only in biologically inert amounts is phosphatidyl glycerol (PG).

In still another aspect, the invention provides methods of enhancing the anti-inflammatory activity of non-steroidal anti-inflammatory drugs. The method comprises proving a non-covalently associated combination of zwitterionic phospholipid with an amount of a non-steroidal anti-inflammatory drug. The amount of NSAID in the composition is again defined as an amount that provides reduced pharmacological activity

in the absence of zwitterionic phospholipid. In some embodiments, the method employs compositions that are essentially free of anionic phospholipids, such as the DPPG or PG, or includes amounts of anionic phospholipid that are biologically and pharmacologically inert.

In particular embodiments, the composition is further defined as comprising an equimolar amount of NSAID and zwitterionic phospholipid. The present inventors' studies demonstrate the described combination of ingredients provides an enhancement of the anti-inflammatory activity and potency of these drugs compared to drug preparations that do not include zwitterionic phospholipid. Also demonstrated is the reduction in anti-inflammatory activity observed where pharmacologically active (i.e., non-biologically insert amounts) amounts of anionic phospholipid, such as PG (PI), are included in the preparation. Hence, preparations that include pharmacologically active amounts of DPPG or PI would not necessarily provide for the same enhanced anti-inflammatory activity, or the afore-described enhanced pharmacological activity (i.e., antipyretic or enhanced reduction in platelet aggregation activity), described in the present methods. Pharmacologically active amounts of other negatively-charged phospholipids would also not be contemplated as particularly useful in view of these results.

Turning now to still a further aspect of the present invention, a method for enhancing the antipyretic potential of sub-therapeutically effective amounts of nonsteroidal anti-inflammatory drug is disclosed. In some embodiments, the method comprises again combining zwitterionic phospholipid with an amount of non-steroidal anti-inflammatory drug to provide a noncovalently associated composition thereof. In some embodiments, the composition is further defined as essentially free of biologically active amounts of anionic (or negatively charged) phospholipid.

As used in the description of the above method, a sub-therapeutically effective amount of NSAID is defined as an amount that provides reduced antipyretic activity in the absence of a zwitterionic phospholipid. The enhancement in activity of low amounts of NSAID illustrated in the various *in vivo* studies disclosed herein demonstrate that while doses of aspirin of about 9 mg/kg in combination with the zwitterionic phospholipid, DPPC, did provide for a fever reducing (anti-pyretic) pharmacologic response, treatment with this same dose of NSAID without phospholipid did not.

The present invention also discloses particular pharmaceutical preparations. These pharmaceutical preparations are further described as suitable for enteral or oral administration, and comprise a non-covalently associated combination of zwitterionic phospholipid, non- steroidal anti-inflammatory drug , and a pharmaceutically acceptable carrier. These compositions are formulated to provide a non-covalently linked composition that is further described as being essentially free of biologically active (pharmacologically active) amounts of anionic phospholipid, and in some embodiments, essentially free of DPPG.

In some applications, the non-steroidal anti-inflammatory drug is one or more of those listed in Table 1, such as naproxen, indomethacin, diclophenac, salicylate, aspirin, or any mixture thereof. In particular embodiments, the non-steroidal anti-inflammatory drug of choice is salicylate. While any variety of zwitterionic phospholipids may be employed alone or in combination with the described drugs, some of the representative phospholipids include phosphatidyl choline, dipalmitoyl phosphatidylcholine, phosphatidyl serine, other zwitterionic phospholipids, or mixtures thereof. The compositions may further include a neutral lipid, such as a triglyceride. Representative triglycerides are described in U.S. Patent 4,950,656, which reference is specifically incorporated herein by reference for this purpose. In particular embodiments, the pharmaceutical preparation is defined as comprising an equimolar amount of zwitterionic phospholipid and NSAID.

In yet another aspect, the present invention provides a method of maintaining and/or enhancing the therapeutic activity of a non-steroidal anti-inflammatory drug in the presence of a drug that reduces or inhibits gastric secretion. In one embodiment, the method comprises administering a combination of a non-steroidal anti-inflammatory agent and a zwitterionic phospholipid to an animal having received an anti-secretory agent. By way of example, drugs that reduce or inhibit gastric acid secretion in an animal include omeprazole.

The present invention also provides methods for maintaining or enhancing therapeutic activity of a non-steroidal anti-inflammatory agent in the presence of an H2 receptor blocker. In some embodiments, the method comprises administering the anti-inflammatory agent in association with a zwitterionic phospholipid to the animal having received an H2 receptor blocker, wherein the pH of the gastric secretions in the animal are

maintained at fasting levels. As used in the description of the present invention, fasting levels are defined as a pH of about a pH 1 to about pH 4. By way of example, H2 receptor blockers include Zantac, Tagamet, nizetidine, as well as those agents listed at Table 7.

The present invention further provides a method for maintaining and/or enhancing the bio-availability of a non-steroidal anti-inflammatory drug in an animal. In some embodiments, the method comprises administering a combination of a non-steroidal anti-inflammatory agent and a zwitterionic phospholipid to an animal having received an anti-secretory agent, wherein the pharmacological activity of the NSAID is greater than the pharmacological activity of the non-steroidal anti-inflammatory agent administered in the absence of a zwitterionic phospholipid. activity in an animal receiving an anti-secretory agent.

The present invention further provides a pharmaceutical preparation comprising a non-covalently associated combination of a non-steroidal anti-inflammatory agent, zwitterionic phospholipid, and an agent that reduces or inhibits gastric acid secretion. Such agents include, by way of example, antisecretory agents. By way of example, such anti-secretory agents include omeprazole. Such agents include both proton pump inhibitors (e.g. lansoprazole), and H2 receptor antagonists.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, anti-oxidant, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions and methods described herein is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

As used in the description of the present invention, the term "sub-therapeutically effective amount", particularly as it is used to described the amount of NSAID employed, is defined as an amount of the NSAID that provides reduced pharmacological (i.e., anti-pyretic) activity in the absence of non-covalent association with a zwitterionic phospholipid.

It is understood that as used in the present disclosure and appended claims, the terms "a" and "an," as in "an element" or "a molecule" are intended to include one or more

items or elements, and in no way limit the description or claimed element to one element or item.

The following abbreviations are employed in the description of the invention:

PI = phosphatidyl inositol

PC = phosphatidylcholine

PG = phosphatidylglycerol

L-NAME = N-nitro-L-arginine Methyl Ester

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1A. Photograph of test tubes containing 30 nM of the sodium salts of one of the five NSAIDs tested in chloroform in the absence and presence of 30 nM DPPC (naproxen, indomethacin, diclofenac, salicylate, aspirin).

FIG. 1B. The concentration of each NSAID in solution increases (quantified by UV absorption) in proportion to the molar equivalents of DPPC dissolved in chloroform (Naproxen = -▲-; Aspirin = -•-; Salicylate = -□-, Diclofenac = -△-; Indomethacin = -○-).

FIG. 2A. The DPPC-induced reduction in ASA's solubility in water was maximal when both reactants were present in equimolar concentration over a 60 min. incubation period at 25°C.

FIG. 2B. Time course at 25°C of the reduction in ASA's solubility in water induced by the presence of equimolar concentrations of DPPC. In contrast, the concentration of ASA in water was not significantly changed over time by the addition of an equimolar concentration of the anionic phospholipid, DPPG as a lipidic suspension (ASA only = -•-; DPPG/ASA = -□-; DPPC/ASA = -○-).

FIG. 3. The passive diffusion of ASA from water into cyclohexane is markedly accelerated by the presence of zwitterionic (DPPC) but not anionic (DPPG) phospholipids in the aqueous solution (ASA/DPPC = -○-; ASA/DPPG = -□-; DPPC = -•-; ASA = -x-).

FIG. 4A. The injurious potential of salicylate and ASA to induce gastric lesions (in rats subsequently challenged with 0.6N HCl), is remarkably decreased when the NSAIDs are intragastrically administered in association with DPPC (without DPPC = clear bar; with DPPC = hatched bar).

FIG. 4B. The injurious potential of non-salicylate NSAIDs to induce GI bleeding (in rats pre- and post-treated with L-NAME to increase the animal's susceptibility to the NSAID) is remarkably decreased when the drugs are intragastrically administered in association with DPPC (without DPPC = clear bar; with DPPC = hatched bar).

FIG. 4C. The anti-pyretic activity of the NSAID (aspirin - dose 90 mg/kg) is not diminished, and is augmented if administered as a lipidic suspension. Asterisk (*) represents a statistically significant difference between the values of rats treated with the NSAID alone (-DPPC) and those treated with the NSAID/DPPC complex (+ DPPC) (Saline = -•-; ASA = -□-; ASA/DPPC = -○-).

FIG. 5. Anti-inflammatory action of ASA and Phospholipon G™ (Phospholipid "G") as determined by the implanted string assay as described. Dosage is 90 mg/kg ASA. Each bar represents data for n=5 rats.

FIG. 6. Antipyretic activity of ASA\DPPC complex at a subthreshold ASA dosage (9.0 mg/kg) (Saline = -□-; ASA (9.0 mg/kg) = -▲-; ASA/DPPC (9 mg/kg) = -○-).

FIG. 7. Antipyretic activity of aspirin (ASA) alone when administered at doses which range from 2.5-90.0 mg/kg. In this and all subsequently figures the test agents were intragastrically administered 18 hrs after the rats were subcutaneously injected with 2g/kg Brewer's Yeast to induce a 0.5-1.0°C increase in body temperature. It can be appreciated that doses of aspirin of <10 mg/kg failed to reduce the fever during the 4 hour study period (Saline = -◇-; ASA, 2.5 mg/kg = -□-; ASA, 5 mg/kg = -○-; ASA, 10 mg/kg = -△-; ASA, 20 mg/kg = -■-; ASA, 45 mg/kg = -•-; ASA, 90 mg/kg = -▲-).

FIG. 8. In contrast to the above pattern aspirin (ASA) when complexed with an equimolar concentration of dipalmitoylphosphatidylcholine (DPPC) effectively reduced fever at a dose of 9.0 mg/kg, whereas the anionic phospholipid, DPPG, failed to augment aspirin anti-pyretic activity at this same sub-threshold dose. This figure also demonstrates that anionic phospholipid is essentially biologically inert in terms of enhancing the anti-pyretic action

of aspirin (ASA, 9.0 mg/kg = -•-; ASA/DPPC, 9.0 mg/kg = -□-; ASA/DPPG, 9.0 mg/kg = -○-; n=5/grp; * = p <0.05 vs ASA; † = p <0.05 vs ASA/DPPG).

FIG. 9. ASA at a subthreshold dose of 4.5 mg/kg, which had no antipyretic activity alone, effectively reduced fever when complexed with an equimolar concentration of DPPC (H_2O = -□-; H_2O/DPPC (4.5 mg/kg) = -▲-; ASA (4.5 mg/kg) = -⊖-; ASA/DPPC (4.5 mg/kg) =--○--).

FIG. 10. Compilation of all the data at subthreshold doses (2.5-9.0 mg/kg) of ASA. ASA alone (open symbols) data demonstrates the enhancement of antipyretic activity when ASA was complexed to DPPC (closed symbols) (Saline = -*-; ASA, 9mM = -□-; ASA, 4.5 mM = -○-; ASA, 2.5 mM = --△--; ASA/DPPC, 9mM = -■-; ASA/DPPC, 4.5mM = -•-; ASA/DPPC, 2.25 mM = --▲--).

FIG. 11. Dose-response analysis of the antipyretic activity of ASA alone and the ASA/DPPC (equimolar ratio) complex 1 hr (FIG. 11) (ASA = -•-; ASA/DDPC = -□-) after intragastric administration. The potency of the ASA, as reflected by the ED_{50} is increased ~ 10 fold when it is administered with the zwitterionic phospholipid.

FIG. 12. Dose response analysis of the anti-pyretic activity of ASA alone and the ASA/DPPC (equimolar ratio) complex 2 hours after intragastric administration.

FIG. 13. Effect of varying the ASA:DPPC ratio from 1:1 on antipyretic activity at 1 hr post intragastric administration. It can be appreciated that the ability of the zwitterionic phospholipid to enhance the antipyretic activity of ASA was lost when the molar concentration of DPPC was increased (from unity) by a factor of 4 or decreased by a factor of 10 (ASA =9.01 mg/kg).

FIG. 14. Effect of varying the ASA:DPPC ratio from 1:1 on the antipyretic activity of the complex 2 hours post intragastric administration (Legends same as in FIG. 13) (ASA = 9.01 mg/kg).

FIG. 15. At subthreshold doses the antipyretic efficacy of ASA could be clearly enhanced (0-120 min. post-administration), if the NSAID was administered as a microemulsion containing DPPC and tripalmitin (TP). In all cases the molar ratio of ASA:DPPC was maintained at 1:1, whereas the TP was administered in excess (weight ratio of DPPC:TP = 1:4) (n = 5/grp; * = p>0.05 vs ASA/DPPC; ASA/DPPC 1 mg/kg = -•-; ASA/DPPC/TP,1 mg/kg = --○--; ASA/DPPC, 9 mg/kg = --▲--; ASA/DPPC/TP, 9 mg/kg = --△--).

FIG. 16. It can be appreciated that the addition of the neutral lipid provided a further enhancement of antipyretic activity, even over that of ASA/DPPC, as can best be seen with the 1 mg/kg subthreshold ASA dose (n = 10/grp; * = p<0.05 vs ASA/DPPC; ASA, 1 mg/kg = -♦-; ASA/DPPC = -○-; ASA/DPPC/TP = --•--).

FIG. 17. The ability of DPPC to promote the movement of aspirin from water into a lipidic cyclohexane phase (as a membrane model) is greatly accelerated by the presence of the neutral lipid, tripalmitin (TP) to form a microemulsion. This may provide an explanation why the presence of neutral lipids further promote the therapeutic potency of the NSAID/DPPC complex (DPPC + TP (1:4), 2.5mM + ASA, 10mM (sonicated) = -○-; DPPC, 2.5mM + ASA, 10mM (sonicated) = -□-; TP, 2.5 mM + ASA, 10 mM = -■-).

FIG. 18. The ability of DPPC to enhance the anti-pyretic activity of ASA was seen with indomethacin (Saline = -•-; indomethacin (10 mg/kg) = -□-; indomethacin (10 mg/kg)/DPPC = -○-).

FIG. 19. Demonstrates the ability of DPPC to enhance the anti-pyretic activity of naproxen (25 mg/kg dose) (Saline = -•-; naproxen (25 mg/kg) = -□-; naproxen (25 mg/kg)/DPPC =-○-).

FIG. 20. Demonstrates the ability of DPPC to enhance the anti-pyretic activity of diclofenac, 10 mg/kg (Saline = -□-; diclofenac (10 mg/kg) = -□-; diclofenac (10 mg/kg)/DPPC = -○-; n = 5/grp; * = p<0.05 vs. DICLO).

FIG. 21. Demonstrates the ability of DPPC to enhance the anti-pyretic activity of salicylic acid (SA) (70 mg/kg dose) (Saline = - -; SA (70 mg/kg) = -○-; SA (70 mg/kg)/DPPC = -•-; n = 5/grp, * = p<0.05 vs. SA/DPPC).

FIG. 22. Antipyretic activity of ASA (18 mg/kg) and ASA/DPPC complex with IP injection of omeprazole (150 mg/kg) two hours prior to NSAID dosage. ASA (saline) = - • -; ASA/DPPC (Sal) = - ○ -; ASA (omeprazole) = --▲--; ASA/DPPC (omep) = --△--; n = 5/grp; * = p<0.05 vs. ASA (omep); † = p<.05 vs. ASA (Sal).

FIG. 23. Antipyretic activity of ASA (18 mg/kg) and ASA/DPPC complex with IP injection of Ranitidine (2 mg/kg) l hour prior to NSAID dosage. ASA (saline) = - • -; ASA (DPPC) = - ○ -; ASA (Ranitidine) = --▲--; ASA/DPPC (Ranitidine) = --△--; n = 5/grp; * = p<0.05 vs. ASA (rani); † = p<0.05 vs. ASA (Sal).

FIG. 24. Demonstrates that aspirin's ability to acutely induce gastric lesions was reduced to a variable degree in rats pretreated with an antisecretory agent (see open bars), with omeprazole and cimetidine being most protective. In contrast, antisecretory drugs consistently protected against aspirin-induced gastric lesions if the NSAID was chemically associated with DPPC (see stippled bars).

Pretreatment of rats with any one of the antisecretory agents, prior to the intragastric administration of phospholipid-complexed aspirin, afforded the animals full protection against NSAID-induced gastric lesion in this ulcer model.

FIG. 25. Indicates that the combination therapy of antisecretory agents with indomethacin/DPPC proved superior to either the acid-inhibitor or the phospholipid alone in the prevention of acute NSAID-induced GI bleeding over an 18 hour period.

FIG. 26A-26C. Indicates that the antipyretic activity of aspirin, administered at a dose of 18 mg/kg, was clearly attenuated if the rats were pretreated with antisecretory doses of omeprazole (FIG. 26A), ranitidine (FIG. 26B), or cimetidine © (FIG. 26C). However, the antisecretory agents' ability to attenuate aspirin's antipyretic activity was completely reversed if the NSAID was preassociated with DPPC, to increase the drug's lipophilic characteristics.

FIG. 27. Demonstrates that the proton pump inhibitor, omeprazole had an effect of reducing the antipyretic activity of indomethacin, administered at a dose of 4 mg/kg, which once again could be overcome if the NSAID was administered as a complex with DPPC.

FIG. 28A-28B. Demonstrate that the analgesic activity of both aspirin and indomethacin was similarly attenuated if rats were pretreated with an antisecretory agent. Under control conditions the "pain pressure threshold" of the inflamed paw increased approximately two-fold, from 40-60 mm Hg to 90- 100 mm Hg, 2 hours after rats were administered the ED_{50-80} dose of either NSAID alone. This analgesic action of both aspirin and indomethacin was clearly reduced or abolished altogether if the rats were pretreated with one of the potent antisecretory agents (compare open-bars). Furthermore, the analgesic activity of both aspirin and indomethacin could be restored in rats pretreated with an inhibitor of gastric acid secretion, if the NSAIDs were administered in the lipid-associated state (compare stippled- to open-bars for each antisecretory agent). An analgesic activity pattern very similar to that described above were observed 26 hours after rats received three consecutive

treatments of aspirin or indomethacin ± DPPC (at 0, 6, and 24 hours) preceded 1 hour earlier with an injection of an antisecretory agent. Once again the NSAID-induced analgesia was significantly reduced by pretreatment with one of the three antisecretory agents which could be overcome if the NSAIDs were chemically associated with DPPC. It is also important to note that at both time-points in the absence of one of the above antisecretory agents, both the antipyretic and analgesic activities of aspirin and indomethacin were at least as good and most cases enhanced when the drug was complexed to DPPC over that observed with the NSAID alone (compare stippled to open-bars of rats treated with saline).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

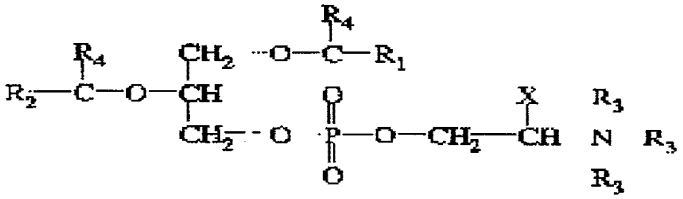
The present invention arises from the discovery that aspirin and other NSAIDs chemically associate with zwitterionic phospholipids, such as dipalmitoyl phosphatidylcholine (DPPC). Important embodiments of the invention include methods of enhancing the various therapeutic activities of NSAID's, such as antipyretic, anti-inflammatory, and analgesic pharmacological activities. Surprisingly, these responses are observed without evidence of gastrointestinal side effects as demonstrated in acute and chronic animal models of NSAID injury in the present disclosure.

The data disclosed herein indicates that NSAIDs have the capacity to chemically associate with zwitterionic phospholipids in both organic and aqueous solvent systems, and in doing so, both classes of molecules undergo profound changes in their physical and chemical properties. Complex formation in aqueous solvent systems is shown to occur more efficiently at pH values at or slightly below the pKa of the NSAID. Therefore, without being bound by any theory, it is contemplated that the intermolecular bonding is not covalent, but is instead both hydrophobic and electrostatic, with the latter association being between the negatively charged carboxyl group of the NSAID and the positively charged nitrogen of the phospholipid. This possible interaction has been supported by computer assisted molecular modeling programs (Quanta and CHARMm), which also indicate that the NSAID/phospholipid complex has a lower molecular free energy (greater thermodynamic stability) than either reactant alone.

According to the present invention, it would be expected that orally administered NSAIDs would chemically associate with the intrinsic zwitterionic phospholipids that coat the luminal aspects of the mucus gel layer of the upper GI tract. A description of luminal aspects of the mucus gel layer is described in Goddard *et al.*, (1990) and Kao et al. (1990). While not intending to be limited to any particular mechanism of action, this intermolecular association is thought to be the basis for the attenuation in surface activity and/or the loss of stability of the interfacial extracellular phospholipid layer, and to culminate in an NSAID induced decrease in mucosal hydrophobicity and barrier properties.

Description of the Lipid Compounds

The phospholipids of the present invention are characterized generally by the formula:



wherein R_1 and R_2 are saturated or unsaturated substitutions ranging from 8 to 32 carbon atoms; R_3 is H or CH_3, and X is H or COOH; and R_4 is = 0 or H_2.

As will be appreciated by those of skill in the art, the foregoing chemical structure defines a zwitterionic phospholipid structure and embraces a wide range of phospholipids, including but not limited to phosphatidyl chorines, phosphatidyl ethanolamines, phosphatidyl serines and various other zwitterionic phospholipids.

Other phospholipids that may be employed in the composition include: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin, and other ceramides, and mixtures thereof.

Description of the NSAID's

A few of the non-steroidal anti-inflammatory agents that may be employed in the methods and compositions disclosed herein include by way of example: pyrazolones,

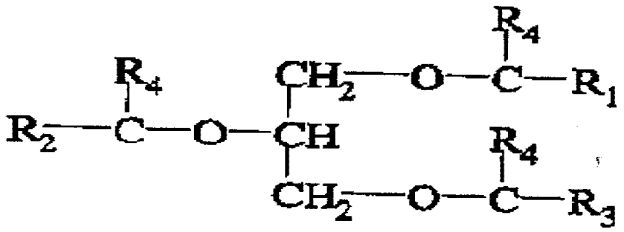
phenylbutazone (4-butyl-1,2-diphenylpyrazolidine-3,5-dion), and oxyphenbutazone (4-butyl-2-(4-hydroxyphenyl)-1-p-phenylpyrazolidine-3,5-dion), salicylic acid derivatives such as salicylic acid salicylic acid amide, acetyl-salicylic acid, benorilate (4-acetamidophenyl-o-acetylsalicylate), and diflunisal (5-(2,4-difluorophenyl)-salicylic acid); Indoles, especially indometacine and its analogs such as indometacine (1-(p-chlorobenzyl)-5-methoxy-2-methylindole acetic acid), glucametacine (1-(p-chlorobenzoyl-5-methoxy-2-methylindole-3-yl acetic acid glucose amide), acemetacine (1-(p-chlorobenzoyl)-5-methoxy-3-methylindole-3-acetic acid-glycolic acid-ester), and sulindac (5-fluor-2-methyl-1-p-(methylsulphenyl)-benzylidene-indene-3-acetic acid); Phenyl acetic acid or phenyl propionic acid derivatives such as ibuprofen (2-(4-isobutylphenyl)-propinic acid); naproxen (2-(6-methoxy-2-naphthyl)-propinic acid), alclofenac (4-allyloxy-3-chlorophenyl-acetic acid), ketoprofen (2-(3-benzylphenyl)benzoic acid), diclofenac (2-(2,6-dichlorophenylamino)-phenylacetic acid), fenoprofen (2-(3-phenyloxyphenyl)-acetic acid), tolmetin (1-methyl-5-(p-toluyl)-pyrrole-2-yl-acetic acid), flurbiprofen (2-2-fluorobiphenyl-4-ye-proprionic acid), and suprofen (p-2-thenoyl-hydratropic acid) phenyl-propionic acid); Anthranilic acids and their nitrogen analogs such as flufenamino acid (N-(m-trifluoromethylphenyl)-anthranilic acid), mefenamino acid (N-(2,3-dimethylphenyl)-anthranilic acid), and niflumin acid (2-(3-trifluoromethylaminolino)-nicotinic acid).

Phospholipid compounds found to be particularly useful in the practice of the present invention are dilinoleoyl phosphatidylcholine (DLL-PC), dipalmitoyl phosphatidylcholine (DPPC) and egg phosphatidylcholine (Egg-PC or PC_e). In DPPC, a saturated phospholipid, the saturated aliphatic substitution R_1 and R_2 are CH_3--$(CH_2)_{14}$, R_3 is CH_3 and X is H. In DLL-PC, an unsaturated phospholipid, R_1 and R_2 are CH_3--$(CH_2)_4$--CH=CH--CH_2--CH=CH--$(CH_2)_7$, R_3 is CH_3 and X is H. In Egg PC, which is a mixture of unsaturated phospholipids, R_1 primarily contains a saturated aliphatic substitution (e.g., palmitic or stearic acid), and R_2 is primarily an unsaturated aliphatic substitution (e.g., oleic or arachidonic acid).

Description of the Neutral Lipids

Neutral lipids form another component of some embodiments of the compositions described herein. This class of lipids include the triglycerides.

The triglycerides useful in the practice of the present invention are generally characterized by the formula:



wherein R_1, R_2 and R_3 are each saturated or unsaturated substitutions ranging from 4 to 32 carbon atoms; and R_4 is either ==0 or H_2.

As will be appreciated, this structure embraces a wide range of triglycerides, both saturated and unsaturated, and include, for example, triglycerides such as tripalmitin (saturated), triolein and trilinolein (both unsaturated). A further listing of saturated and unsaturated fatty acids that can be esterified or ether-linked to the triglyceride in question is provided in U.S. 5,032,585, which is specifically incorporated herein by reference.

In a particular anticipated pharmaceutical preparation, the preparation will be provided in a pill form suitable for human ingestion, and contain about 2 to about 300 mg per kg aspirin or salicylate, together with an equimolar amount of PC, DPPC, or a combination thereof, or any other zwitterionic phospholipid. In the described methods, the compositions include the NSAID and the zwitterionic phospholipid in molar ratios ranging from about 1:0.1 to about 1:20, and preferably from about 1:0.5 to about 1:2. In a most preferred embodiments, the ingredients are included in a molar ratio of about 1:1.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit aid scope of the invention.

EXAMPLE 1
GI LEGIONARY AND BLEEDING - EFFECT OF NSAID AND ZWITTERIONIC PHOSPHOLIPID COMPOSITIONS

The present example demonstrates the utility of the present invention for providing compositions that reduce the GI-related side-effects commonly associated with NSAIDS.

In order to retard the ability of NSAIDs to interact with the extracellular phospholipid lining of the mucus gel layer, several NSAIDs were preassociated with zwitterionic phospholipids and the effect was determined in various rat ulcer models. The present example shows that ability of the NSAIDs to induce acute and/or chronic GI lesions and bleeding was remarkably decreased when the drugs were administered as a complex with DPPC or related phospholipids. Surprisingly, the anti-pyretic and anti-inflammatory activity of aspirin appeared to be consistently enhanced when associated with zwitterionic phospholipids. This is in contrast to previous side effects of other formulations that reportedly suffer from reduced therapeutic efficacy or onset (Alpsten *et al.,* 1982; Mojaverian *et al.,* 1987).

Ulcer Models

Gastric lesions were acutely induced in rats in accordance with the following techniques. For the salicylate-based NSAIDs, fasted male Sprague Dawley rats (150-200g) were intragastrically injected with saline (control), ASA or salicylate, or the drugs preassociated with an equimolar concentration of DPPC (all solutions adjusted to a pH of 3.1). Ten minutes later, the rats were intragastrically challenged with 1 ml of 0.6 N HCl. Gastric lesions were macroscopically scored 60 minutes later in accordance with a previously outlined method (Lichtenberger *et al.,* 1983, incorporated herein by reference).

In order to investigate the effects of non-salicylate NSAIDs to induce GI bleeding, fasted rats were subcutaneously injected with N-nitro-L-arginine Methyl Ester (L-NAME), 1 hr. before and 1 and 6 hrs. after intragastrically receiving 1 ml of the NSAIDs; indomethacin, diclofenac, and naproxen administered alone and in association with an equimolar concentration of DPPC. The Nitric Oxide synthesis inhibitor, L-NAME, is administered before and after the NSAID to increase the rat's sensitivity to the drug in accordance with the method of Chen et. al., *Gastroenterology* **104**: A53, 1993). Eighteen

hours after receiving the NSAID, the distal half of the intestine was excised and flushed with 10 ml of saline. The hemoglobin (Hb) concentration of the intestinal perfusate was measured as an estimate of GI bleeding in accordance with a previously described method (Lichtenberger *et al.,* 1983).

Rats were treated with ASA over a two week period to investigate the chronic effects of NSAID exposure (in the presence and absence of phospholipids) on hematocrit, gastric mucosal hydrophobicity, and granuloma formation. In order to ensure that the rat had an empty stomach prior to receiving the daily intragastric dose of ASA, they were placed on a reverse lighting schedule (9AM/lights off; 5PM/lights on), and were only provided access to chow during the day (dark period). The test solutions (saline, ASA and ASA/Phospholipon 90G complex) were intragastrically administered between 8AM - 9AM daily during the two week study period. In these chronic exposure experiments, ASA was complexed with an equimolar concentration of Phospholipon 90G (purified soya lecithin, prepared and obtained from Nattermann GmbH of Cologne, Germany) instead of DPPC. At the completion of the study period, blood was collected into a capillary tube for the determination of hematocrit and the stomach was excised for contact angle analysis.

One of a number of NSAIDs was intragastrically administered to rats alone or preassociated with an equimolar concentration of DPPC or Phospholipon 90 G (purified soya lecithin, prepared by Nattermann GmbH of Cologne Germany) in several models of acute and chronic injury of the upper GI tract. Two contrasting animal models were employed to determine the ability of DPPC to protect against acute NSAID injury to the GI tract. For salicylate-based NSAIDs, which primarily induce stomach injury, gastric lesions were scored in fasted rats who were initially treated with aspirin or salicylate alone or complexed with DPPC and challenged 10 minutes later with a supra-physiological dose of HCl. For non-salicylate NSAIDs (indomethacin, diclofenac and naproxen) which predominantly induce injury to the mid-distal regions of the small intestine, the quantity of intraluminal blood was assessed in the distal half of the small intestine of rats who were pre- and post-treated with the Nitric Oxide synthetase inhibitor, L-NAME (N-nitro-L-arginine methyl ester).

The results, shown in FIG. 4A and FIG. 4B, respectively, clearly demonstrate (in rats sensitized to the drugs) that the injurious potential of both salicylate-based and non-

salicylate NSAIDs to induce acute GI lesions and bleeding is significantly reduced, by >85%, if the NSAID is preassociated with the zwitterionic phospholipid prior to administration. Similarly, daily administration of aspirin to rats over a 2 week period resulted in a significant fall in both hematocrit and gastric mucosal hydrophobicity, which was prevented in rats that received the aspirin/Phospholipon G complex (Table 2).

Table 2. Effect of DPPC on Aspirin's Chronic GI Side Effects

Group	Gastric Mucosal Hydrohobicity (degrees, Contact Angle)[b]	Hematocrit
Saline (Control	52.2 ± 2.7(6)	51.7 ± 0.4 (10)
Aspirin (90 mg/kg)	15.8 ± 6.6[c](6)	47.8 ± 0.2[c](10)
Aspirin + Phospholipon G	55.3 ± 5.8[c,d](6)	53.1 ± 0.4[c,d](10)

[b] Gastric mucosal hydrophobicity was measured by contact angle analysis as described in Hills *et al.*, 1983; Goddard *et al.*, 1987; Goddard *et al.*, 1990.

[c] $p<0.05$ in comparison to saline-treated control values.

[d] $p<0.05$ in comparison to values of aspirin-treated rats.

(n) number of rats/group

EXAMPLE 2 - SOLUBILITY STUDIES; COMPLEX FORMATION BETWEEN NSAIDS and ZWITTERIONIC PHOSPHOLIPIDS

To demonstrate the effect of DPPC on the solubility of the sodium salts of the five NSAIDs (naproxen, indomethacin, diclofenac, salicylate and aspirin) in chloroform, each NSAID was added to chloroform at a 30 nM final concentration. DPPC was dissolved in the chloroform that was contained in half the tubes at a final concentration which ranged between 5 - 40 nM, prior to the addition of the NSAID salt. DPPC, as well as PC and other zwitterionic phospholipids useful in the practice of the invention, may also be dissolved in other organic solvents, such as ethanol, in the practice of the present invention. The tubes were gently mixed at 25°C for 16 hrs, after which they were photographed and/or centrifuged (2,000g for 15 min) and the supernatant collected to determine the concentration of NSAID in solution. The latter was assessed by measuring the NSAID's

UV absorbance at 290 nm, and comparing these readings to the appropriate standard curves for each NSAID. It should be noted that for each of the five NSAIDs a linear relationship existed between the drug's concentration (nM) in water and the UV absorbance reading. The equations for the regression lines for each of the NSAIDs were as follows: aspirin, y = 2.603x -0.004, r= 0.999; salicylate, y = 8.780x + 0.123, r = 0.999; indomethacin, y = 18.325x + 0.156, r= 0.999; diclofenac, y = 21.523x + 0.008, r = 0.999; and naproxen, y = 3.732x + 0.005, r =0.999. In all cases the readings for the unknowns fell within the linear portion of the standard curves. Further, the presence of DPPC in the solvent did not interfere with these analyses, as it failed to contribute to the W absorbance reading in the absence of the NSAID.

To assess the effect of DPPC on the solubility of the sodium salt of ASA in water, ASA was dissolved in water at a final concentration of 30 nM (pH adjusted to 6.0), and its intrinsic fluorescence (290 nm/excitation; 406 nm/emission) was monitored. DPPC was present as a lipidic suspension in half the tubes at a final concentration which ranged between 15 - 60 nM. The tubes were gently mixed at 25°C for the desired incubation period, after which they were centrifuged (2,000g for 15 min) and the supernatant collected to determine the concentration of ASA in solution. Once again a linear relationship (y = 14.02 + 0.353, r = 0.999) was found between the fluorescent reading and the concentration of aspirin in solution.

It can be appreciated from FIG. 1A and FIG. 1B that the sodium salts of these NSAIDs are insoluble in chloroform unless an equimolar or greater concentration of DPPC is added to the organic solvent, at which point complete solvation takes place. Conversely the sodium salts of the NSAIDs are readily soluble in either saline or water, and are removed from solution as a complex within minutes after an equimolar concentration of DPPC is added as a lipidic suspension. The solubility of aspirin in saline can be followed either by monitoring its intrinsic fluorescence or radioactivity (employing ^{14}C-labeled aspirin). FIG. 2A and FIG. 2B demonstrate that the injection of DPPC into an aqueous solution results in the precipitation of the NSAID, presumably as a complex with the phospholipid. The rapid change in solubility of the NSAID in both the organic and aqueous solvent systems does not occur if DPPC is substituted by the anionic phospholipid, dipalmitoylphosphatidylglycerol (DPPG).

EXAMPLE 3 - GRANULOMA FORMATION AND ENHANCED ANTI-INFLAMMATORY ACTIVITY

The present example demonstrates the utility of the present methods for enhancing the anti-inflammatory activity of ASA accomplished when ASA, or other non-steroidal anti-inflammatory drug complexed with Phospholipon 90G. A foreign-body granuloma model widely used by those of skill in the art to assess anti-inflammatory action was employed.

The inventors used the rat model of foreign-body granuloma formation. This model is recognized by those of skill in the art as a representative model for granuloma formation and anti-inflammatory activity, as described in Ucelay *et al.*, (1988); and Castro *et al.*, (1980). These references are specifically incorporated herein for the purpose of providing details associated with the use of this model.

Methods

The above model has been successfully employed in both rats and invertebrates to quantify this most basic component of a tissue's response to injury (Ucelay *et al.*, 1988; Castro *et al.*, 1980; Clatworthy *et al.*, 1994, each incorporated herein by reference). Sterile tared cotton string was surgically implanted (bilaterally) under the abdominal skin of ether anesthetized rats on day 1 of the study period, and then randomly placing rats in a group to be daily treated with saline, aspirin or the NSAID complexed with Phospholipon 90G over a two-week period.

At the completion of the study period the string with the adherent granuloma tissue was surgically dissected from the euthanized rats and dried in a vacuum for several days at room temperature, until a baseline dry weight was obtained. The difference between this value and the initial dry weight of the string prior to implantation divided by the latter value provided an estimate of the weight of granuloma tissue. This technique proved to be very reproducible and accurate, as determined both by the close agreement between the changes in weight of the two pieces of string that were implanted contralaterally in each animal (< 12.5% difference in values between the right and left string); and the low variance (< 8%) in values of granuloma formation within a group of animals.

Improvement in the anti-inflammatory efficacy of ASA was observed over a 2 week study period. This effect is seen when the NSAID was administered as a complex with Phospholipon G at concentrations below the maximally effective dose for this particular drug (see Table 3, FIG. 5).

Table 3. Effect of Aspirin ± DPPC Formulations on Foreign-Body Granuloma Formation.

Group	± DPPC	n	Granuloma Formation[a]
Saline (Control	-	5	3.20 ± 0.10
ASA (90 mg/kg)	-	5	1.90 ± 0.10[b]
	+	5	1.4 ± 0.10[b,c]
ASA (140 mg/kg)	+	6	1.00 ± 0.15[b]

[a] The values represent the dry weight of the string with adherent granuloma tissue -dry weight of string/dry weight of the string.

[b] $p<0.05$ in comparison to saline-treated control values.

[c] $p<0.05$ in comparison to values of rats treated with NSAIDs alone.

EXAMPLE 4 - CONTACT ANGLE ANALYSIS

The present example is provided to describe the model which was used to examine the surface tension reducing action of the compositions of a combination of non-steroidal anti-inflammatory agent and zwitterionic phospholipid.

Contact angle analysis was performed with the use of a goniometer on excised gastric mucosal tissue, that was lightly blotted and dried, as previously outlined (Hills *et al.*, 1983; Goddard *et al.*, 1987; Goddard *et al.*, 1990, each incorporated herein by reference). Briefly, this was accomplished by applying a droplet of water (~5μl) to the tissue surface, and employing the telescopic eyepiece of the goniometer to measure the maximal angle that is dissected at the triple point, where the solid/liquid/ and air interface meet.

EXAMPLE 5 - ANTIPYRETIC ACTIVITY

An established rat fever model was used in the present example to demonstrate the utility of the invention for enhancing the anti-pyretic activity of a NSAID by combining these class of agents with a phospholipid.

The rat fever model involves the injection of rats with Brewer's yeast (2g/kg, s.c.) to induce an increase in fever of 0.5 -1.5°C. These models are described in Adams *et al.* (1968) and Ucelay *et al.* (1988), which references are specifically incorporated herein for this purpose. The animals were intragastrically treated (instilled) with either saline, 90 mg/kg ASA, or 90 mg/kg ASA preassociated with an equimolar concentration of DPPC. Similar antipyretic analyses were performed with the sodium salts of the following NSAIDs: diclofenac (10 mg/kg), indomethacin (10 mg/kg) and naproxen (30 mg/kg), alone and complexed with an equimolar concentration of DPPC. All test solutions were titrated to a pH of 4.5 prior to intragastric administration. Rectal temperatures were monitored in conscious, restrained rats at all indicated times. This technique provided a very reliable and reproducible estimate of the antipyretic activity of NSAID formulations as indicated by the fact the variance within a group was low, with the standard errors < 5% of the mean values, and the fact that the difference in mean temperature values for a given group varied < 2 % between separate experiments.

EXAMPLE 6 - PHARMACEUTICAL COMPOSITIONS

The complexes described in the present example are preferably for oral administration, for example, with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds (i.e., NSAID and Zwitterionic phospholipid and/or neutral lipid) may be incorporated with excipients or carriers and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain enough active NSAID compound to achieve an effective plasma level of the active drug. Aspirin, for example, would be provided in doses of from about 10 mgm, or about 20 mg, or 32.5 mg or even up to 60 mg, or 300 mgs/kg would be contained in each tablet or dose, where employed for

administration to animals having a weight of about 60 kg - 70 kg. The dose will vary depending on the NSAID or combinations of NSAIDs used. The amount of the NSAID in particular preparations is more generally described as an amount of NSAID or combination of NSAIDs effective to provide a pharmacologically active plasma concentration of the drug when used in combination with zwitterionic phospholipid. These amounts are determinable by one of ordinary skill in the pharmaceutical arts given the data disclosed herein, and general pharmaceutical references such as Remingtons Pharmaceutical Sciences.

The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose, aspartame or saccharin may be added, or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both.

A syrup of elixir may contain the active compounds sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor, again formulated for oral administration. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

The present compositions may also be formulated as injectable formulations, or as formulations suitable for enteral administration, according to those techniques known to those of ordinary skill in the medicinal arts.

EXAMPLE 7
LIPID-PERMEABILITY OF NSAID/PHOSPHOLIPID COMPLEX

The present example illustrates the utility of the invention for providing compositions and methods for enhancing the big-absorption and big-availability of

NSAID's. The diffusion of aspirin (alone and complexed with phospholipid) from water into cyclohexane was used as a model to estimate the membrane permeability of the drug.

Permeability Analysis

The sodium salt of ASA (also salicylate) was dissolved in 5 ml of water at a final concentration of 100mM (pH adjusted to 6.0) and gently stirred at 25°C. An equal volume of cyclohexane was layered over the aqueous solution and the entry of the NSAID into the organic phase was monitored fluorometrically over time. In order to determine the effect of phospholipid association on the lipid permeability of the NSAID, ASA (or salicylate) at the above concentration was sonicated in the presence of 0.5 mM phospholipid (DPPC or DPPG) in water (adjusted to a pH of 7), and its rate of diffusion into the cyclohexane phase was measured fluorometrically. This was accomplished by removing 1 ml of the top phase chloroform solution by pipette, injecting it into the cuvette to obtain the fluorescence reading, and returning the sample to the incubation vessel to assure that the volume did not change. This entire process could be completed in < 30 seconds.

In these studies, the concentration of NSAID:phospholipid in water was adjusted to a molar ratio of 200:1, creating a large driving force to promote NSAID flux into the hydrocarbon phase, and minimizing the turbidity encountered with high phospholipid concentrations.

Results

Under neutral conditions, the passive diffusion of aspirin across an aqueous - hydrocarbon interface, as assessed fluorometrically, was negligible unless it was chemically associated with the zwitterionic phospholipid, DPPC (See Figure 3). Furthermore, this increase in the flux rate into the organic phase, was simply not a consequence of liposomal encapsulation since the NSAID failed to enter cyclohexane if the anionic phospholipid, DPPG was substituted for DPPC. These studies also revealed that DPPC promoted the flux of sodium-salicylate from the aqueous to the organic phase in a similar manner.

EXAMPLE 8
ANTI-INFLAMMATORY AND ANTI-PYRETIC ACTIVITY OF NSAID/PHOSPHOLIPID COMPLEX

This example demonstrates the utility of the present invention for enhancing the fever-reducing potential of the NSAID (20 mg/kg dose) when chemically associated with a zwitterionic phospholipid. The ability of NSAIDs (administered alone and complexed with DPPC) to reduce fever in rats was determined. Fever was induced 18 hrs prior to drug treatment by the subcutaneous administration of Brewer's yeast (Adams *et al.*, 1968; Ucelay *et al.*, 1988).

The results (See FIG. 4C) indicate that the anti-pyretic activity of the ASA/DPPC complex was significantly greater than that of the NSAID alone, at all time periods examined.

The enhancement in anti-pyretic activity of the NSAID/DPPC complex compared to the NSAID alone over the first three hours after intragastric administration, was observed to a lesser degree with sodium salts of the following drugs; diclofenac (-0.29°C), indomethacin (-0.28°C), and naproxen (-0.30°C).

EXAMPLE 9
ENHANCEMENT OF ANTIPYRETIC ACTIVITY OF ASA/DPPC COMPLEX AT SUBTHRESHOLD ASA DOSAGE

The antipyretic activity of the ASA/DPPC complex was determined in rats as in Example 8 (supra), this time at much lower doses, more than 2 times lower than in Example 8. In the present example, a dosage of 9.0 mg/kg ASA was administered either alone or complexed with DPPC. The data from this study is summarized in FIG. 6.

As can be seen in FIG. 6, ASA alone does not have significant antipyretic activity at this dosage level, however ASA complexed with DPPC does have significant antipyretic activity over a five hour period. The dosage level in the present example is a 10-fold reduction of the standard dosage of 90 mg/Icg, as used in Example 8, and reported in FIG. 4C.

Based on the recommended human dosages of 90 mg/kg for juvenile rheumatoid arthritis, or 325 to 650 mg for antipyretic or analgesic treatment in adults (See pages 1110-1111, Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Company,

Easton, Pennsylvania, 1990, incorporated herein by reference), it is expected that ASA complexed with DPPC, or other zwitterionic phospholipid, at a 10-fold lower dosage, i.e. approximately 9 mg/kg for juvenile rheumatoid arthritis or 32.5 to 65 mg for antipyretic activity in adults, would be as effective as the normal dosage of ASA alone to provide fever-reducing activity.

EXAMPLE 10
ANALGESIC EFFECT OF COMPLEXED-NSAIDS

It is contemplated that, in light of the demonstrated enhancement of the effects of NSAIDs (anti-inflammatory and antipyretic) when complexed with a zwitterionic phospholipid, or even further administered in combination (i.e., in a mixture) with a neutral lipid (triglyceride) that the analgesic effects of these drugs will also be enhanced when complexed with the phospholipids. Two established pain tests, using rats, may be employed to demonstrate enhancement of this effect. Tail retraction occurs in response to a noxious stimulus that generates pain. Tail retraction response may be observed and timed in rats given a placebo in order to determine a base line reaction time. If after receiving either DPPC-complexed or uncomplexed aspirin the time latency between pain induction (with a laser heat source) and tail retraction increases, this is a direct reflection of the analgesia. Retraction times will be compared between placebo and aspirin-treated rats. The test will be conducted with varying dosages and varying times after the administration of the placebo, aspirin, and/or other NSAIDs, or complex to determine if dosage or duration have some effect on the strength of the analgesia.

A model for analgesic effect in rats involves animals injected with formalin. The rats are injected under the dorsal surface of the right hind paw with a 0.05 ml volume of 15% formalin and saline solution (Helmstetter and Fanselow, 1987). The treated rats are then given the aspirin, and/or other NSAIDs, aspirin/DPPC or PC complex, or placebo at various times before and after injection with the irritating solution. The rats are then placed in a cage and their behavioral responses to the painful stimulus are be observed (employing a video camera system) and graded as follows: (1) freezing - an absence of all activity other than respiration; (2) paw lifting - the rat holds its treated paw close to its body; (3) paw licking - the rat either licks the treated paw or has some other type of mouth contact with

it; and (4) general activity - this involves any other type of general movement. The behavioral response of the rat is indicative of its sensitivity. The formalin test, along with the tail flick test, comprises two distinct and separate tests to evaluate pain sensitivity and analgesia effectiveness.

EXAMPLE 11
EFFECTS OF LOW DOSE ASPIRIN (9 MG/KG) ALONE AND AS A COMPLEX WITH PHOSPHOLIPID/NEUTRAL LIPIDS ON FOREIGN-BODY GRANULOMA FORMATION IN RATS[A]

The present example is provided to demonstrate the utility of the present invention for reducing inflammation. The string granuloma model described herein was again used to demonstrate the activity of the presently disclosed methods for treating this condition.

Sterile tared string was surgically implanted. Five days later rats were intragastrically administered twice a day with either saline, ASA alone, or ASA lipid mixtures. Rats were sacrificed after each rat received 5 doses of the test compounds and the string and granuloma excised and weighed.

*$p<0.05$ vs. ASA alone. ASA = Aspirin;
DPPC = Dipalmitoylphosphatidylcholine,
TP = Tripalmitin; TO = Triolein

TABLE 4

Saline	ASA	ASA DPPC/TP	ASA PhospholiponG/TO
4.08(8)	3.89(8)	3.44(8)	3.49(8)*
±25	±0.06	±0.24	±0.17

Table 4. As demonstrated in the data provided in Table 4, a phospholipid (DPPC)/neutral lipid (TO) microemulsion appeared to modestly enhance the anti-inflammatory activity of ASA when the NSAID was administered at a subthreshold dose (9 mg/kg) for this activity.

EXAMPLE 12
EFFECT OF DPPC ON ASPIRIN'S INHIBITORY EFFECT ON PLATELET AGGREGATION

The present example demonstrates the ability of Non-Steroidal anti-inflammatory Drugs (NSAIDs) to inhibit cellular activation resulting in either platelet aggregation or the synthesis and release of inflammatory mediators. The activity is shown to be enhanced if the NSAIDs are administered as a complex with zwitterionic phospholipids alone or together with neutral lipids.

TABLE 5

High Ristocetin (1.5 mg/ml) Test		
	% Platelet Aggregation	
ASA Conc	-DPPC	+DPPC
0.00	93%	100%
0.01mM	100%	100%
0.1 mM	83%	87%
1.0 mM	95%	35%
1.0 mM[a]	100%	84%
Low Ristocetin (0.75) mg/ml Test		
ASA Conc	-DPPC	+DPPC
0.00	75%	95%
0.01mM	65%	12%
0.1mM	26^%	20%
1.0mM	80%	6%
1.0mM[a]	39%	15%

Abbreviations:ASA= aspirin

DPPC = dipalmitoylphosphatidylcholine

DPPC was added at a conc. equimolar to the aspirin.

In the absence of aspirin, DPPC was added at a final concentration of 1 mM.

[a] Second study.

The advantage of this invention is it will allow the NSAIDs to be administered at a lower than normal dose, due to their enhanced efficacy and potency-thus increasing their effectiveness and minimizing their side-effects on the GI tract and other organ systems.

A number of embodiments of the invention would include the combination of NSAIDs with zwitterionic phospholipids alone and together with neutral lipids. These combinations would both increase the efficacy and potency of NSAIDs to inhibit the activation of: 1) platelets; 2) neutrophils; 3) monocytes/macrophages; 4) lymphocytes; 5) Pans and 6) other bone-marrow derived cell types.

Considering the interest in the pharmaceutical industry in the role of NSAIDs in the prevention of cardiovascular disease and tissue/joint inflammation, the presently described pharmaceutical preparations would provide alternative clinical management protocols with a improved bioavailability at lower doses of the sometimes irritative NSAID regimen.

This list would include at minimum the 20-40 pharmaceutical companies presently marketing an NSAID.

EXAMPLE 13
ANTI-SECRETORY AGENTS TOGETHER WITH NSAID AND LIPIDS

The present example demonstrates the utility of the present invention for compositions that include an anti-secretory drug, such as Tagamet, or other histamine type 2 receptor antagonist, or Omeprazole (Prilosec™, or other proton-pump inhibitor or H^+/K^+ATPase inhibitors), either before or along with an NSAID complexed with phospholipid and/or neutral lipid (such as a triglyceride). These embodiments of the invention are further described in Example 14.

While not intending to be limited to any particular mechanism of action, by including a phospholipid and/or neutral lipid, the poor absorption of NSAID's that sometimes results with an anti-secretory agent administered therewith, may to some degree be prevented or lessened. Hence, the bioavailability and therapeutic action of the NSAID when administered together with an anti-secretory agent may be maintained, and in some cases enhanced.

The present example also demonstrates that the therapeutic (antipyretic) activity of aspirin, and other NSAIDs, is attenuated if animals are pre-treated with an agent that

inhibits gastric acid secretion. Omeprazole (sold under the name Prilosec™ is in the class of "proton pump inhibitors", also called "H^+/K^+ ATPase inhibitors" that act by irreversibly binding to and inhibiting H+/K+ ATPase of the parietal cell, the rate limiting enzyme in gastric HCl secretion. Ranitidine (sole under the name Zantac™ is in the class of "H_2 receptor antagonists" that prevents histamine from binding to its type-2 receptor on the parietal cell to inhibit gastric acid secretion. It can be appreciated from figures 22 and 23 that the blocking effect of these two classes of antisecretory drugs on the therapeutic actions of the NSAID, however, is overcome if the NSAID is complexed with a zwitterionic phospholipid. As demonstrated in Figures 23 and 23, aspirin at a dose of 20 mg/kg failed to reduce fever in rats if the NSAID was administered in conjunction with either the proton pump inhibitor (Prilosec™ or a histamine blocker, Ranitidine (Zantac™). This block in therapeutic activity due to inhibition of gastric acid secretin was overcome if the NSAID was administered as a complex with phospholipid, DPPC.

EXAMPLE 14
THERAPEUTIC REGIMENS

The present example is provided to demonstrate various therapeutic combination regimens for treating fever, inflammation and pain. These regimens include the administration of NSAIDs together with phospholipid and/or neutral lipid.

Agents that include phospholipid, such as lecithin - tablets and the like, may be used as part of the regimens disclosed herein for the enhancement of NSAID activity.

Therapeutic Regimen

For use as an improved regimen (e.g., anti-pyretic, platelet aggregation, analgesic), the present invention contemplates an initial administration of the phospholipid, such as in a tablet or phospholipid-containing agent, such as lecithin tablets, that contain phospholipid (e.g., phosphatidyl choline).

Either at the same time or following the administration of a phospholipid or phospholipid containing agent, the patient would then be given an NSAID. The NSAID may also be administered in combination with the phospholipid as a single composition,

or alternatively as a combination with both a phospholipid and a neutral lipid, such as a triglyceride (e.g., TO).

The present inventors also propose regimens that include the administration of an NSAID and phospholipid and/or neutral lipid, either before or at the same time as an anti-secretory drug, such as Tagamet™ and Prilosec™. This is because antisecretory drugs are observed by the present inventor to reduce the anti-pyretic action of NSAIDs. It is expected that the inclusion of phospholipid and/or neutral lipid will improve the observed reduced absorption of NSAID's observed when NSAIDs are administered with an antisecretory agent alone. Typically, this reduced absorption required that a higher dose of the NSAID be administered to the patient in order to provide the desired therapeutic effect.

Combinations of NSAIDs with zwitterionic phospholipids alone or in combination with neutral lipids will promote the ability of this family of drugs to influence certain target cells, such as neutrophils, platelets, eosinophils, macrophages, and others. In doing so, said phospholipids will increase the efficacy and potency of the NSAIDs to inhibit the cellular cyclo-oxygenase and the formation of arachidonic acid-derived products and other agents involved in cellular aggregation, adhesion, and the synthesis and release of inflammatory mediators and/or cytokines.

EXAMPLE 15
GI SIDE-EFFECTS OF NSAIDS ARE REDUCED BY ANTISECRETORY AGENTS

Rodent model systems were employed in the present study to investigate the effects of combination therapy on the GI toxicity and therapeutic activity of aspirin and indomethacin. A comparison was then made of these results to phospholipid-associated NSAIDs.

Methods

Fasted rats were pretreated with either saline or an antisecretory dose of omeprazole, ranitidine or cimetidine and 1-2 hours later were intragastrically administered saline, aspirin or indomethacin. In ulcer models, aspirin-treated rats were post-challenged with intragastric HCl and gastric lesions measured, whereas indomethacin treated rats were pre- and post-challenged with L-NAME, and GI bleeding assessed. For antipyretic and

analgesic activity rectal body temperature in febrile rats was measured, and the rat's pain sensitivity to pressure applied to an inflamed limb respectively.

Results

NSAID-induced GI ulceration and bleeding were reduced in rats pretreated with antisecretory agents and abolished in rats administered phospholipid-associated NSAIDs in combination with inhibitors of acid secretion. The antipyretic and analgesic activity of both NSAIDs were attenuated in rats pretreated with an antisecretory agent. This pH-dependent block in therapeutic activity was overcome if the NSAID is preassociated with a phospholipid to enhance the drug's lipophilic characteristics. The present example demonstrates that the combination therapy of antisecretory agents and NSAIDs, chemically associated with phospholipids, has distinct advantages with regards to both low GI toxicity and restored therapeutic activity.

EXAMPLE 16
PROTON PUMP INHIBITORS AND H2 RECEPTOR ANTAGONISTS, EFFECTS ON ACUTE GI TOXICITY, ANTIPYRETIC, AND ANALGESIC ACTIVITY OF NON-STEROIDAL ANTI-INFLAMMATORY AGENTS

In the present study, rat model systems were also employed to investigate the effects of both proton pump inhibitors and H2 receptor antagonists on the acute GI toxicity, antipyretic and analgesic activity of aspirin and indomethacin. Parallel studies were performed with these NSAIDs coupled to phospholipids to demonstrate this as an effective strategy to increase the bioavailability of the NSAID in the presence of an anti-secretory agent, while providing enhanced protection against the drugs' injurious actions.

Materials and Methods

Animals

Male Sprague Dawley rats weighing 150-175 g were purchased from Harlan Sprague Dawley Inc (Indianapolis, IN) and housed in our institution's Animal Care Center 5-10 days before study, during which time they had *ad libitum* access to water and Harlan Teklab F-6 Rodent Diet (Madison, WN). All animal protocols employed exceeded NIH guidelines in the treatment and welfare of laboratory animals.

Gastric pH

Fasted rats were injected intraperitoneally with saline (control) or various doses of omeprazole (Astra Hässle AB, Molndal, Sweden), cimetidine or ranitidine (Sigma Chemical Co., St. Louis, MO). One hour later, the rats were ether anesthetized and after a midline incision, the stomachs were exteriorized, and 2 ml of H_2O was injected into the gastric lumen and collected for pH analysis.

Ulcer Models and Chemical Association of NSAIDs with DPPC

Rats were fasted overnight and then injected i.p. with an acid-inhibitory dose of omeprazole (400, μmoles/kg or 140 mg/kg) ranitidine (5 mg/kg) or cimetidine (50 mg/kg). One hour later, the rats were intragastrically administered an ED_{50-80} dose of aspirin (ASA, 20 mg/kg) and challenged 10 min later with an intragastric dose of 0.6 N HCl as outlined previously. In this and all subsequent studies, the NSAIDs were administered either alone or chemically associated with an equimolar concentration of dipalmitoylphosphatidylcholine (DPPC).

The NSAID/DPPC complex was prepared, by initially dissolving the required amount of DPPC in chloroform, followed by exhaustive overnight evaporation of the organic solvent under vacuum. The NSAID salt, which was dissolved in water and subsequently titrated to the desired pH, was added to the tube containing the lipid film, followed by 15 min of sonication in a bath type sonicator (Laboratory Supplies Co. Inc., Hicksville, N.Y.). Sixty min after administration of ASA±DPPC, the rats were euthanized by CO_2 asphyxiation and the stomachs removed, and gastric lesions scored in accordance to a previously described method as described in Lichtenberger *et al.* (1995) (Natural Medicine, 1:154-158), which reference in specifically incorporated herein by referenced for this purpose.

The second animal model was based on a method described in Lichtenberger *et al.* (1995), in which fasted rats were administered N^G-nitro-L-arginine methyl ester (L-NAME, 20 mg/kg, i.p.) 1 hour before and 3 and 6 hours after the animals intragastrically received indomethacin± DPPC, at a dose of 10 mg/kg or saline (control). In this study the antisecretory agents were administered (i.p.) 90 min prior to indomethacin (or saline) treatment. Eighteen hours after the administration of the NSAID (or saline), the rats were

euthanized (CO_2 asphyxiation) and the distal half of the intestine was flushed with 10 ml of saline which in turn was collected for hemoglobin analysis to provide an estimate of GI bleeding as outlined in Lichtenberger *et al.* (1995).

Antipyretic Activity

The antipyretic activity of NSAIDs was evaluated and compared in conscious rats who were made febrile by the subcutaneous injection of 2 g/kg Brewers yeast and fasted thereafter, in accordance to the method described by Adams *et al.*, (1968) J. Pharm. Pharmac. 20:305-312 (incorporated herein by reference). Eighteen hours later, the fasted febrile rats (body temperature increased by 0.5-1.0.°C) were injected with an antisecretory agent or an equivalent volume of vehicle injected by the same route. One hour later, the rats intragastrically received 1 ml of an approximate ED_{50-80} dose of either aspirin (18 mg/kg) or indomethacin (18 mg/kg) alone or the drugs preassociated with DPPC (adjusted to a pH of 7.0). Rectal body temperature was monitored over the subsequent 4 hour period.

Analgesic Activity

The ability of NSAIDs to reduce the hyperalgesia (i.e., increased sensitivity to pain) associated with an inflamed hindpaw (induced 4 days previously by the injection of 0.05 ml complete Freund's Adjuvant) was assessed. A modified version of the Randall-Selitto test was used to assess pain sensitivity of each hindpaw. (Lichtenberger *et al.* (1995) (Randall *et al.* (1957) Arch. Int. Pharmacolog. 111:409-419.) Rats were restrained in Plexiglass tubes and external pressure was applied sequentially to the uninflamed and inflamed hindpaws (0-250g at a rate of 16 g/sec with an Analgesymeter (Life Sciences Inst., Woodland Hills, CA). The "pain pressure threshold" was defined as the pressure at which an animal exhibits paw withdrawal. In these studies, rats were fasted overnight and pretreated with an antisecretory agent or saline, 1 hour before receiving an ED_{50-80} dose of the NSAID alone (10 mg/kg aspirin, 4 mg/kg indomethacin), or the NSAID preassociated with DPPC as described above. Two hours later, pain pressure thresholds were assessed on the both hindpaws. The dosing regimen described above was repeated at 6 and 24 hours, and the sensitivity to externally applied pressure was measured 2 hours after the animals received the last NSAID dosage.

The results were analyzed with the analysis of variance (ANOVA) procedure. If the ANOVA procedure yielded a significant interaction between variables, post *ad hoc* comparisons were made with Fisher's LSD test ($p<0.05$). Data are expressed as mean ± standard error of the mean, with $p \leq 0.05$ being considered statistically significant.

Results

Table 6 reveals that in the conscious rats, gastric acid secretion was maximally inhibited by the injection of omeprazole at a dose of 400 μmoles/kg, with gastric pH not being significant from neutrality. The H_2 - receptor blockers, ranitidine and cimetidine, at doses of 5 and 50 mg/kg respectively, were somewhat less efficacious in inhibiting gastric acid secretion in the rat, raising the gastric pH to values >6.0 in 1-2 hours.

Table 6. Ability of Antisecretory Drugs to Neutralize Gastric Juice pH in Rats[a]

Groups	Dose	Gastric Juice pH
Saline	0	3.45±0.05
Omeprazole[b]	40 μmoles/kg	4.15±0.60
	400 μmoles/kg	7.21±0.15*
Ranitidine[c]	5 mg/kg	6.03±0.27
	20 mg/kg	6.30±0.24*
Cimetidine[c]	20 mg/kg	3.58 ±0.46
	50 mg/kg	6.13 ±0.32*

[a]Fasted rats were injected i.p. with omeprazole, ranitidine, cimetidine or saline. 2 hours later under other anesthesia a laparotomy was performed. Clamps were then placed at the esophageal and duodenal ends and 2 ml. of water was injected through the gastric wall into the lumen. After mixing, he gastric fluid was collected for pH analysis.

[b]Omeprazole was suspended in 1 part 10 mg% polyethylene glycol in 5 parts 19mM $NaHCO_3$.

[c]Ranitidine and cimetidine were solubilized in distilled H_2O.

*=$p<0.05$ vs. The gastric juice pH of control rats injected with saline.

Proton pump inhibitors and H2 - receptor antagonists are presently being recommended for both the prevention and treatment of gastroduodenal ulcers associated with NSAID usage based upon encouraging clinical findings.[4-8] Pretreatment of rats with antisecretory doses of omeprazole, ranitidine and cimetidine were moderately effective in reducing GI injury and bleeding induced by acute exposure to aspirin or indomethacin. The GI toxicity of the above potent antisecretory agents were used in combination with phospholipid-associated NSAIDs. The results presented above clearly indicate that this strategy proved the most effective in eliminating the acute GI side-effects of both aspirin and indomethacin.

The results presented here based upon rodent model systems, indicate that the powerful anti-secretory agents - omeprazole, ranitidine and cimetidine appear to attenuate the therapeutic activity of anionic NSAIDs to reduce fever, pain and/or inflammation. This apparent reduction in bioavailability was best observed when the antisecretory agents were administered at a dose to increase gastric juice pH > 6.0 and the NSAIDs were administered at an ED_{50-80} dose to induce anti-pyretic, anti-inflammatory/analgesic activity. These findings thereby establish that although combination therapy of antisecretory agents with NSAIDs appears to effectively reduce the GI toxicity of NSAIDs, due to neutralization of gastric acidity, it may be contra-indicated in clinical situations where relief from pain, inflammation and fever is needed.

The mechanism by which omeprazole, ranitidine and cimetidine blocks the therapeutic activity of both aspirin and indomethacin most likely relates to their common ability to neutralize gastric acidity and block the acid-dependent conversion of the NSAIDs into their membrane-permeable lipidic state. Orally administered NSAIDs are absorbed across the upper GI tract primarily in their undissociated lipidic state (McCormack, K & Brune, K).[28] The present inventors' view of the data presented here thus propose that pharmacological neutralization of the gastric juice to pH > pKa of the NSAID would limit the absorption of the drugs across the gastroduodenal mucosa. This block in the gastric absorption of the drugs would result in the movement of the NSAIDs into, and possibly their absorption from, lower regions of the small intestine where they could be exposed to metabolism by both pancreatic and/or brush border enzymes.

The data presented in this invention demonstrated that the antipyretic, and anti-inflammatory activity of the complex was superior to that of the NSAID alone. This apparent enhancement in therapeutic activity may be attributable to an increase in the lipid permeability/solubility of the phospholipid-associated NSAID. It was predicted that ionic binding between the DPPC and the NSAID may shield the NSAID from undergoing pH dependent changes in charge, and that unlike the free-NSAID, the complex would remain lipophilic even as the intragastric pH approached neutrality. Evidence reported here appears to support this assertion, as the block in the NSAIDs' antipyretic and anti-inflammatory/analgesic activities observed with the three potent antisecretory test agents could be overcome if the NSAIDs were administered to rats as a preformed complex with DPPC. It was also of interest to note that even in the absence of the antisecretory agents, both NSAIDs when administered as a lipidic complex appeared to have equivalent or greater therapeutic efficacy to that of the NSAID alone in reducing fever and pain, confirming the present findings on the ability of DPPC to enhance the therapeutic activity of NSAIDs.

Table 7. Inhibitors of Gastric Acid Secretion
H2-Receptor Antagonists

Chemical Name	Brand Name
Cimetidine	Tagamet®
Ranitidine	Zantac®
Famotidine	Pepsid®
Nizatidin	Axid®
Proton-Pump Inhibitors	
Omeprazole	Prilosec®
Lansoprazole	Prevacid®

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred

embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically, pharmacologically, and/or physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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WHAT IS CLAIMED IS:

1. A method for maintaining or enhancing therapeutic activity of a non-steroidal anti-inflammatory drug in the presence of an H2 receptor blocker in an amount comprising:

administering an anti-inflammatory agent in association with a zwitterionic phospholipid,

wherein the pH is increased above fasting levels in the animal.

2. The method of claim 1 wherein the H2 receptor blocker is ranitidine or cimetidine.

3. The method of claim 1 wherein fasting levels are about pH 1 to about pH 4.

4. A method for maintaining and/or enhancing the big-availability of a non-steroidal anti-inflammatory agent in the presence of an anti-secretory agent, comprising:

administering a combination of a non-steroidal anti-inflammatory agent and a zwitterionic phospholipid to an animal having received an anti-secretory agent.

wherein the pharmacological activity of the non-steroidal anti-inflammatory agent is greater than the pharmacological activity of the non-steroidal anti-inflammatory agent in the absence of a zwitterionic phospholipid to an animal receiving an anti-secretory agent.

5. The method of claim 4 wherein the composition is further defined as comprising an equimolar amount of zwitterionic phospholipid and non-steroidal anti-inflammatory drug.

6. The method of claim 1or 4 wherein the non-steroidal anti-inflammatory drug is salicylate, naproxen, indomethacin, diclofenac, aspirin, or a mixture thereof.

7. The method of claim 1 or 4 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidyl choline.

8. The method of claim 1 or 4 wherein the composition further comprises a neutral lipid.

9. The method of claim 8 wherein the neutral lipid is a triglyceride.

10. A pharmaceutical preparation comprising a non-covalently associated combination of nonsteroidal anti-inflammatory agent, zwitterionic phospholipid, and anti-secretory agent.

11. The pharmaceutical preparation of claim 10 wherein the zwitterionic phospholipid is dipalmitoyl phosphatidyl choline and the neutral lipid is tripalmitin.

12. The pharmaceutical preparation of claim 10 wherein the antisecretory agent is omeprazole.

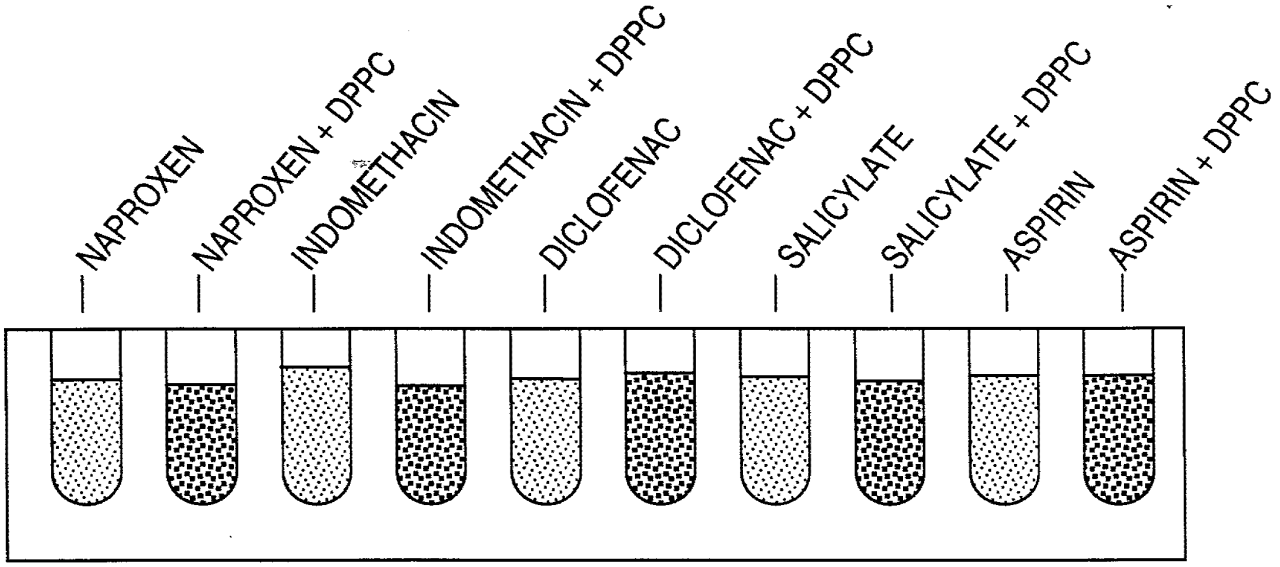

NAPROXEN
NAPROXEN + DPPC
INDOMETHACIN
INDOMETHACIN + DPPC
DICLOFENAC
DICLOFENAC + DPPC
SALICYLATE
SALICYLATE + DPPC
ASPIRIN
ASPIRIN + DPPC

Fig. 1A

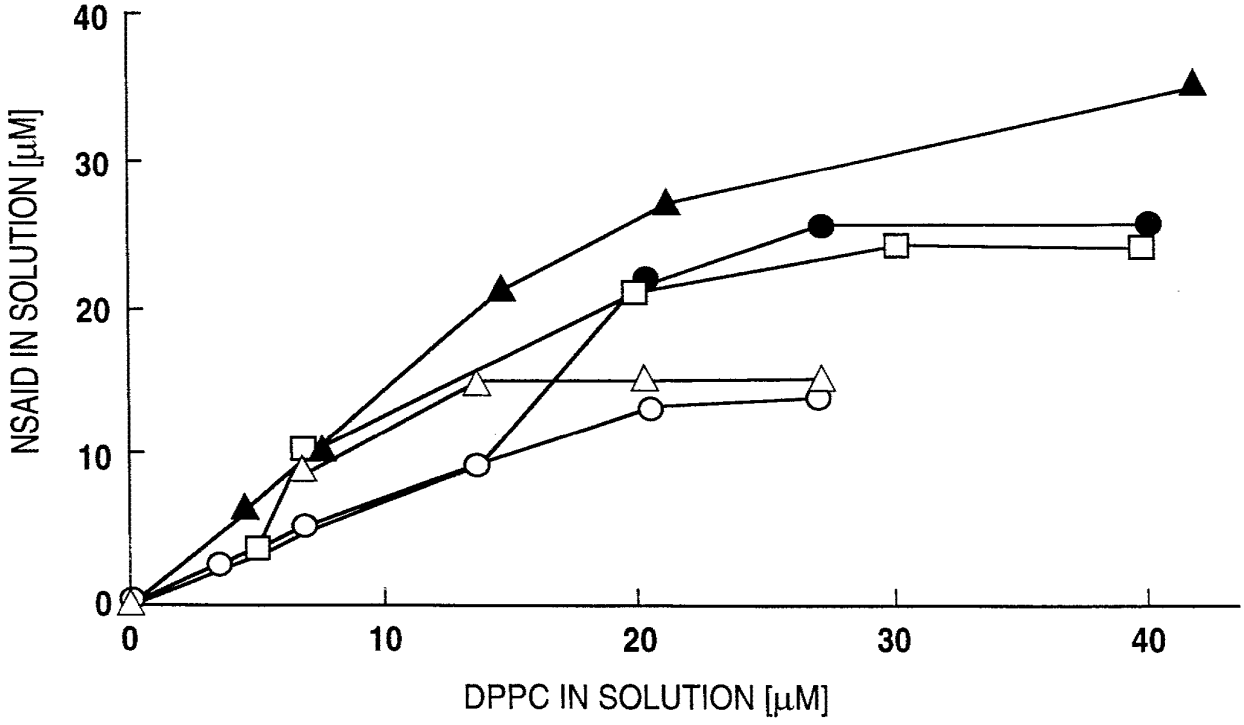

NSAID IN SOLUTION [μM]
0
10
20
30
40
DPPC IN SOLUTION [μM]

Fig. 1B

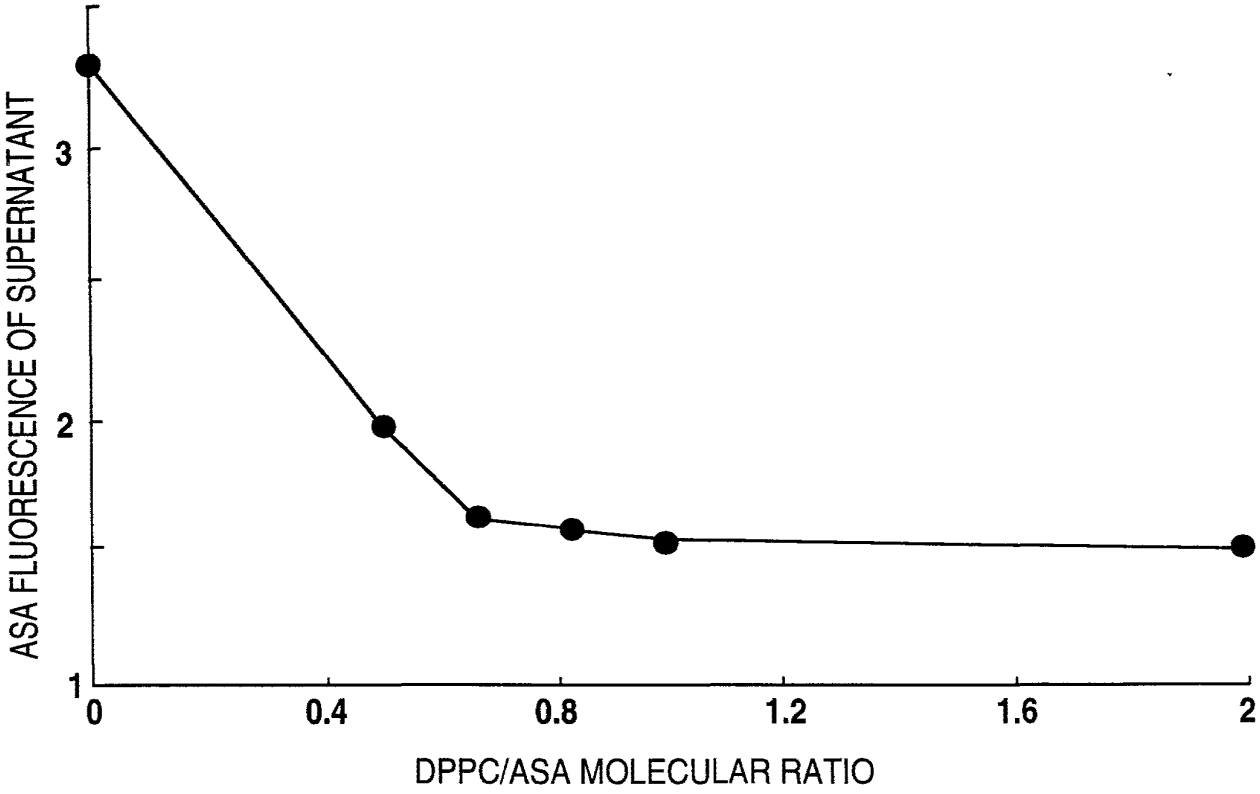

ASA FLUORESCENCE OF SUPERNATANT
1
2
3
0
0.4
0.8
1.2
1.6
2
DPPC/ASA MOLECULAR RATIO

Fig. 2A

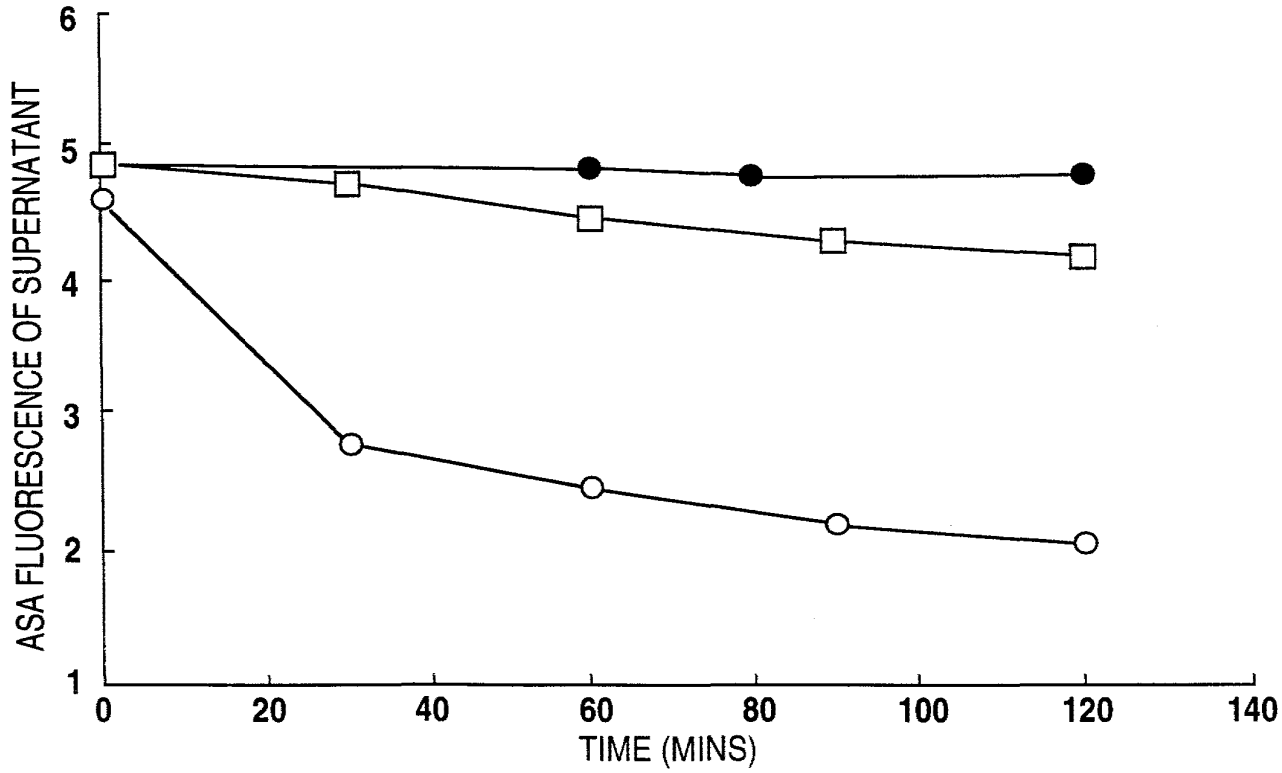

ASA FLUORESCENCE OF SUPERNATANT
1
2
3
4
5
6
0
20
40
60
80
100
120
140
TIME (MINS)

Fig. 2B

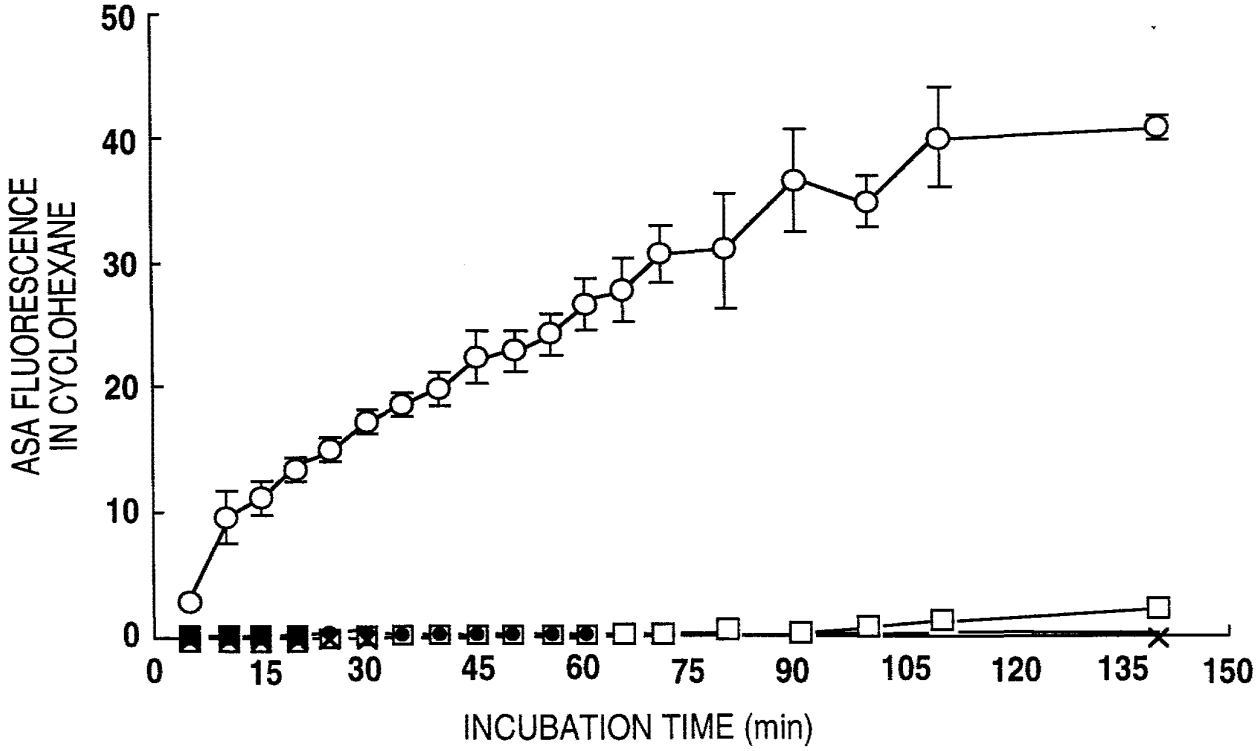

ASA FLUORESCENCE IN CYCLOHEXANE
50
40
30
20
10
0
0
15
30
45
60
75
90
105
120
135
150
INCUBATION TIME (min)

Fig. 3

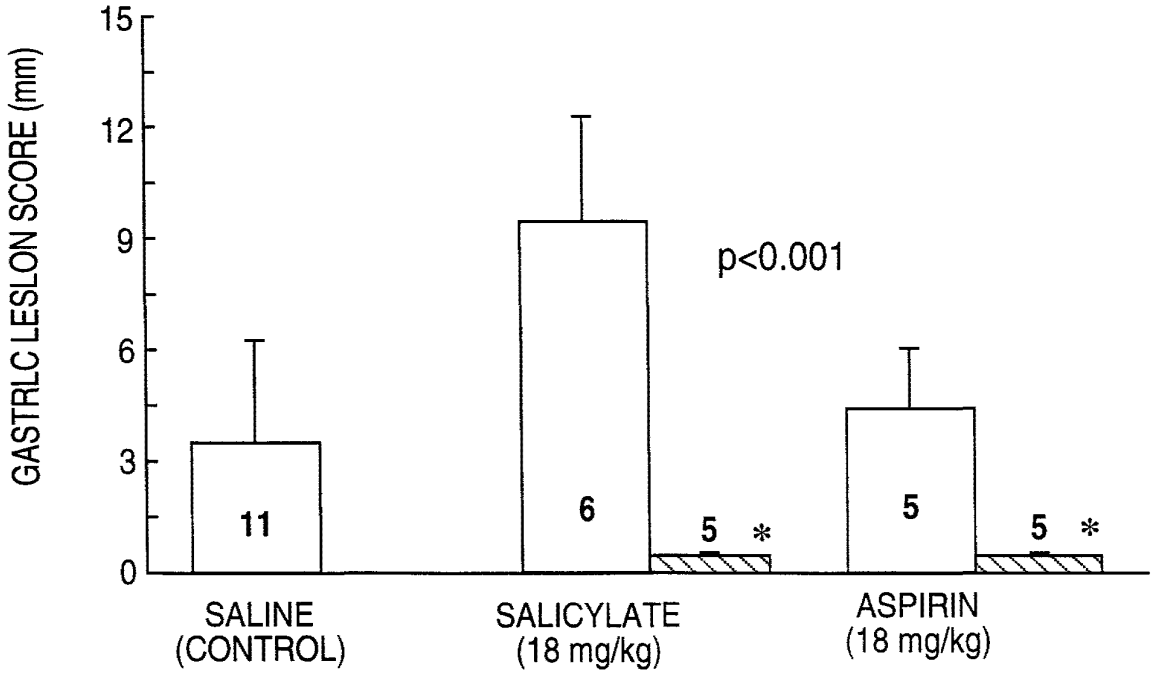

GASTRLC LESLON SCORE (mm)
15
12
9
6
3
0
11
6
5
*
p<0.001
5
5
*
SALINE (CONTROL)
SALICYLATE (18 mg/kg)
ASPIRIN (18 mg/kg)

Fig. 4A

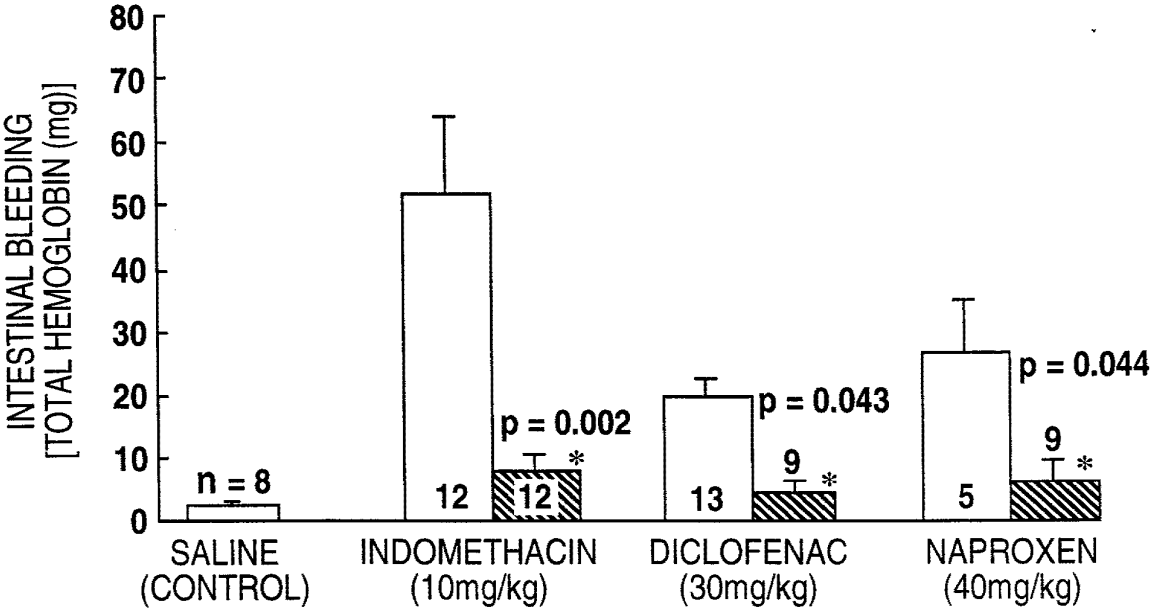

INTESTINAL BLEEDING
[TOTAL HEMOGLOBIN (mg)]
80
70
60
50
40
30
20
10
0
n = 8
12
12
p = 0.002
*
13
9
p = 0.043
*
5
9
p = 0.044
*
SALINE
(CONTROL)
INDOMETHACIN
(10mg/kg)
DICLOFENAC
(30mg/kg)
NAPROXEN
(40mg/kg)

Fig. 4B

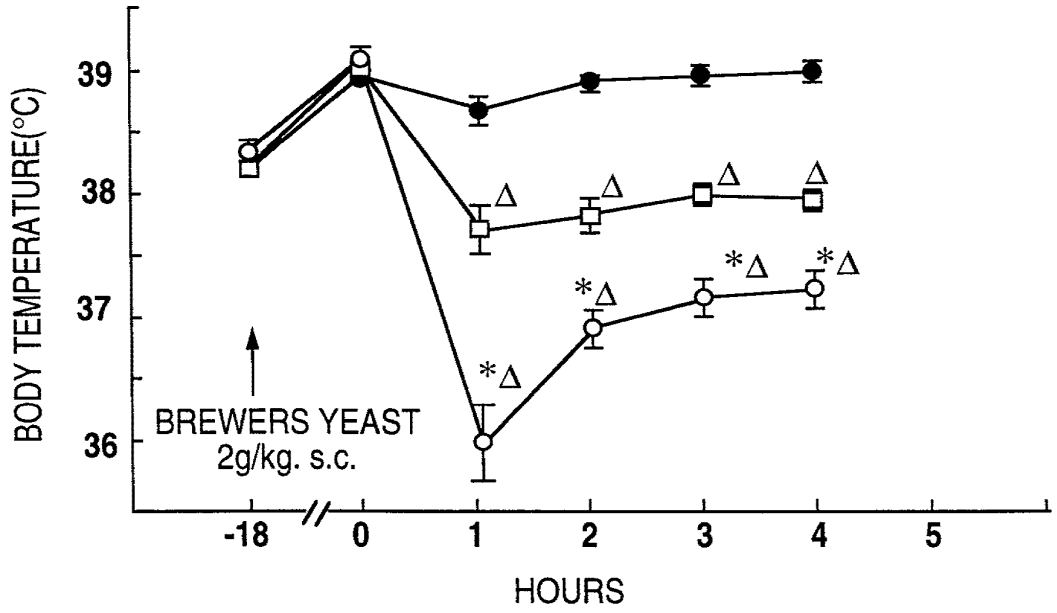

BODY TEMPERATURE(°C)
39
38
37
36
BREWERS YEAST
2g/kg. s.c.
-18
0
1
2
3
4
5
HOURS
Δ
*Δ

Fig. 4C

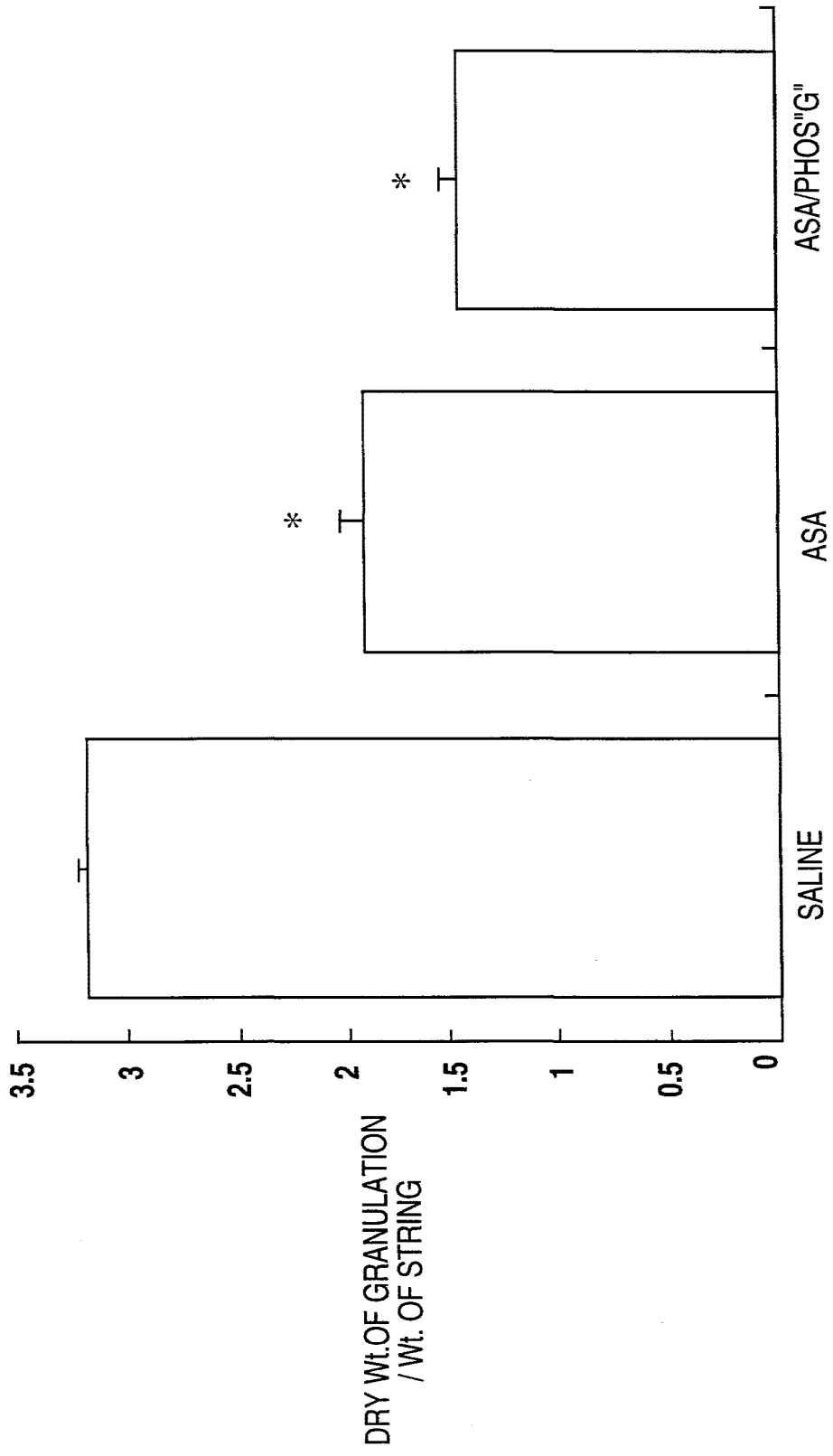

3.5
3
2.5
2
1.5
1
0.5
0
DRY Wt.OF GRANULATION
/ Wt. OF STRING
SALINE
ASA
ASA/PHOS"G"
GROUP
*
*

Fig. 5

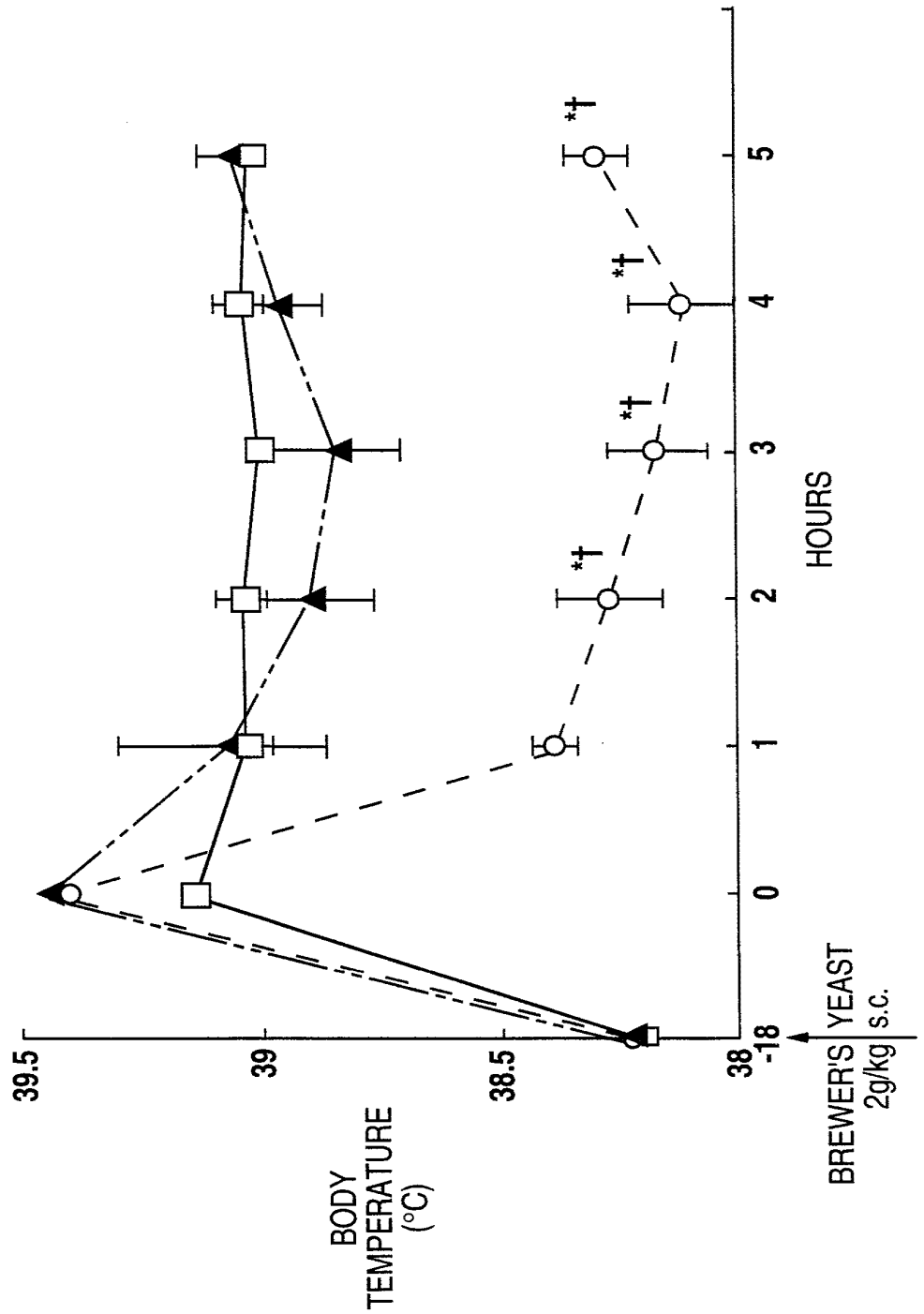

39.5
39
38.5
38
-18
0
1
2
3
4
5
HOURS
BODY TEMPERATURE (°C)
BREWER'S YEAST 2g/kg s.c.
*†

Fig. 6

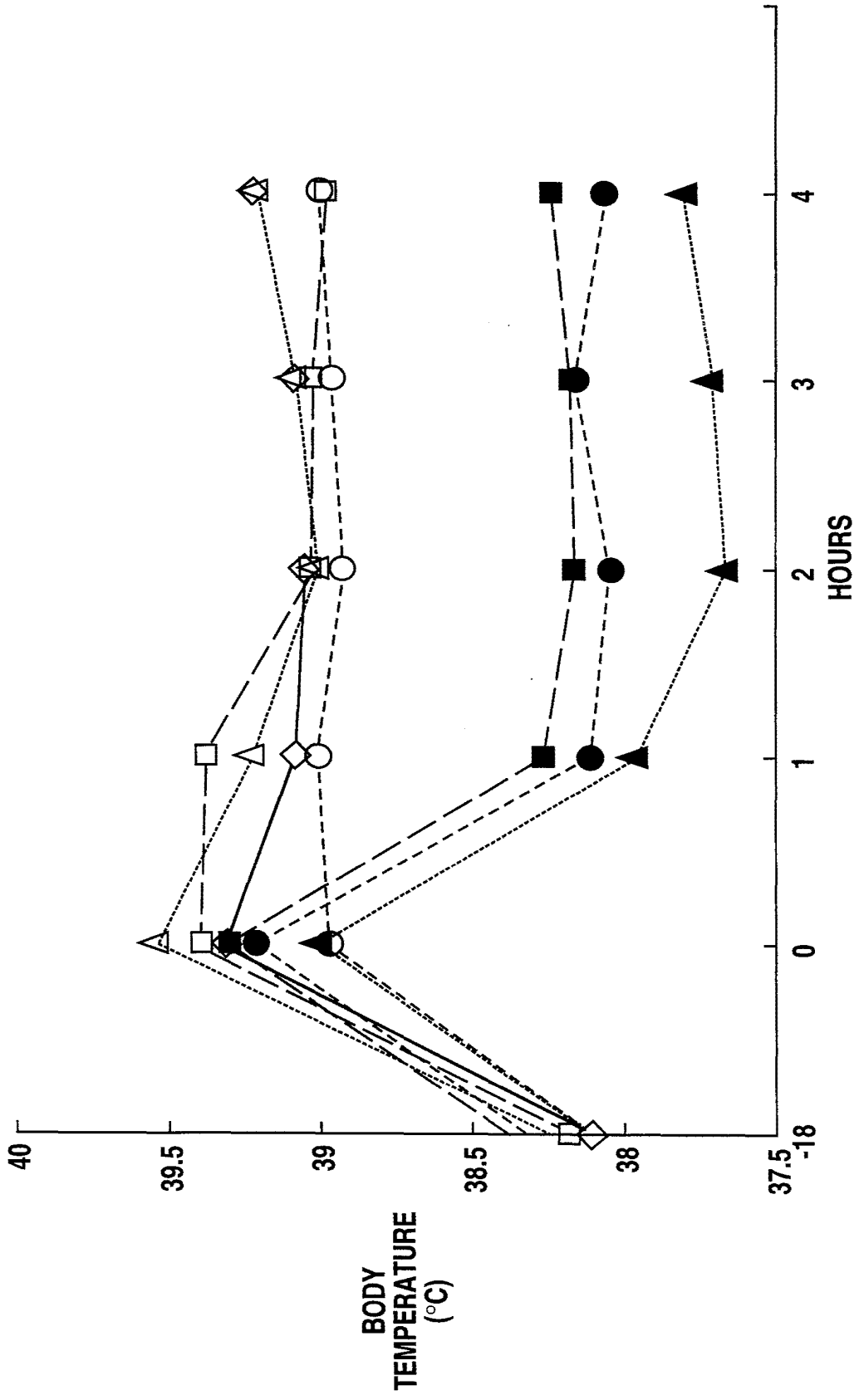


Fig. 7

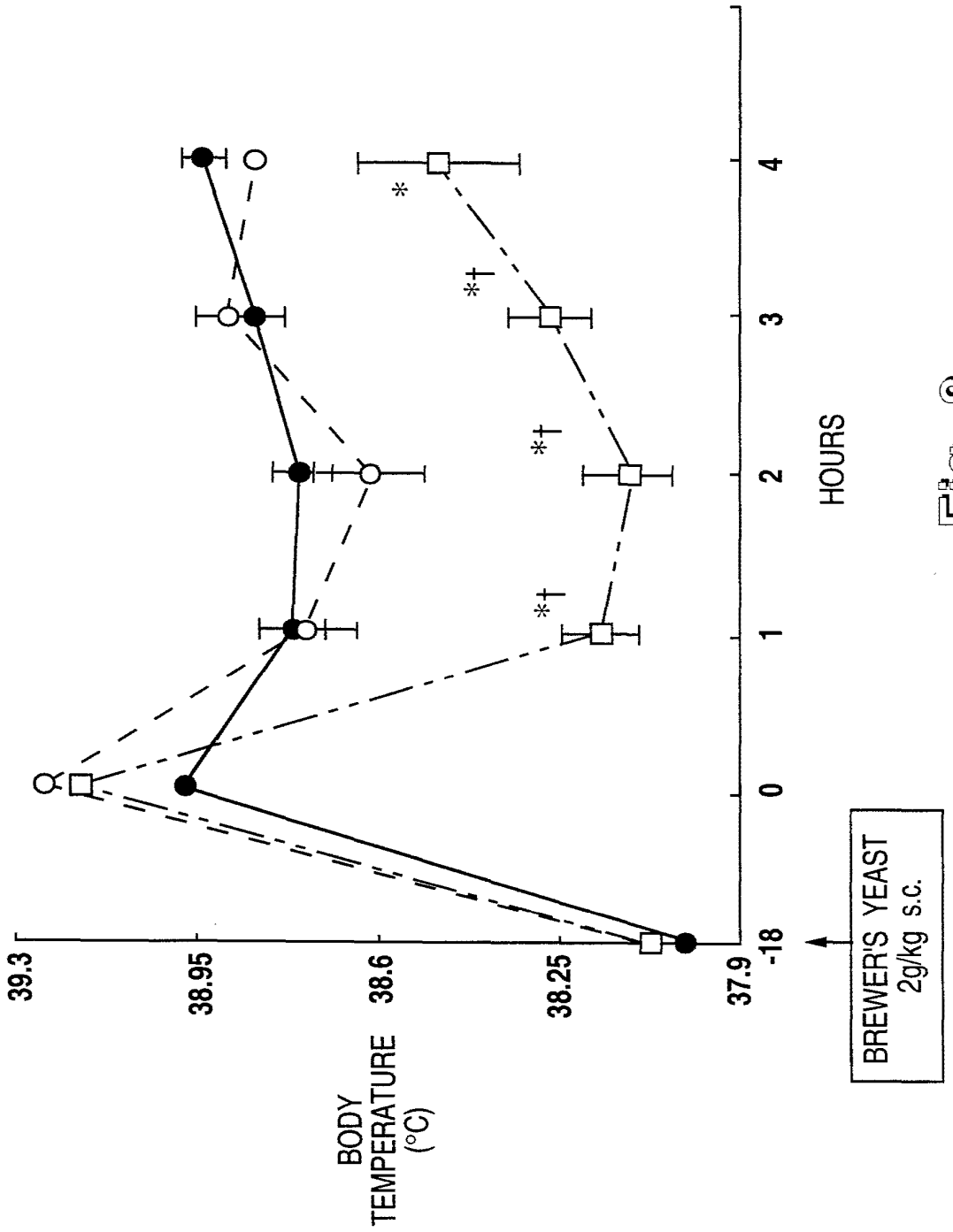

39.3
38.95
38.6
38.25
37.9
BODY TEMPERATURE (°C)
-18
0
1
2
3
4
HOURS
BREWER'S YEAST 2g/kg s.c.
*
*†
*†
*†

Fig. 8

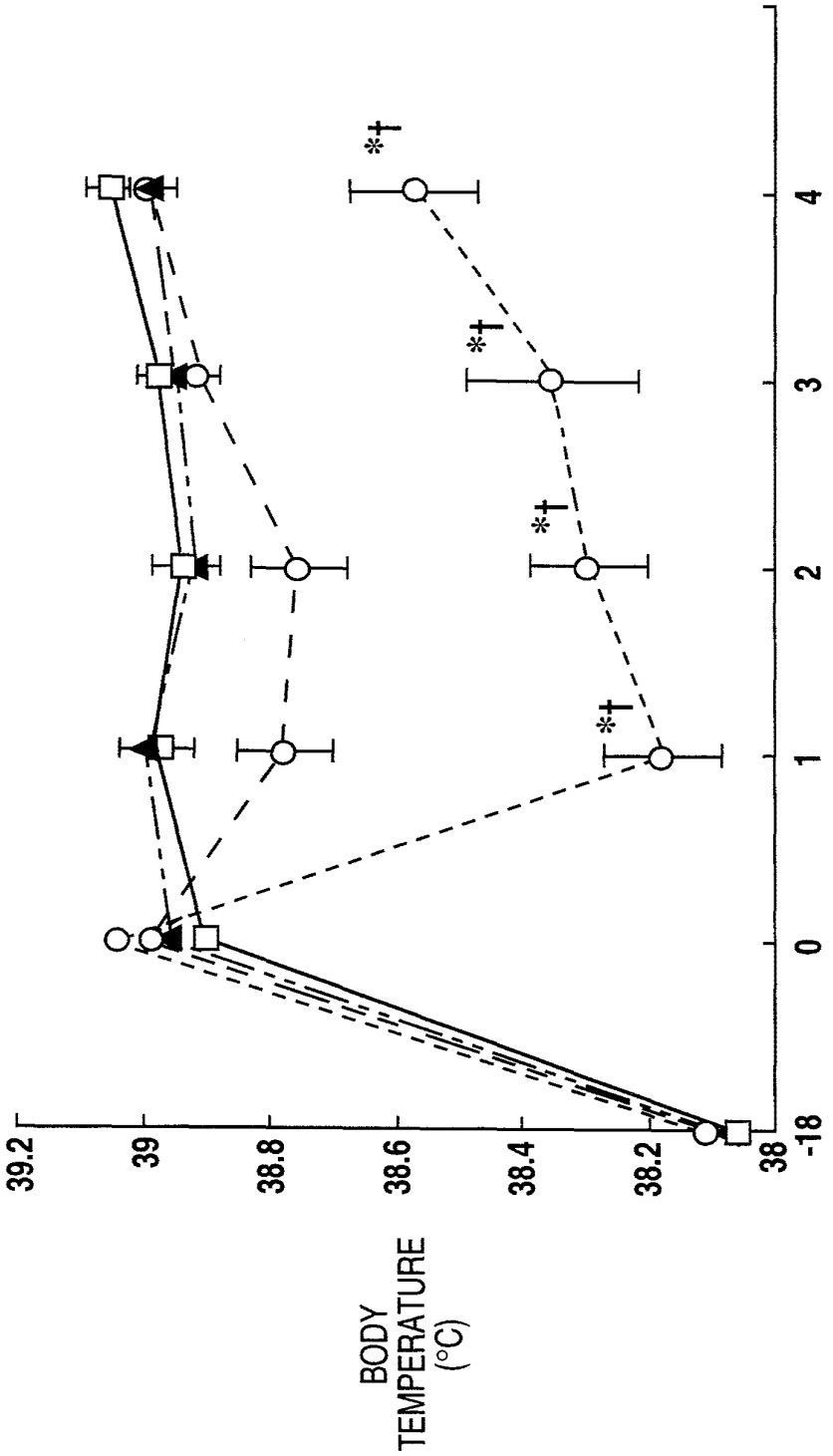


Fig. 9

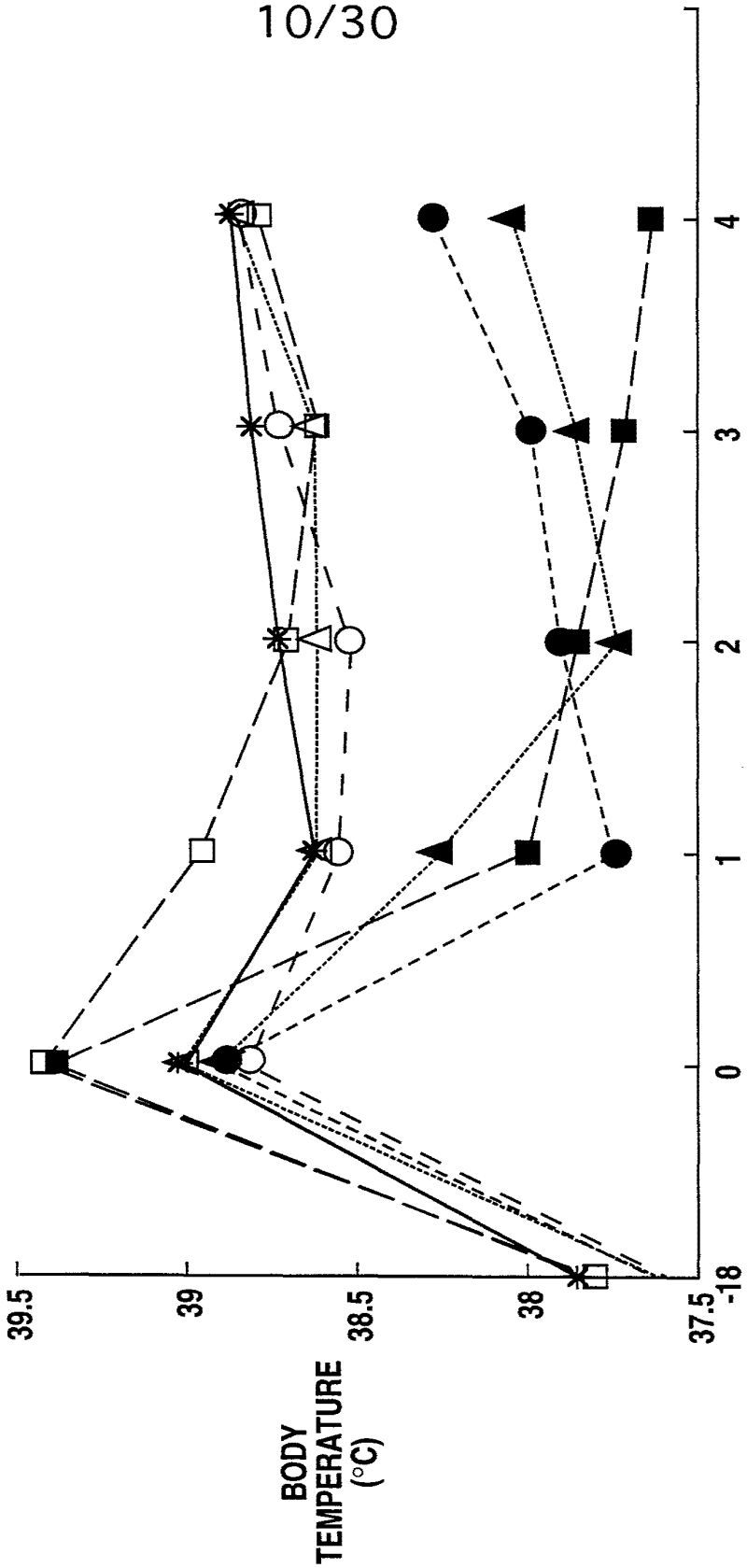


Fig. 10

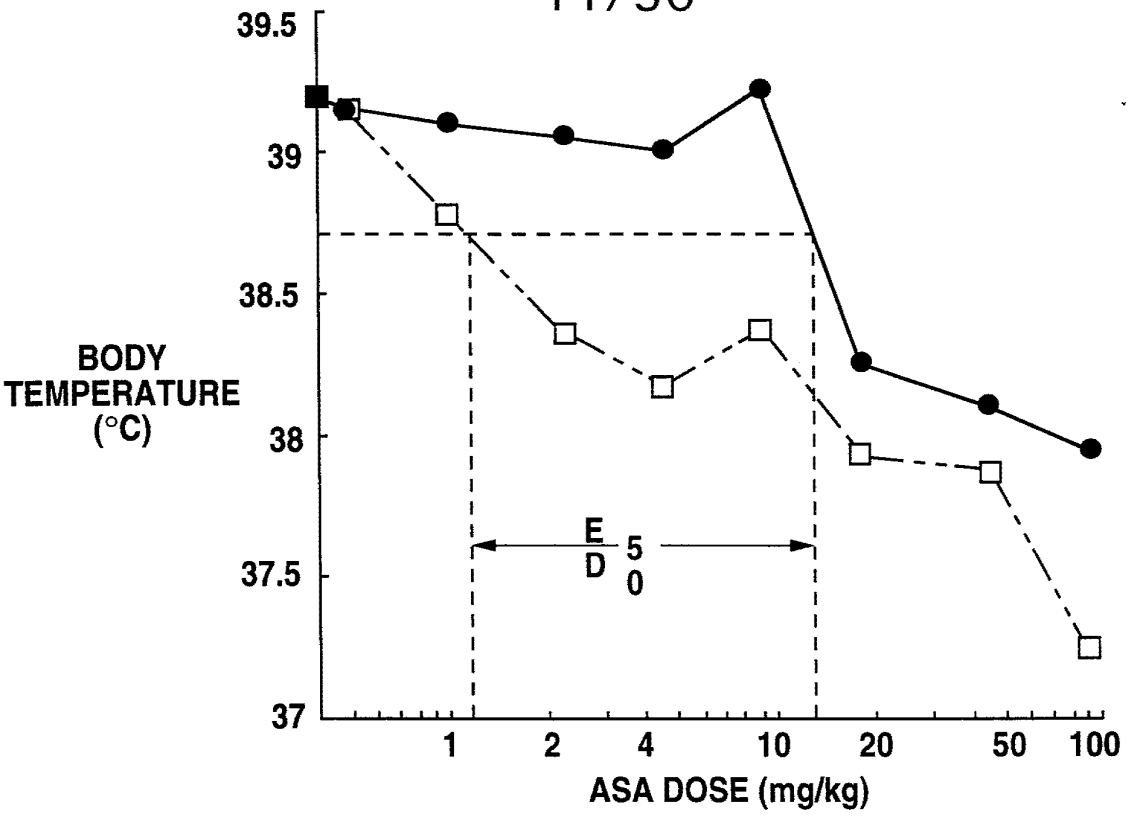

39.5
39
38.5
38
37.5
37
BODY TEMPERATURE (°C)
E D 5 0
1
2
4
10
20
50
100
ASA DOSE (mg/kg)

Fig. 11

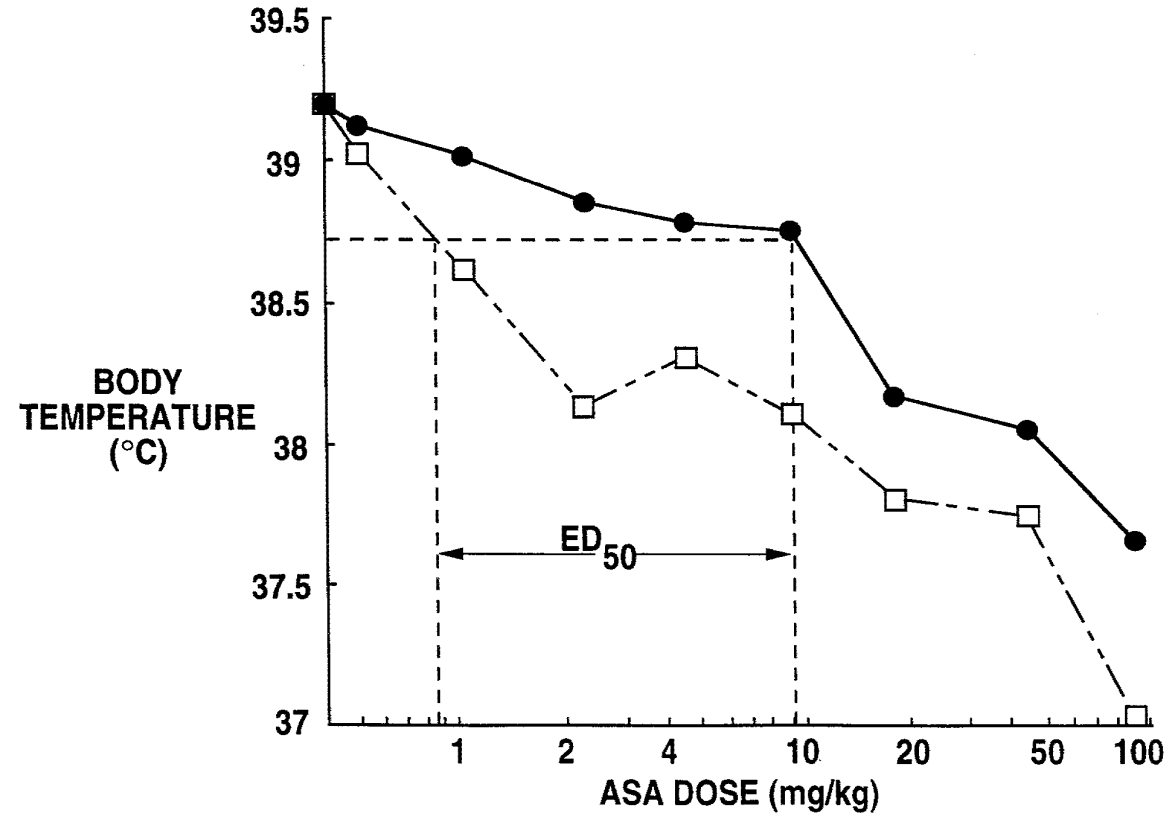

39.5
39
38.5
38
37.5
37
BODY TEMPERATURE (°C)
ED50
1
2
4
10
20
50
100
ASA DOSE (mg/kg)

Fig. 12

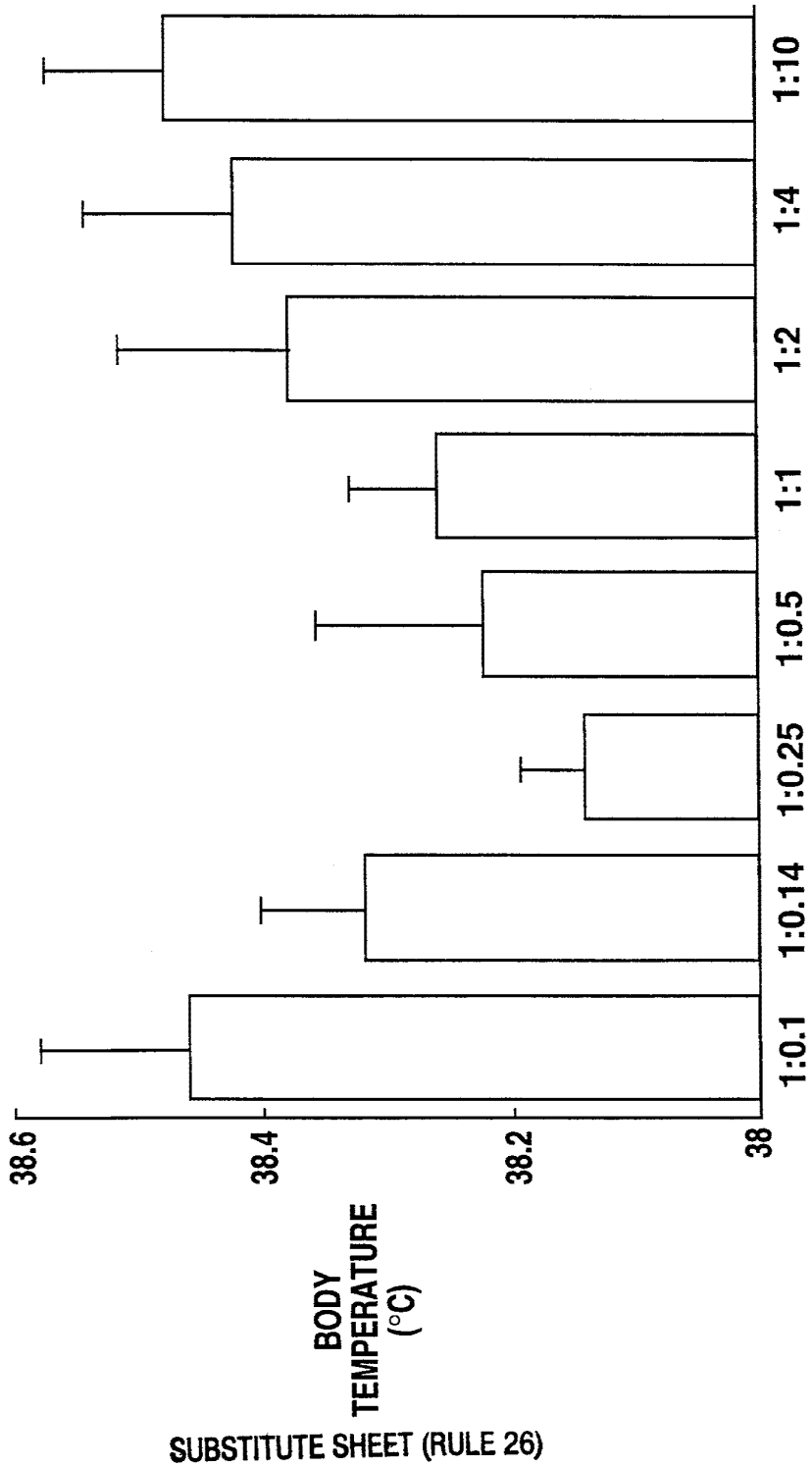

38.6
38.4
38.2
38
BODY TEMPERATURE (°C)
1:0.1
1:0.14
1:0.25
1:0.5
1:1
1:2
1:4
1:10
ASA to DPPC Ratios

Fig. 13

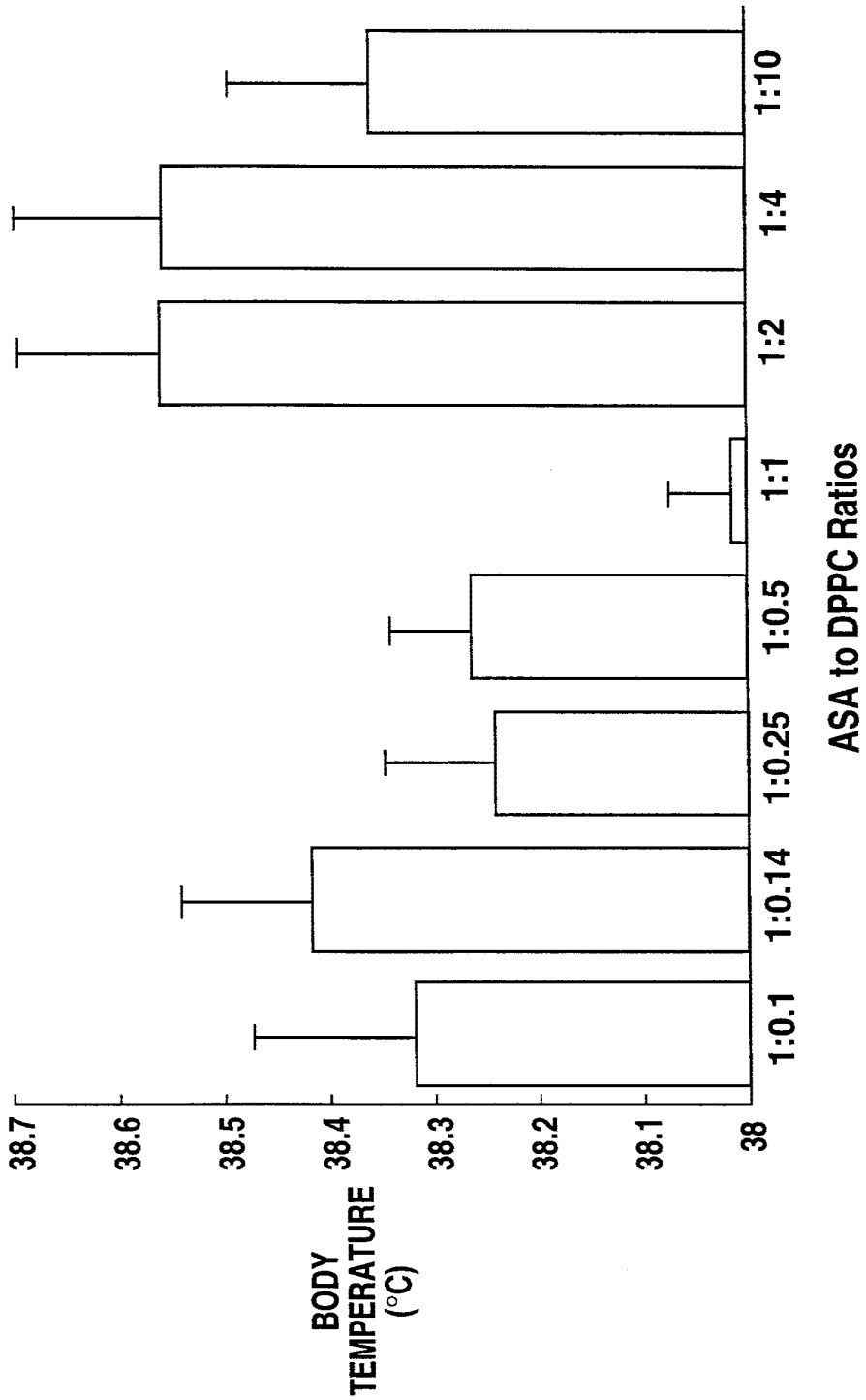

BODY TEMPERATURE (°C)
38.7
38.6
38.5
38.4
38.3
38.2
38.1
38
1:0.1
1:0.14
1:0.25
1:0.5
1:1
1:2
1:4
1:10
ASA to DPPC Ratios

Fig. 14

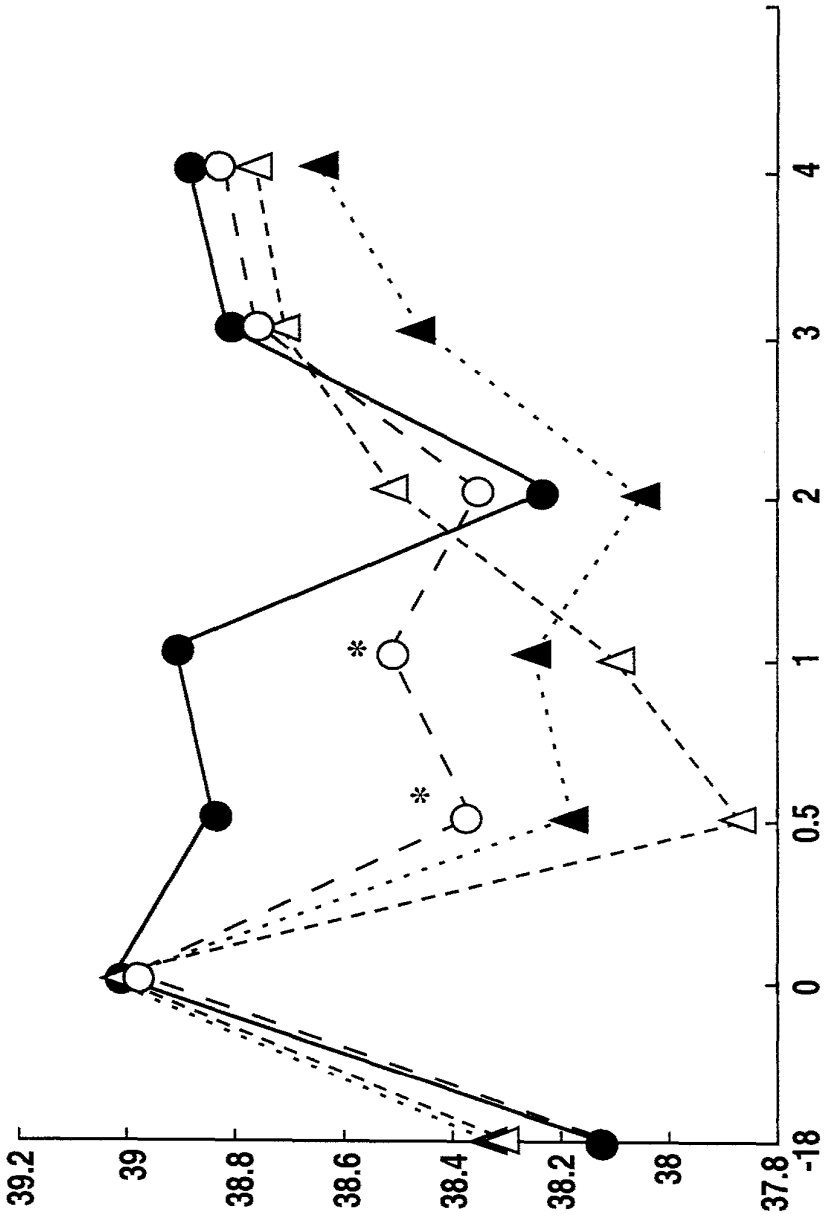

BODY TEMPERATURE (°C)
39.2
39
38.8
38.6
38.4
38.2
38
37.8
-18
0
0.5
1
2
3
4
HOURS
*
*

Fig. 15

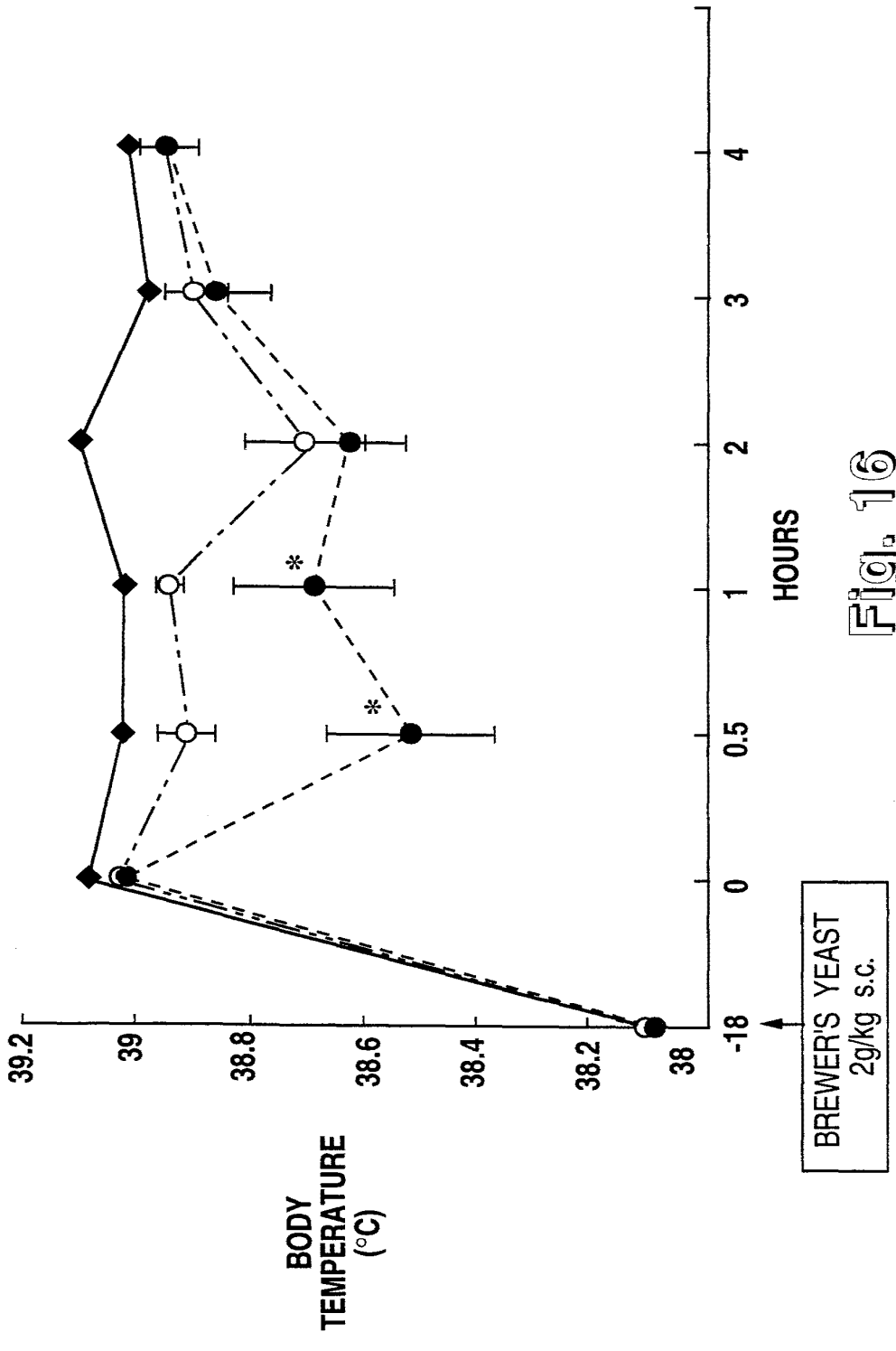

BODY TEMPERATURE (°C)
39.2
39
38.8
38.6
38.4
38.2
38
-18
0
0.5
1
2
3
4
HOURS
BREWER'S YEAST 2g/kg s.c.
*
*

Fig. 16

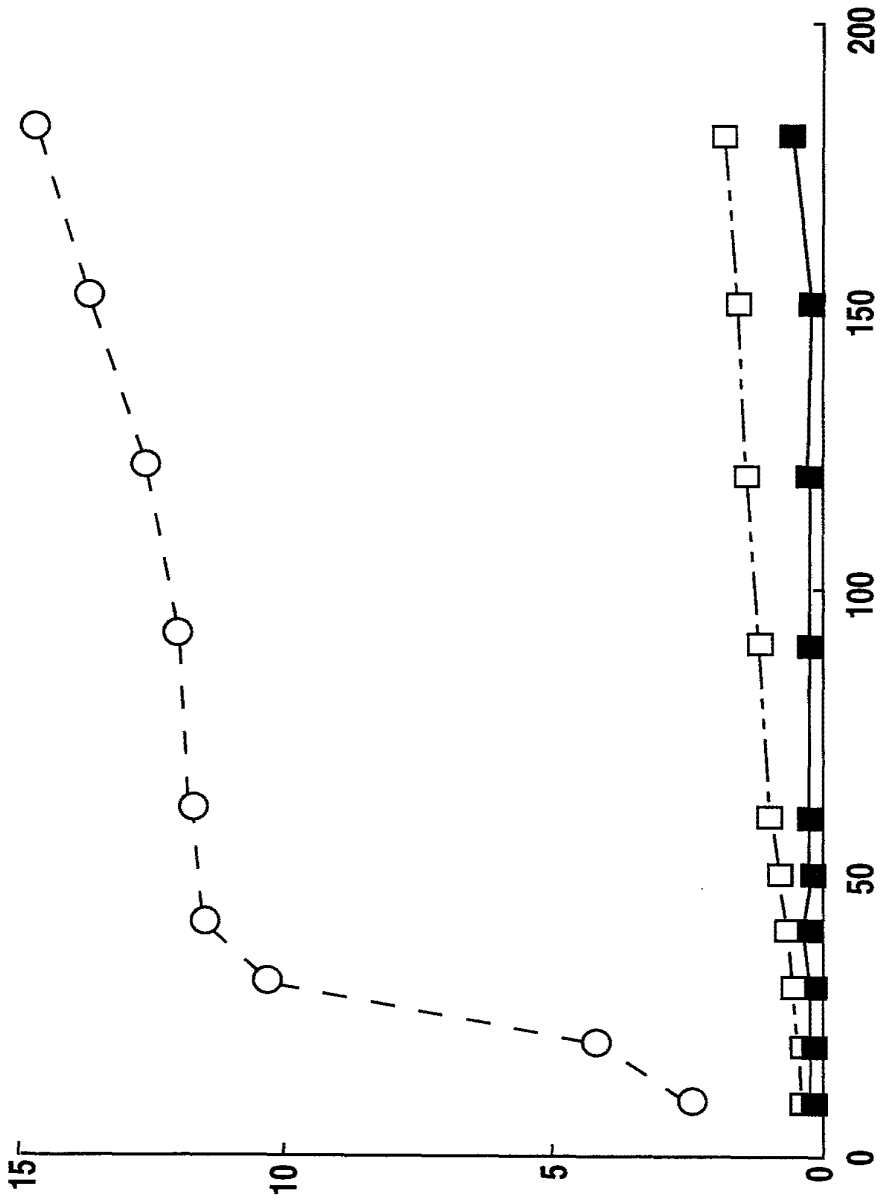

FLUORESCENCE OF ASPIRIN
IN CYCLOHEXANE
15
10
5
0
0
50
100
150
200
TIME (MINUTE)

Fig. 17

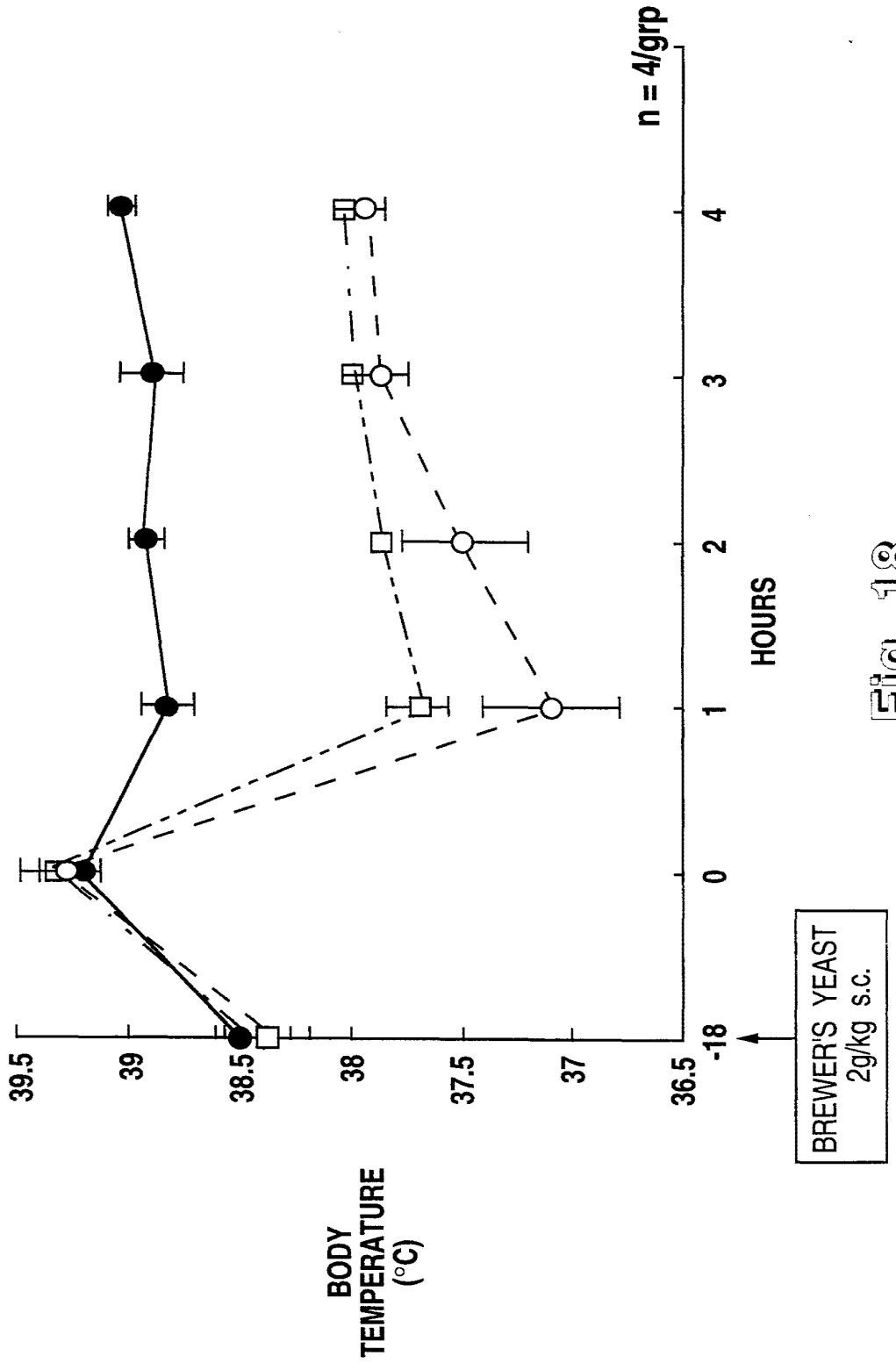

n = 4/grp
BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
HOURS
BREWER'S YEAST 2g/kg s.c.

Fig. 18

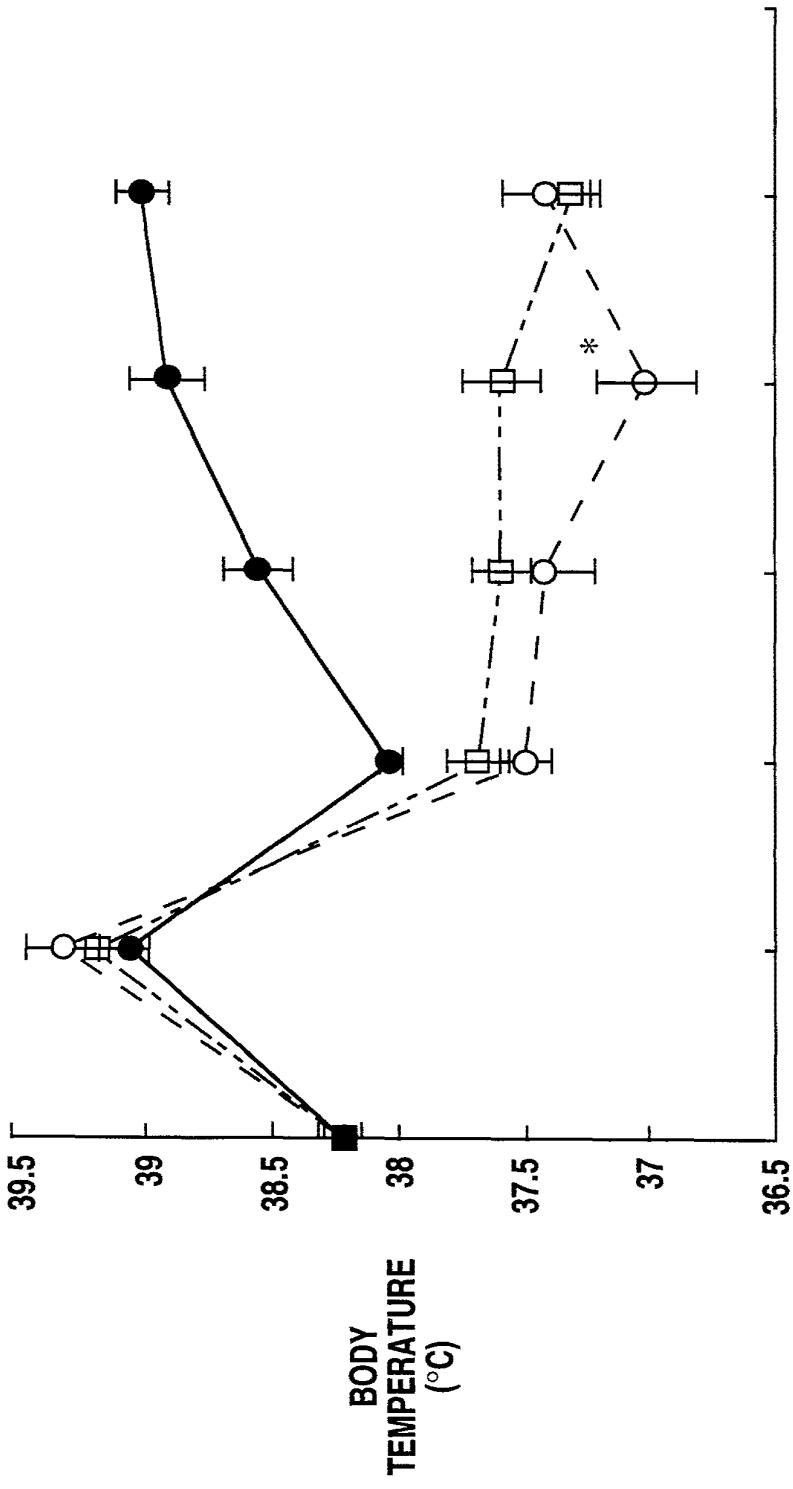

BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
HOURS
*
BREWER'S YEAST 2g/kg s.c.

Fig. 19

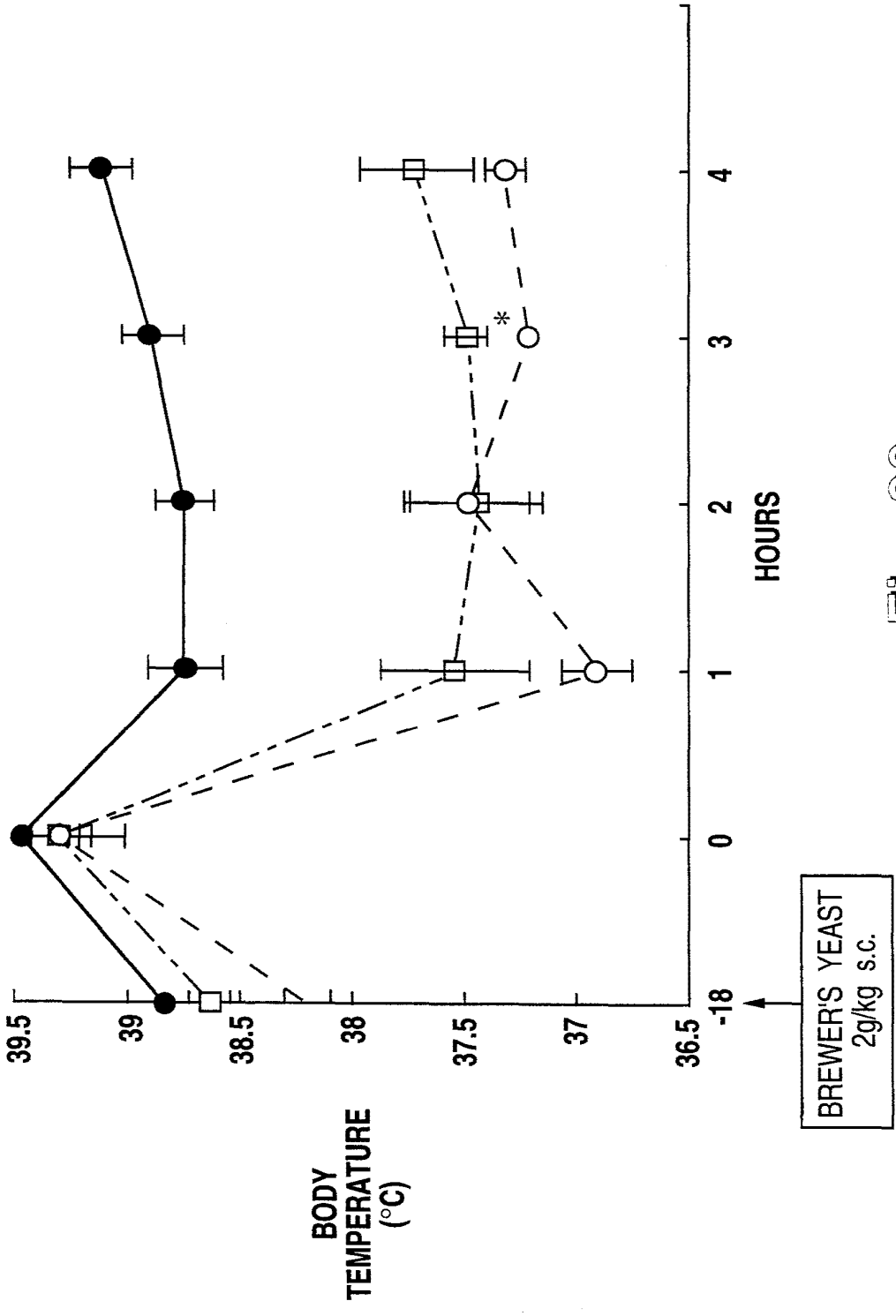

BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
HOURS
*
BREWER'S YEAST 2g/kg s.c.

Fig. 20

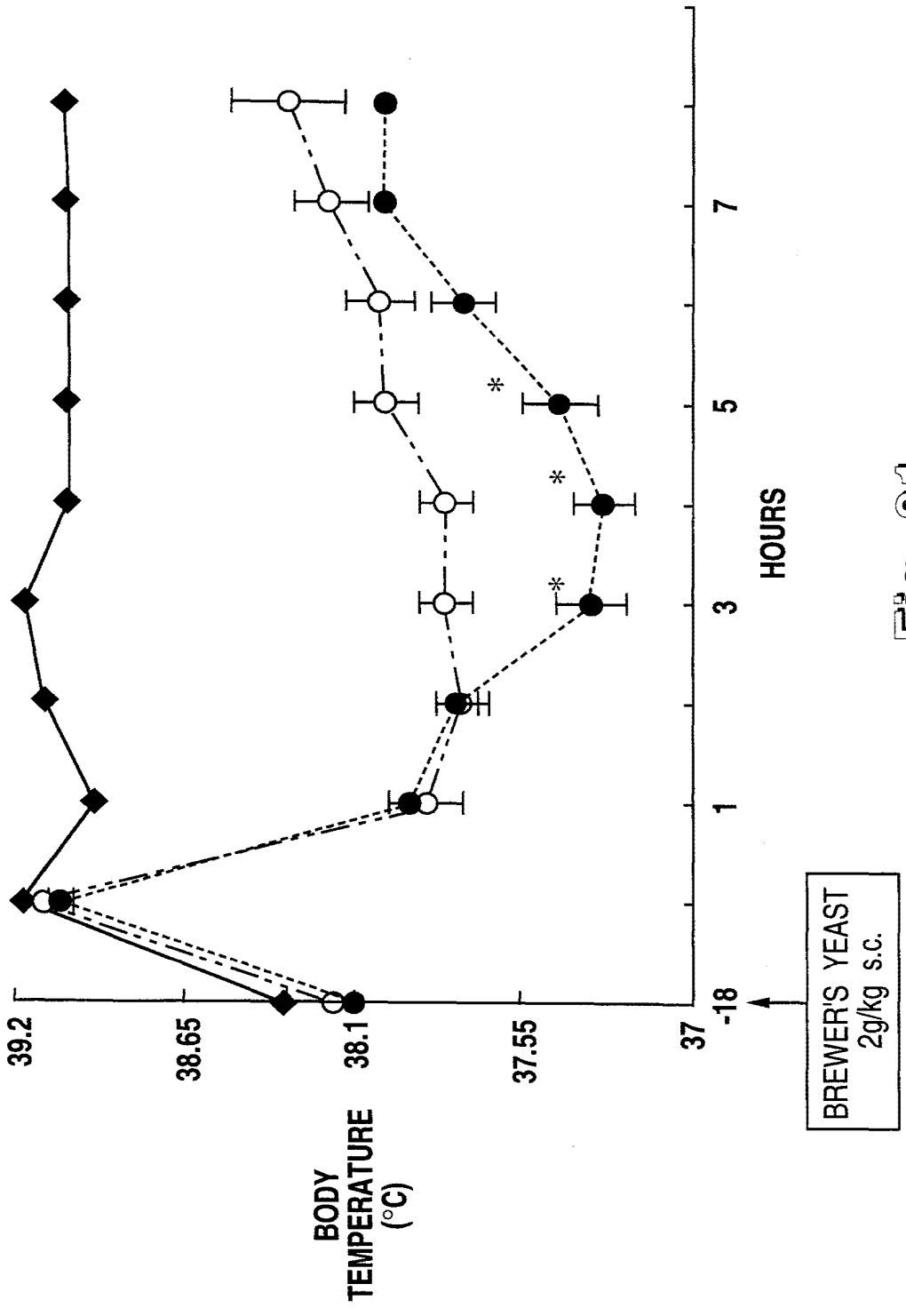

BODY TEMPERATURE (°C)
39.2
38.65
38.1
37.55
37
-18
1
3
5
7
HOURS
BREWER'S YEAST 2g/kg s.c.
*
*
*

Fig. 21

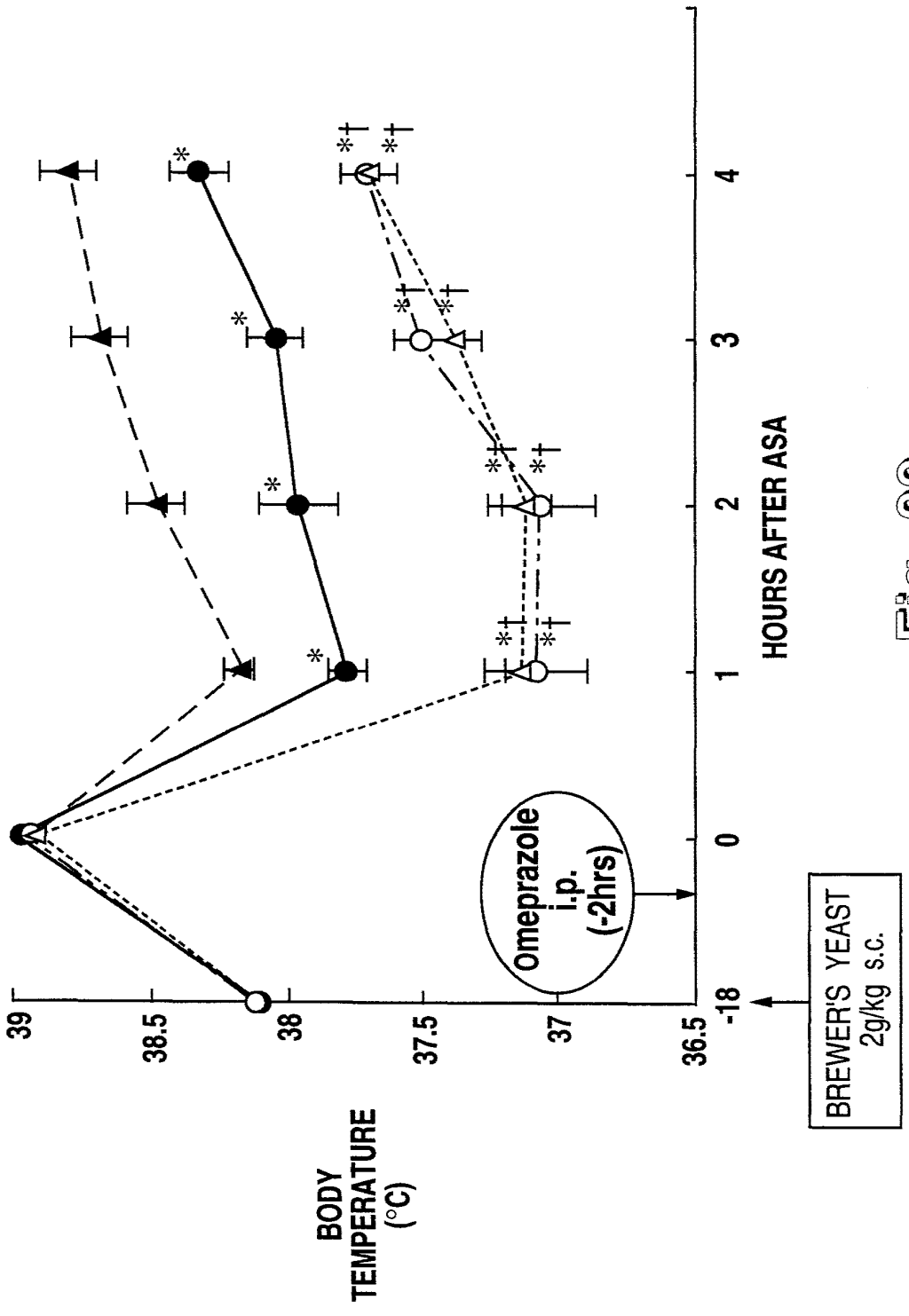

BODY TEMPERATURE (°C)
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
HOURS AFTER ASA
Omeprazole i.p. (-2hrs)
BREWER'S YEAST 2g/kg s.c.

Fig. 22

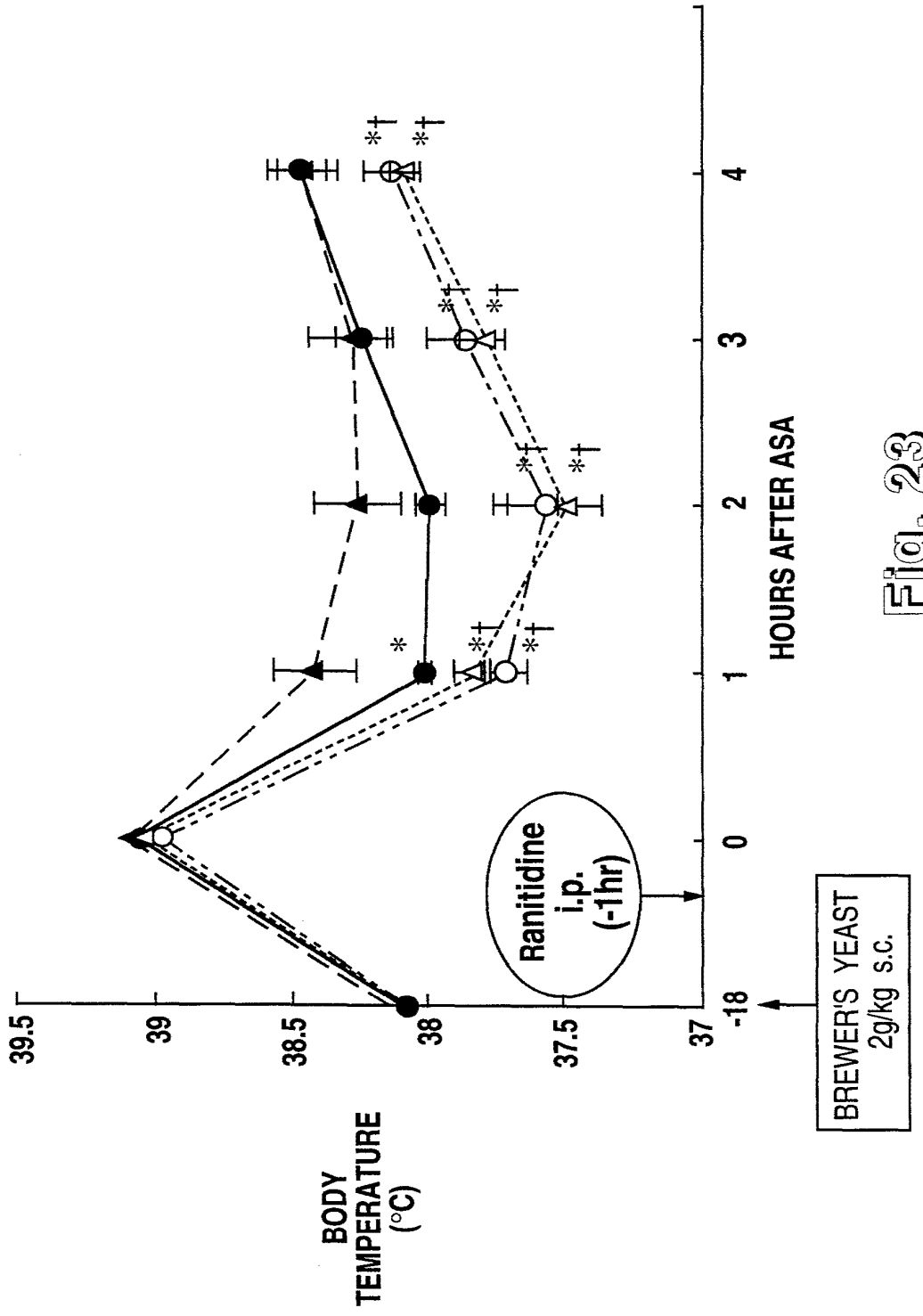

BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
-18
0
1
2
3
4
HOURS AFTER ASA
Ranitidine i.p. (-1hr)
BREWER'S YEAST 2g/kg s.c.

Fig. 23

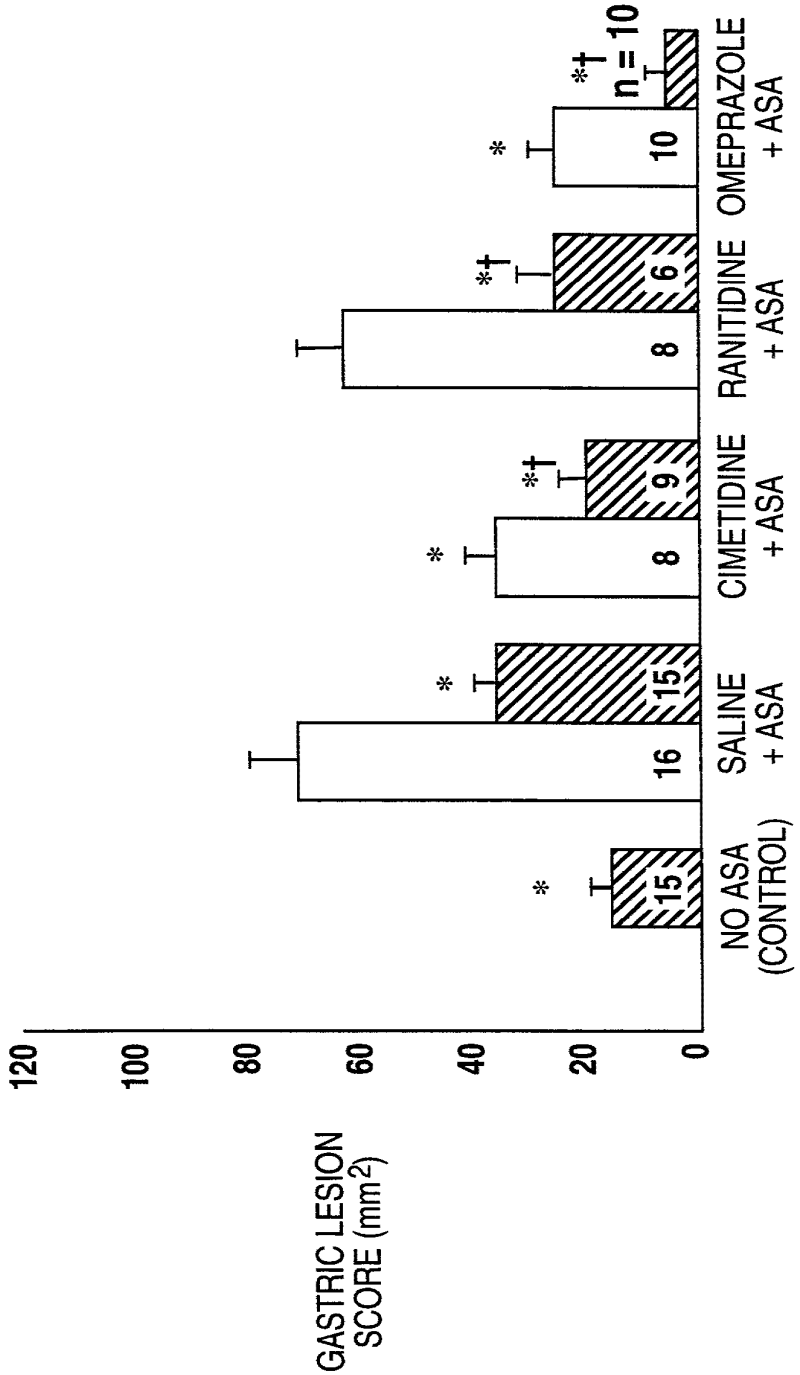

GASTRIC LESION SCORE (mm2)
120
100
80
60
40
20
0
NO ASA (CONTROL)
SALINE + ASA
CIMETIDINE + ASA
RANITIDINE + ASA
OMEPRAZOLE + ASA
15
16
15
8
9
8
6
10
n = 10
*
*†

Fig. 24

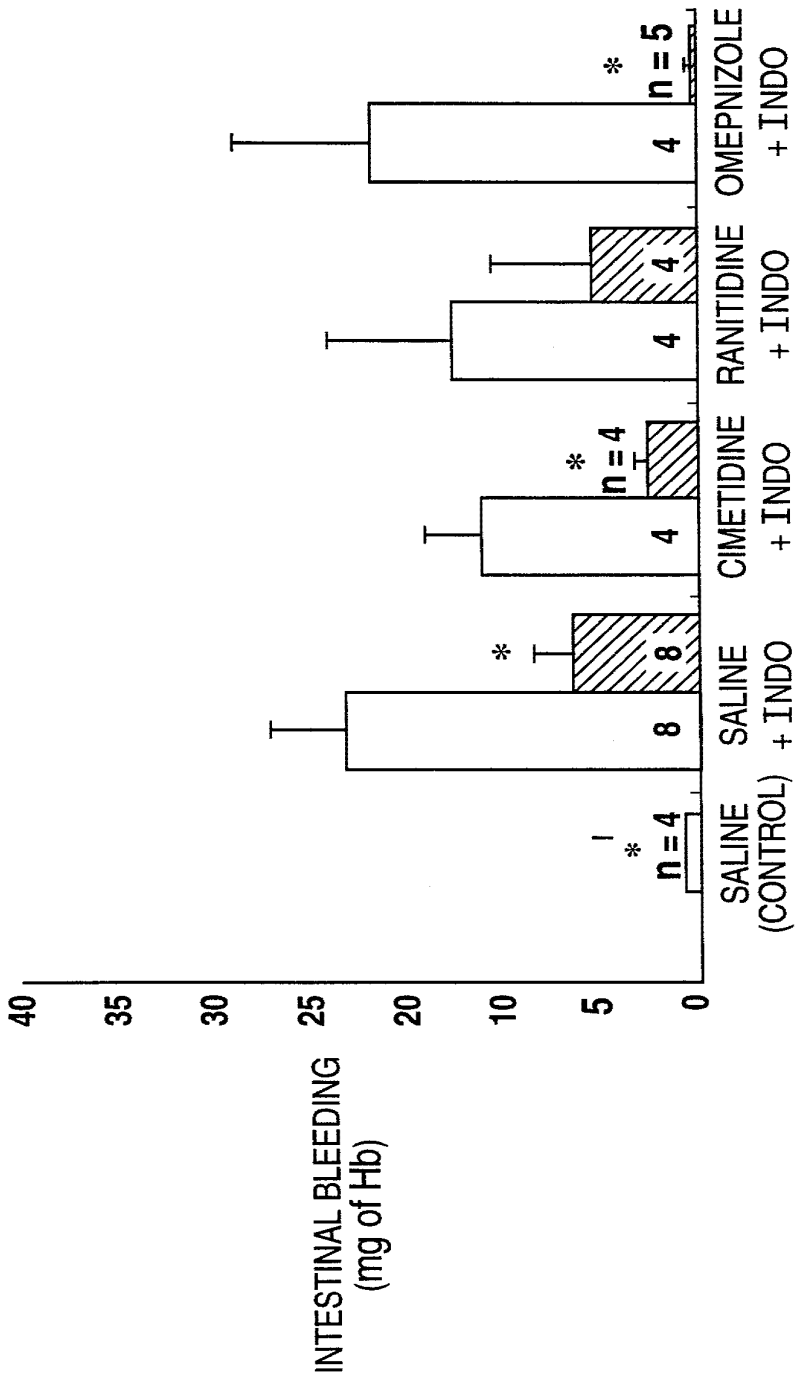

INTESTINAL BLEEDING
(mg of Hb)
0
5
10
20
25
30
35
40
n=4
8
8
4
n=4
4
4
4
n=5
SALINE
(CONTROL)
SALINE
+ INDO
CIMETIDINE
+ INDO
RANITIDINE
+ INDO
OMEPNIZOLE
+ INDO
*

Fig. 25

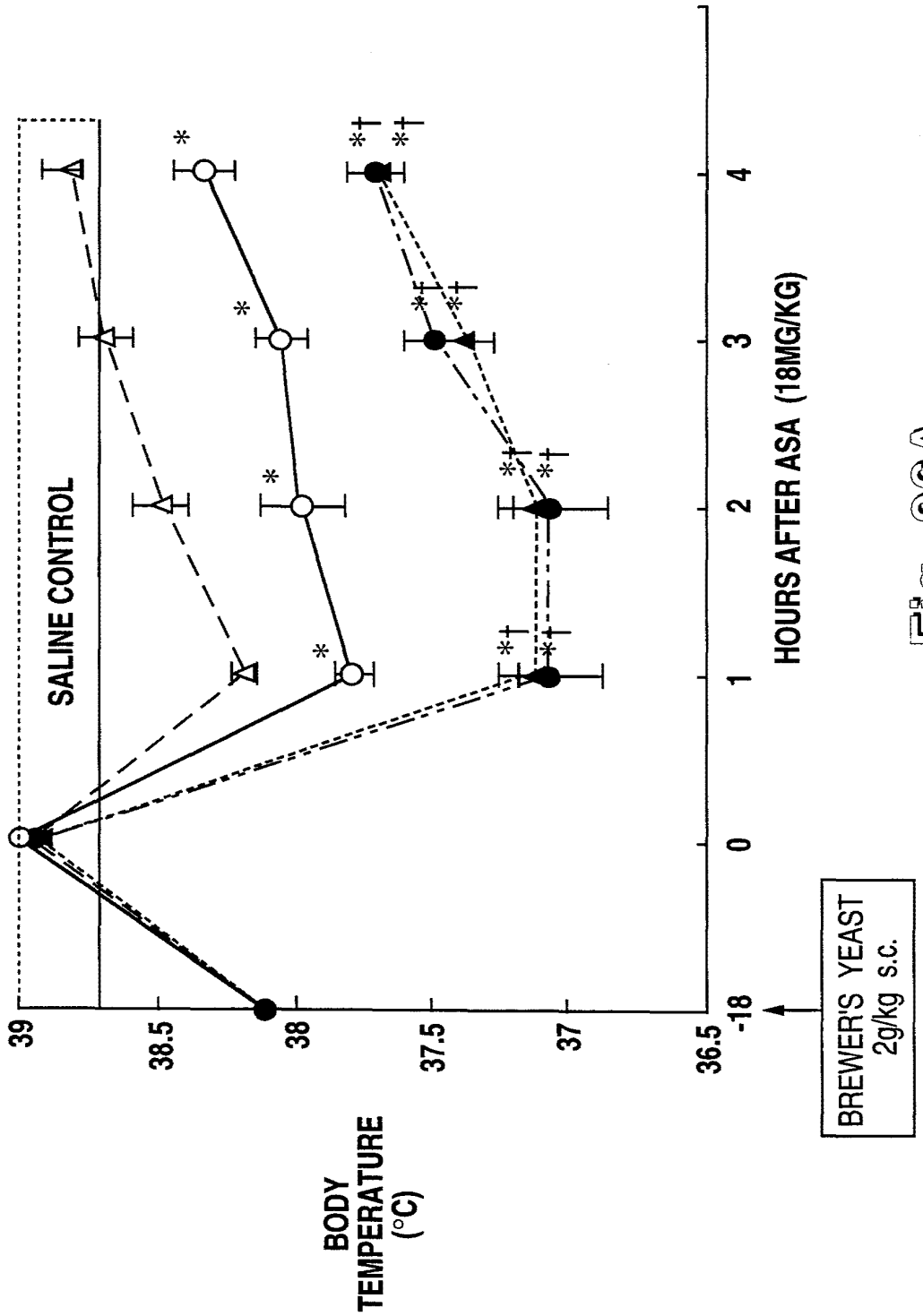

SALINE CONTROL
BODY TEMPERATURE (°C)
39
38.5
38
37.5
37
36.5
-18
0
1
2
3
4
HOURS AFTER ASA (18MG/KG)
BREWER'S YEAST 2g/kg s.c.

Fig. 26A

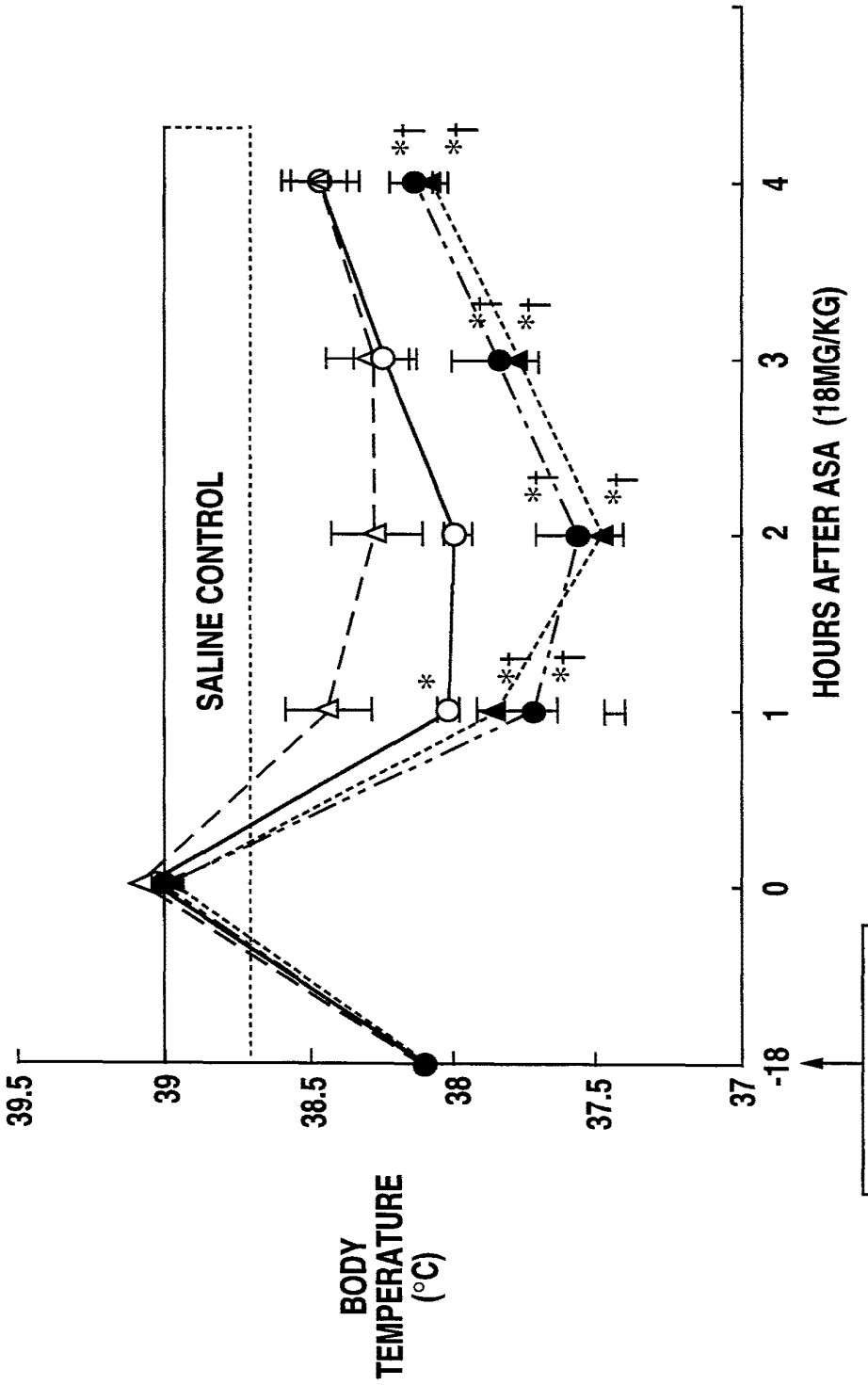

BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
37
SALINE CONTROL
-18
0
1
2
3
4
HOURS AFTER ASA (18MG/KG)
BREWER'S YEAST 2g/kg s.c.

Fig. 26B

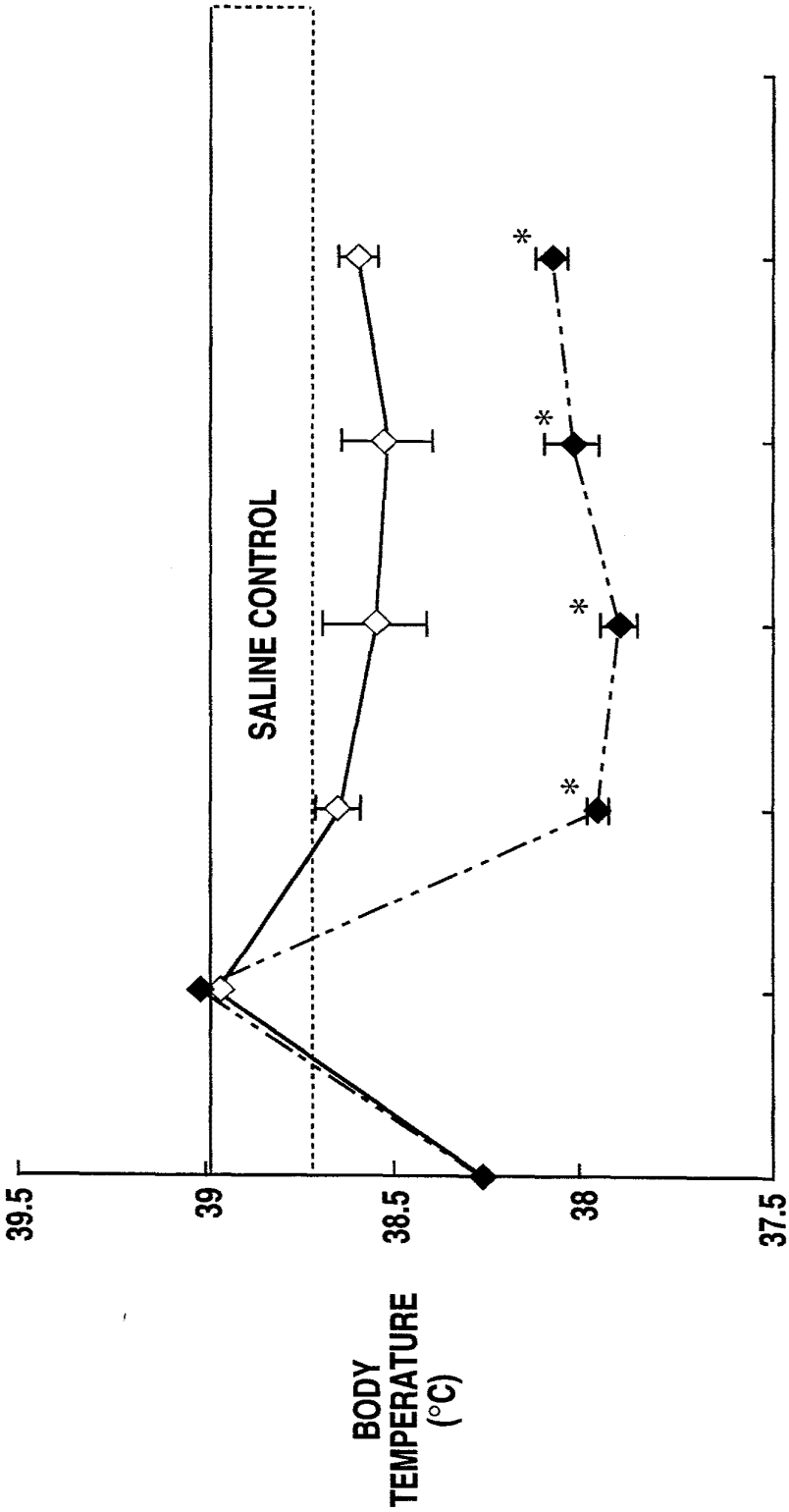

SALINE CONTROL
BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
-18
0
1
2
3
4
HOURS AFTER ASA (18MG/KG)
BREWER'S YEAST 2g/kg s.c.
*

Fig. 26C

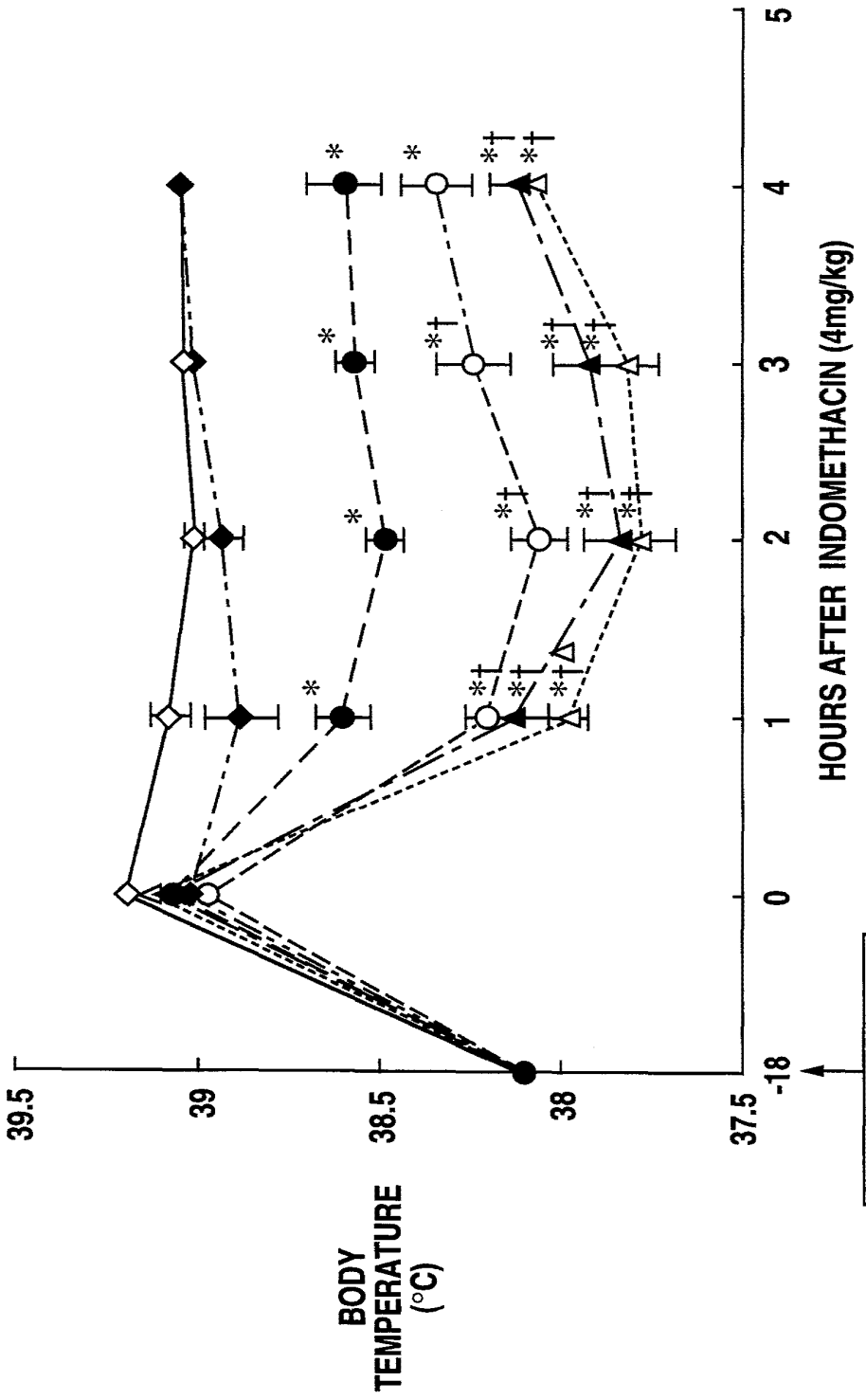

BODY TEMPERATURE (°C)
39.5
39
38.5
38
37.5
-18
0
1
2
3
4
5
HOURS AFTER INDOMETHACIN (4mg/kg)
BREWER'S YEAST 2g/kg s.c.

Fig. 27

FIG. 28 **A. Aspirin (ASA)**

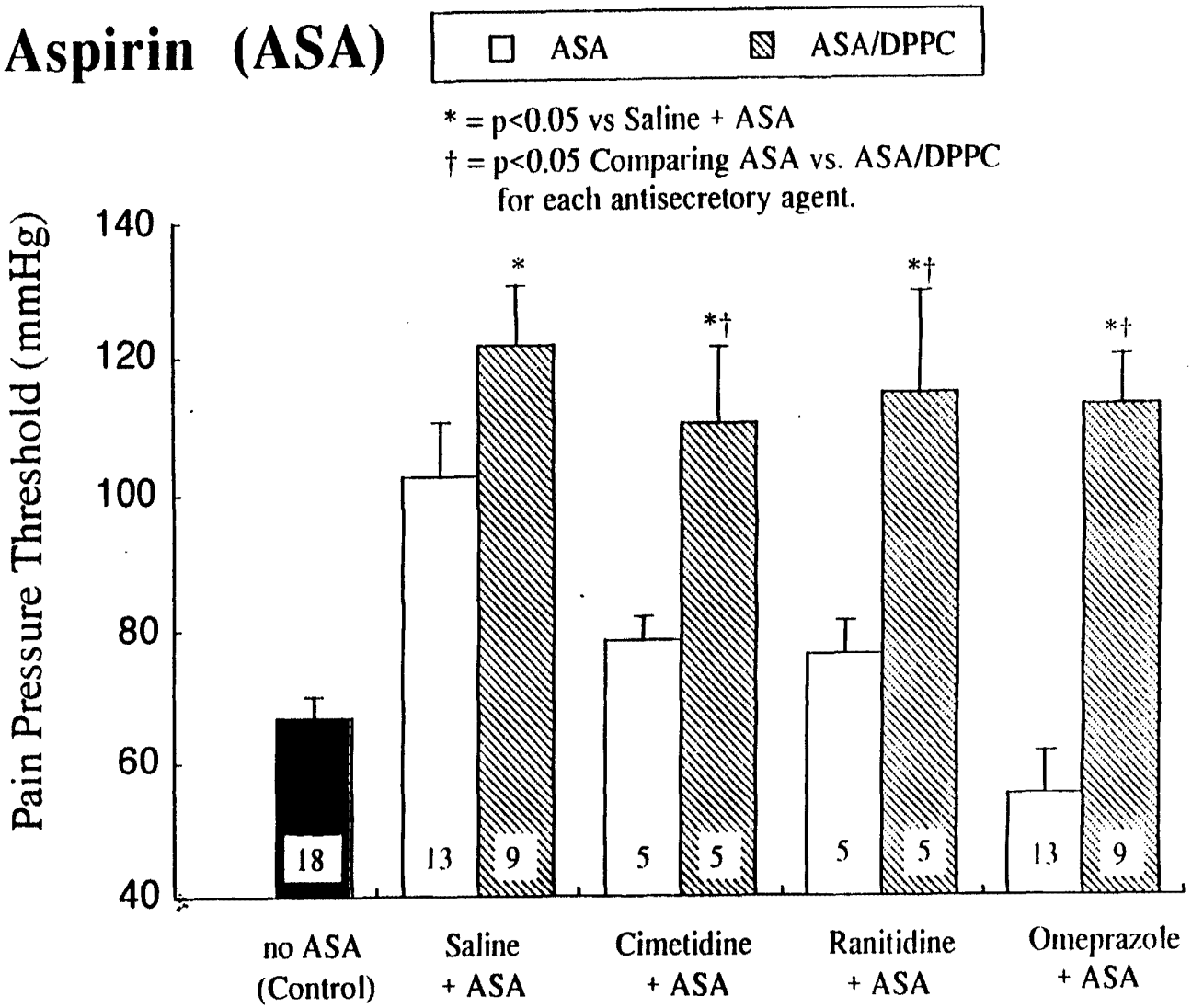
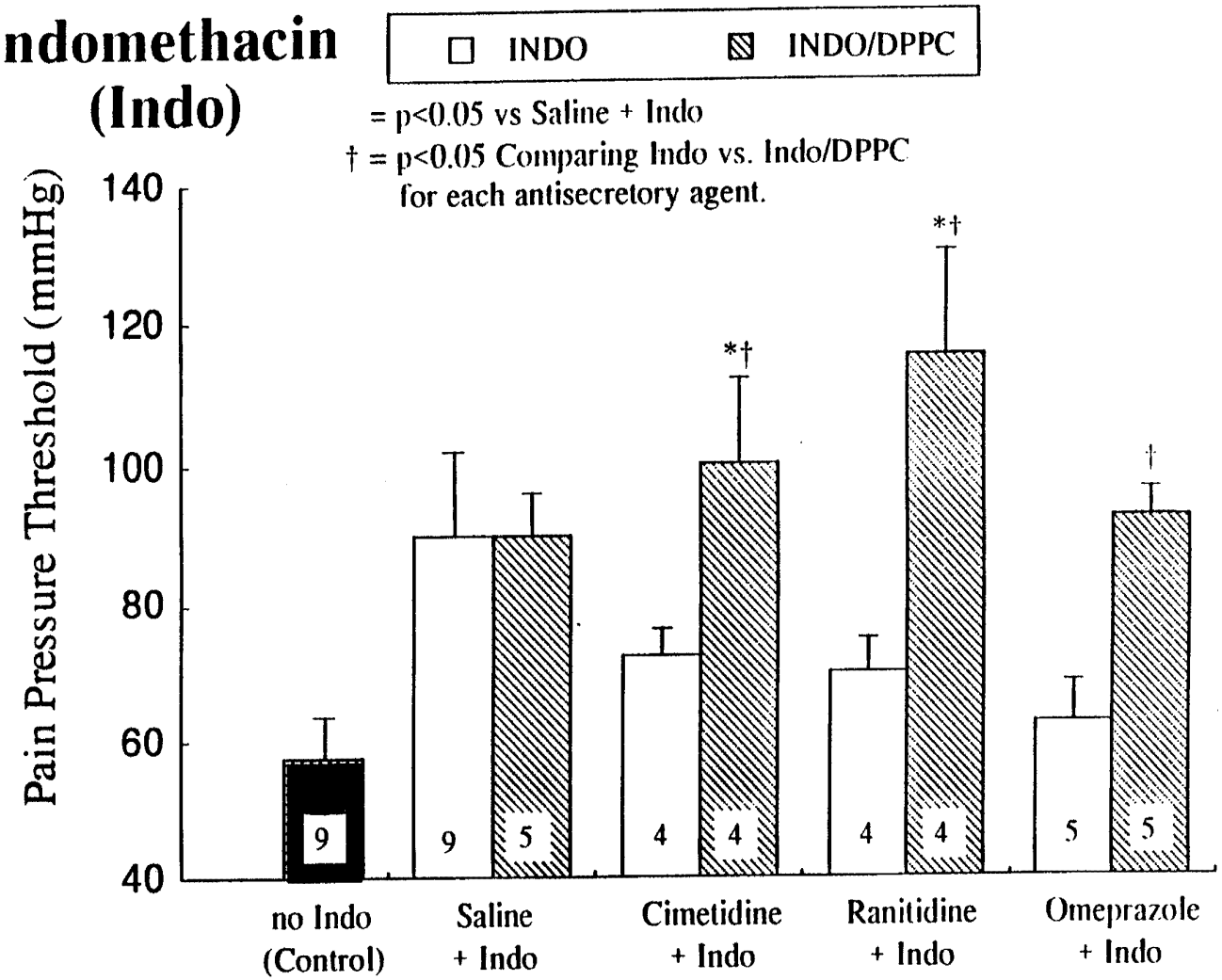


FIG 28 **B. Indomethacin (Indo)**



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A. CLASSIFICATION OF SUBJECT MATTER

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B. FIELDS SEARCHED

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,918,063 A (LICHTENBERGER) 04 April 1990, see entire document.	1-12
Y	Database Chemical Abstracts on STN, AN 1989:107568, Liversidge et al, 'Influence of indomethacin amphoteric gel on gastric ulcerogenicity and absorption of indomethacin in rats,' Pharm. Res. (1989), 6(1), pp. 44-8. See entire abstract.	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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(57) Abstract

The present invention relates to methods and compositions for treating a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising bismuth, a gastropathic amount of a non–steroidal anti–inflammatory drug, and a therapeutically effective amount of each of one or more antimicrobials. The inventions may further comprise therapeutically effective amounts of one or more anti–secretory drugs.

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COMPOSITIONS FOR THE TREATMENT OF GASTROINTESTINAL DISORDERS CONTAINING BISMUTH, AND NSAID AND ONE OR MORE ANTIMICROBIALS

BACKGROUND OF THE INVENTION

Upper abdominal pain and other gastrointestinal disorders are common and chronic problems for a vast number of the population. Of the individuals examined and diagnosed by their physicians, many can be shown to have diseases such as peptic or other ulcers, or non-ulcer dyspepsia. Until the mid 1980s, these conditions were thought to be caused by stress, diet or other environmental factors. Research now indicates that *Helicobacter pylori*, (hereinafter referred to as "*H. pylori*") a bacterium found exclusively in the gastric mucus of humans, plays a major role in the pathogenesis of these diseases and other gastrointestinal disorders.

Various methods and agents have been used to treat and/or eradicate gastrointestinal disorders caused by *H. pylori*. These include the administration of antacids, H_2 antagonists, and antimicrobials such as antibiotics. U.S. Patent No. 5,256,684 to Marshall, issued October 26, 1993 discloses a method for treating an infectious upper gastrointestinal tract disorder resulting from *Campylobacter pyloridis* comprising the administration of bismuth and an antimicrobial. U.S. Patent No. 5,476,669 to Borody, issued December 19, 1995 discloses a method for preventing the recurrence of duodental ucler associated with *Campylobacter pylori* infection comprising the administration of bismuth, metronidazole, and either tetracycline or penicillins.

In addition, speculation on the benefits of other methods for treating *H. pylori* is also available in the art. An example of such is found in Tanaka, S., et al., "Gastroprotective Effect of Ranitidine Bismuth Citrate Is Associated With Increased Mucus Bismuth Concentration In Rats", Gut, 39:164-171 (1996). However, given the prevalence and incidence of infection with *H. pylori,* and the difficulty in treating many patients suffering from such gastrointestinal disorders caused or mediated by *H. pylori*, a continuing need exists for safe and effective treatments against *H. pylori*, preferably which would be effective as mass treatment therapies in large populations of *H. pylori* carriers.

Compositions and methods have been discovered by the present invention for the treatment of gastrointestinal disorders caused or mediated by *H. pylori* comprising the administration of bismuth salts, (other than salts formed between an H_2 receptor antagonist and a complex of bismuth with a carboxylic acid), a non-steroidal anti-inflammatory drug, and one or more antimicrobials. The present invention also comprises the optional administration of one or more antisecretory agents. It is believed that the administration of bismuth with a non-steroidal anti-inflammatory drug enhances gastric mucus bismuth concentrations. Thus, an object of the present invention is to provide safe and effective compositions and methods of treating gastrointestinal disorders caused or mediated by *H. pylori*.

SUMMARY OF THE INVENTION

The present invention relates to a composition for treating a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising from about 50 milligrams to about 5000 milligrams, per day, of bismuth; a gastropathic amount of a non-steroidal anti-inflammatory drug; a therapeutically effective amount of each of one or more antimicrobials; and pharmaceutically acceptable carriers.

The present invention also relates to a method for treatment of a human or lower animal subject having a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising administering to the subject from about 50 milligrams to about 5000 milligrams of bismuth, per day, for from about 1 to about 42 days, a gastropathic amount of a non-steroidal anti-inflammatory drug for up to 14 days, and a therapeutically effective amount of each of one or more antimicrobials for from about 1 to about 21 days.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods and compositions for treating a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising bismuth, a non-steroidal anti-inflammatory drug and one or more antimicrobials. The inventions may optionally comprise therapeutically effective amounts of one or more antisecretory agents. The compositions also comprise pharmaceutically acceptable carreiers. The present invention and the essential and optional components therein are described fully below.

Helicobacter pylori

H. pylori, are spiral bacteria which reside in the stomach. When first identified in the early 1980s, *H. pylori* was referred to by the name *Campylobacter pyloridis*. In recent years, these bacteria have been implicated as a causative factor for gastritis, non-ulcerative dyspepsia, and various ulcers of the gastrointestinal tract. These organisms are described in detail in the following publications, all of which are incorporated herein

by reference in their entireties: Korman, M.G., Tygat, G.N., "*Helicobacter pylori* and Peptic Ulcer", Scandinavian Journal of Gastroenterology, Suppl., 210:92-96 (1995); Marshall, B. J., "*Helicobacter pylori*", American Journal of Gastroenterology, 89(8 Suppl):S116-128 (Aug. 1994); Calam, J., "*Helicobacter pylori*", European Clinical Investigation, 24(8):501-510 (Aug. 1994); NIH Consensus Conference, "*Helicobacter pylori* in Peptic Ulcer Disease. NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease", JAMA, 272(1):65-69 (July 6, 1994); and Marshall, B. J., Warren, J. R., "Unidentified Curved Bacilli in the Stomach of Patients with Gastritis and Peptic Ulceration", The Lancet, 1311-1315 (1984).

Gastrointestinal Disorder

The term "gastrointestinal disorder", as used herein, encompasses any infection, disease or other disorder of the body, typically of the upper and/or lower gastrointestinal tract, caused or mediated by *H. pylori*. An individual having such a gastrointestinal disorder may be symptomatic or asymptomatic. Such disorders include, for example, *H. pylori* disorders not manifested by the presence of ulcerations in the gastric mucosa, including chronic active or atrophic gastritis, non-ulcer dyspepsia, esophageal reflux disease and gastric motility disorders; and peptic ulcer disease, i.e., *H. pylori*-mediated pre-pyloric, marginal, gastric, duodenal and/or jejunal ulcers.

In the present invention, the presence of a gastrointestinal disorder caused or mediated by *H. pylori* is preferably determined by any of the diagnostic methods recognized and utilized by the medical community. Details concerning such methods are described more fully in the following publications, all of which are incorporated herein by reference in their entireties: Megraud, F., "Diagnosis of *Helicobacter pylori* Infection", Scandinavian Journal of Gastroenterology, Supplement, 214: 44-46, 57-60 (1996); Cutler, A. F., "Testing for *Helicobacter pylori* In Clinical Practice", American Journal of Medicine, 100(5A): 35S-39S, 39S-41S (May 20, 1996); Megraud, F., "Diagnosis of *Helicobacter pylori*", Baillieres Clinical Gastroenterology, 9(3): 507-518 (Sept. 1995); and Feldman, R. A., et al., "Accuracy of Diagnostic Methods Used for Epidemiological Studies of *Helicobacter pylori*", Alimentary Pharmacology and Therapeutics, 9 Suppl. 2:21-31 (1995).

Bismuth

The present invention involves administration of bismuth. As used herein, the quantity of bismuth is by weight of elemental bismuth.

In the present inventions, bismuth may be in the form of a pharmaceutically-acceptable salt, or may be in the form of an organic complex which contains bismuth as an active ingredient. Such organic complexes include 2,2'-spirobi[1,3,2-benzodoxabismole]. Salts formed between an H_2 receptor antagonist and a complex of

bismuth with a carboxylic acid are not included for use in the present inventions. Preferably, bismuth is administered in the present methods as a pharmaceutically-acceptable salt. Such bismuth salts include bismuth aluminate, bismuth subcarbonate, bismuth subcitrate, bismuth citrate, tripotassium dicitrato bismuthate, bismuth subgallate, bismuth subnitrate, bismuth tartrate, bismuth subsalicylate, and mixtures thereof. Bismuth citrate, bismuth subcitrate, tripotassium dicitrato bismuthate, bismuth tartrate, bismuth subsalicylate, and mixtures thereof are preferred bismuth salts for use in this invention.

The bismuth useful herein may be administered alone, or in combination with other pharmaceutically-acceptable components in a bismuth-containing composition. A variety of such compositions containing bismuth salts are commercially available. Such compositions include DeNol, containing tripotassium dicitrato bismuthate (by Brocades); Bislumina, containing bismuth aluminate (by Mazuelos); Roter, containing bismuth subnitrate (by Roterpharma); Devrom®, containing bismuth subgallate (by The Parthenon Co., Inc.); and Pepto-Bismol®, containing bismuth subsalicylate (by The Procter & Gamble Company).

In general, bismuth may be administered in an amount of from about 50 milligrams to about 5000 milligrams per day, and preferably from about 50 milligrams to about 2500 milligrams, per day, for from about 1 to about 42 days, preferably for up to about 28 days, and most preferably for up to about 14 days.

Non-Steroidal Anti-Inflammatory Drugs

The term "NSAID", as used herein, refers to any agent which has anti-inflammatory, antipyretic and analgesic properties. Examples of NSAIDs are fully described in U.S. Patent 4,985,459 to Sunshine et al., issued January 15, 1991, incorporated by reference herein in its entirety. For detailed disclosure of the chemical structure, synthesis, side effects, etc. of non-steroidal anti-inflammatory agents, references may be had to standard texts, including Anti-Inflammatory and Anti-Rheumatic Drugs, K. D. Rainsford, Vol. I-III, CRC Press, Boca Raton (1985), and Anti-Inflammatory Agents, Chemistry and Pharmacology, 1 R. A. Scherrer, et al., Academic Press, New York (1974), both of which are incorporated by reference herein.

Specific NSAIDs useful in the present invention include, but are not limited to: the oxicams, such as piroxicam, isoxicam, tenoxicam, sudoxicam, and CP-14,304; the salicylates, such as acetylsalicylic acid, disalcid, benorylate, trilisate, safapryn, solprin, diflunisal, and fendosal; the acetic acid derivatives, such as diclofenac, fenclofenac, indomethacin, sulindac, tolmetin, isoxepac, furofenac, tiopinac, zidometacin, acematacin, fentiazac, zomepiract, clidanac, oxepinac, and felbinac; the fenamates, such as mefenamic, meclofenamic, flufenamic, niflumic, and tolfenamic acids; the propionic

acid derivatives, such as ibuprofen, naproxen, benoxaprofen, flurbiprofen, ketoprofen, fenoprofen, fenbufen, indoprofen, pirprofen, carprofen, oxaprozin, pranoprofen, miroprofen, tioxaprofen, suprofen, alminoprofen, and tiaprofenic; and the pyrazoles, such as phenybutazone, oxyphenbutazone, feprazone, azapropazone, and trimethazone. Mixtures of these NSAIDs may also be employed, as well as the pharmaceutically-acceptable salts and esters of these agents.

Another class of NSAIDs are disclosed in U.S. Patent No. 4,708,966, Loomans, et al., issued November 24, 1987. This patent discloses a class of non-steroidal anti-inflammatory compounds which comprise specifically substituted phenyl compounds, especially substituted 2.6-di-tert-butyl phenol derivatives. For example, compounds selected from 4-(4'-pentyn-3'-one)-2,6-di-t-butylphenol; 4-(5'-hexynoyl)-2,6-di-t-butylphenol; 4-((S)-(-)-3'-methyl-5'-hexynoyl)-2,6-di-t-butyl-phenol; 4-((R)-(+)-3'-methyl-5'-hexynoyl)-2,6-di-t-butylphenol; and 4-(3',3'-dimethoxypropionyl)-2,6-di-t-butylphenol are useful in the present invention.

Examples of preferred NSAIDs useful in the present invention include, but are not limited to: acetylsalicylic acid, ibuprofen, fenbuprofen, fenoprofen, flurbiprofen, indomethacin, ketoprofen, naproxen, their pharmaceutically-acceptable salts, enantiomers thereof, and mixtures thereof. Ibuprofen, indomethacin, acetylsalicylic acid, and naproxen are especially preferred for use in the present invention.

NSAIDs are administered in a gastropathic amount. The term "gastropathic amount", as used herein, refers to a level and frequency of administration of NSAID which is sufficient to produce gastropathy, e.g. mucosal damage as judged by fiberoptic endoscopy, in normal subjects after a one week course of therapy. Such an amount will vary depending on the particular NSAID being administered, the size and/or condition of the subject receiving treatment and/or other medical factors determined by the administering physician. The gastropathic amounts for specific NSAIDs are known in the art. For example, acetylsalicylic acid administered at a levels of about 2.4 to 3.9 grams per day for one week will consistently produce mucosal injury without causing complications. Gastropathic amounts for other NSAIDs are levels which produce comparable gastropathy to the gastropathy produced by the acetylsalicylic acid levels disclosed herein.

The following publications provide greater detail on gastropathy and NSAIDs, and are incorporated herein by reference in their entireties: Heigh, R. I., "Use of NSAIDs. An Assault on the Upper Gastrointestinal Tract", Postgraduate Medicine, 96(6):63-68 (Nov. 1, 1996); Levi, S., et al., "Non-Steroidal Anti-Inflammatory Drugs: How Do They Damage the Gut?", British Journal of Rheumatology, 33(7):605-612

(July 1994); and Bower, P. R., "Non-Steroidal Anti-Inflammatory Drugs", British Journal of Rheumatology, 32 Suppl. 4:35-38 (June 1993).

In the present invention, the duration of NSAID administration is for up to about 14 days, and preferably for from 1 about to about 10 days. The duration of administration should be less than that associated with the development of complications. Therefore, the most preferred duration of administration of the NSAID is from about 1 to about 7 days. In addition to the publications mentioned in the preceding paragraph, complications associated with NSAID usage are discussed in Fenn, G. C., "Review Article: Controversies in NSAID-induced Gastroduodenal Damage--Do They Matter?", Alimentary Pharmacology and Therapeutics, 8(1):15-26 (Feb. 1994), incorporated herein by reference in its entirety.

Antimicrobial

The present inventions also include administration of a theraputically effective amount of each of one or more antimicrobials, per day. As used herein, the term "antimicrobial" refers to one or more antimicrobial agents, other than and in addition to bismuth, which are effective against *H. pylori*. The term "therapeutically effective amount", as used herein, refers to a level which is commonly known in the art and recognized and utilized by the medical community.

Typically, according to the present invention, each of the one or more antimicrobials is administered at a level of from about 100 milligrams to about 10,000 milligrams, per day, for from about 1 to about 28 days. Preferably, each of the one or more antimicrobials is administered at a level of from about 100 milligrams to about 8000 milligrams per day, and more preferably at from about 100 milligrams to about 5000 milligrams per day. It is also preferred that each of the antimicrobials is administered for from about 1 to about 21 days, more preferably for from about 1 to about 14 days, and most preferably for from about 7 to about 10 days.

The specific dosage of antimicrobial(s) to be administered, as well as the duration of antimicrobial(s) treatment, are mutually dependent, and will also depend upon such factors as the specific antimicrobial used, the number of antimicrobials used in the treatment, the resistance pattern of the infecting organism to the antimicrobial used, the ability of the antimicrobial to reach minimum inhibitory concentrations at the site of the infection, the nature and extent of other infections (if any), the personal attributes of the subject, compliance with the treatment regimen, and the presence and severity of any side effects of the treatment. Therefore, in the case of prevention or treatment with more than one antimicrobial, the duration of administration should depend on the type of antimicrobial rather than the administration of the antimicrobials for the same number of days.

A wide variety of antimicrobials are useful in this invention. As used herein, the term "antimicrobial" refers to any naturally-occurring, synthetic or semi-synthetic compound or composition or mixture thereof, which is safe for human use as used in the methods of this invention, and is effective in killing or substantially inhibiting *H. pylori* when used according to the present inventions. Antibiotics are preferred for use herein.

Antibiotics can be generally classified by chemical composition, into the following principal groups: the aminoglycosides, such as gentamicin, neomycin, kanamycin, and streptomycin; the macrolides, such as erythromycin, clindamycin, and rifampin; the penicillins, such as penicillin G, penicillin V, ampicillin and amoxycillin; the polypeptides such as bacitracin and polymyxin; the tetracyclines such as tetracycline, chlortetracycline, oxytetracycline and doxycycline; the cephalosporins such as cephalexin and cephalothin; quinolones such as ciprofloxacin, norfloxacin and ofloxacin; and such miscellaneous antibiotics as chloramphenicol and clindamycin. These antibiotics can generally be said to function in one of four ways: inhibition of cell wall synthesis, alteration of cell wall permeability, inhibition of protein synthesis or inhibition of nucleic acid synthesis.

Other antimicrobials useful herein include the sulfonamides; nitrofurans, such nitrofurazon, nitrofurantoin, and furozolidone; metronidazole, tinidazole, and nimorazole. Antimicrobials among those useful herein are described in Remington's Pharmaceutical Sciences, 18th Edition, pp. 1173-1232 (1990), which is incorporated herein by reference.

While any of these antimicrobials may be used, penicillin, erythromycin, metronidazole, doxycycline, tinidazole, amoxycillin, ampicillin, tetracycline, nitrofurantoin, and mixtures thereof are among the preferred antimicrobials for use in the present invention.

As stated above, the specific preferred quantity of antimicrobial and duration of treatment used in the methods of this invention will, in addition to other factors, depend upon the particular antimicrobial used and its pharmacology. In general, though, the tetracyclines are preferably administered at a level of from about 100 milligrams to about 2,000 milligrams per day. Macrolides (such as erythromycin) are preferably administered at a level of from about 1,000 milligrams to about 4,000 milligrams per day. Penicillins are preferably administered at a level of from about 500 milligrams to about 3,000 milligrams per day. The aminoglycosides (such as neomycin) are preferably administered at a level of from about 100 milligrams to about 8,000 milligrams per day. Nitrofurans (such as nitrofurantoin) are administered preferably at levels of from about 100 milligrams to about 800 milligrams per day. Preferably,

metronidazole is administered at a level of from about 500 to about 2,000 milligrams per day.

The specific method of administering the antimicrobial, according to the processes of this invention, may depend upon such factors as the particular antimicrobial(s) used, the site of infection, the amount of antimicrobial(s) to be administered per day, the presence of any adverse side effects, and the interactions (if any) between the antimicrobial(s) and the bismuth. Thus, the antimicrobial(s) may be administered under the process of this invention by single daily doses, or by administration in two, three, four, or more doses per day.

Antisecretory Agents

The present invention can optionally include one or more antisecretory agents. The term "antisecretory agent", as used herein, refers to agents selected from the group consisting of H_2 receptor antagonists, proton pump inhibitors, and mixtures thereof. These agents are administered in a therapeutically effective amount. The term "therapeutically effective amount", as used herein, refers to a level which is commonly known in the art and recognized and utilized by the medical community. Such an amount will vary depending on the particular agent(s) administered, the size and/or condition of the individual receiving treatment or other medical factors determined by the administering physician.

H_2 receptor antagonists are disclosed fully in U.S. Patent No. 5,294,433 to Singer et al., issued March 15, 1994, incorporated herein by reference in its entirety. Preferred H_2 receptor antagonists include cimetidine, etintidine, ranitidine, ICIA-5165, tiotidine, ORF-17578, luptidine, donetidine, famotidine, rozatidine, pifatidine, lamtidine, BL-6548, BMY-25271, zaltidine, nizatidine, mifentidine, BMY-52368,SKF-94482, BL-6341A, ICI-162846, ramixotidine, Wy-45727, SR-58042, BMY-25405, loxidine, DA-4634, bisfentidine, sufotidine, ebrotidine, HE-30-256,D-16637, FRG-8813, FRG-8701, impromidine, L-643728, HB-4-08, and mixtures thereof..

Preferred for use in the present invention are cimetidine, ranitidine, famotidine, roxatidine, nizatidine, mifentidine, and mixtures thereof. Most preferred are cimetidine and ranitidine.

Proton pump inhibitors are described in greater detail in the following publications, which are incorporated by reference herein in their entireties: U.S. Patent No. 4,786,505 to Lovgren, issued November 22, 1988; U. S. Patent No. 4,255,431 to Junggren, issued March 10, 1981; and U.S. Patent No. 4,853,230 to Lovgren, issued August 1, 1989. Preferred for use in the present invention are omeprazole, lansoprazole, pantoprazole, and mixtures thereof. Most preferred is omeprazole.

Antisecretory agents may be administered for from about 1 to about 42 days, preferably for up to about 28 days, and most preferably for up to about 14 days.

Pharmaceutically Acceptable Carriers

The compositions of the present invention may contain optional components which affect the physical and therapeutic characteristics of the present compositions. In particular, a variety of pharmaceutically-acceptable carriers and excipients may be included, depending upon the particular dosage form to be used. Various oral dosage forms can be used, including such solid forms as tablets, capsules, granules and bulk powders. Tablets can be compressed, tablet triturates, enteric-coated, sugar coated, film-coated or multiple compressed, containing suitable binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents and melting agents. Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions, and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules, containing suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, melting agents, coloring, and flavoring agents.

Specific examples of pharmaceutically-acceptable carriers and excipients that may be used to formulate oral dosage forms of the present invention are described in U. S. Patent 3,903,297, Robert, issued September 2, 1975, incorporated by reference herein. Techniques and compositions for making dosage forms useful herein are described in the following references, all incorporated by reference herein: 7 Modern Pharmaceutics, Chapters 9 and 10 (Banker and Rhodes, editors, 1979); an Lieberman, et al., Pharmaceutical Dosage Forms: *Tablets* (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms (2d edition, 1976).

The compositions of this invention may be used according to the methods of this invention by administering the composition from 1 to 4 times per day, and preferably from 1 to 2 times per day; for from 1 to 28 days, preferably for from about 1 to about 21 days, and most preferably for from about 1 to about 14 days. The specific frequency of administration will depend upon such factors as the specific NSAID, bismuth compound or composition and antimicrobial(s) used, the levels at which the components are incorporated in the composition, the nature and severity of the condition to be treated, and the nature of any concurrent therapy, if any.

Method of Use

The methods of the present invention comprise the treatment of a human or lower animal subject having a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising administering to the subject bismuth, a non-steroidal

anti-inflammatory drug, and one or more antimicrobials. The present method may further comprise the administration of one or more antisecretory agents.

As used herein, the term "administering" refers to any method which, in sound medical practice delivers the compounds or compositions used in this invention to the subject to be treated in such a manner so as to be effective in the treatment of the gastrointestinal disorder. Preferably, the bismuth, NSAID, antimicrobial(s) and antisecretory agent(s), if present, are administered orally.

The present invention encompasses methods wherein the administering of bismuth, the NSAID, the antimicrobial(s) and optionally the antisecretory agent(s) are performed simultaneously (beginning and ending on the same day), concurrently (overlapping but not of the same duration of administration), or consecutively (sequential, but where the course of treatment is substantially continuous). Preferably, the bismuth, NSAID and antimicrobial are administered concurrently and administration for bismuth, the NSAID and the antimicrobial is commenced on the same day. Additionally, if one or more antisecretory agents are also present, it is preferred that the bismuth and the antisecretory agent(s) are administered simultaneously.

The following non-limiting examples illustrate the composition and methods of use of the present invention.

EXAMPLE I

An asymptomatic young volunteer identified as having *H. pylori* infection through the results of a mass screening, is treated by a method of the present invention. The subject is orally administered approximately 2500 milligrams of bismuth in the form of bismuth subcitrate ("DeNol", sold by Brocades) in four equal doses, for 28 days; approximately 100-200 milligrams of indomethacin daily, in four equal doses, for about 14 days; and approximately 1 gram of erythromycin daily, in two equal doses, for about 14 days. One to two months later, a diagnostic test performed on the volunteer shows no evidence of *H. pylori*.

In the above example, tripotassium dicitrato bismuthate, bismuth tartrate, bismuth citrate, and bismuth subnitrate are substituted, respectively, for bismuth subsalicylate, with substantially similar results.

EXAMPLE II

A human subject is suffering from chronic active gastritis. A diagnostic test reveals the presence of *H. pylori*. The individual is treated by orally administering approximately 2100 milligrams of bismuth daily, in the form of bismuth subsalicylate, ("Pepto-Bismol®", sold by The Procter & Gamble Company), in four equal doses, for about 14 days; approximately 3.9 grams of acetylsalicylic acid daily, in three equal doses, for about 14 days; approximately 20 milligrams of omeprazole daily, for 14 days;

approximately 1000 milligrams of metronidazole daily, in four equal doses, for 14 days; and approximately 2000 milligrams of tetracycline daily in four equal doses, for 14 days. Administration of all agents are commenced on the same day. One to two months later, the diagnostic test is repeated. The results show no evidence of *H. pylori*.

EXAMPLE III

A human subject is suffering from non-ulcer dyspepsia. A biopsy of the gastric mucosa is taken from the stomach of the subject. Analysis of the biopsy sample indicates the presence of urease in the sample and the presence of *H. pylori* in the stomach of the subject. The subject is given approximately 1200 milligrams of bismuth daily, (administered as bismuth subsalicylate in the composition Pepto-Bismol®, sold by The Procter & Gamble Company), in four equal doses, for about 21 days; 1200-3200 milligrams of ibuprofen daily, in three to four equal doses, for about 7 days; 150 milligrams of ranitidine daily, in two equal doses, for about 21 days; and 500 milligrams of metronidazole daily, in four equal doses, for about 14 days. Administration of all agents are commenced on the same day. A biopsy sample taken and analyzed one to two months later shows no evidence of *H. pylori*.

WHAT IS CLAIMED IS:

1. A composition for treating a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising:
   a) from 50 milligrams to 5000 milligrams, per day, of bismuth;
   b) a gastropathic amount of a non-steroidal anti-inflammatory drug;
   c) a therapeutically effective amount of each of one or more antimicrobials; and
   d) pharmaceutically acceptable carriers.

2. The composition of Claim 1 further comprising a therapeutically effective amount of one or more antisecretory agents selected from the group consisting of H_2 receptor antagonists, proton pump inhibitors and mixtures thereof.

3. The composition of Claim 1 or 2 wherein the antisecretory agents are selected from the group consisting of cimetidine, ranitidine, famotidine, roxatidine, nizatidine, mifentidine, omeprazole, lansoprazole, pantoprazole, and mixtures thereof.

4 The composition of any of Claims 1-3 wherein the bismuth is selected from the group consisting of bismuth aluminate, bismuth subcarbonate, bismuth subcitrate, bismuth citrate, tripotassium dicitrato bismuthate, bismuth subgallate, bismuth subsalicylate, bismuth tartrate, and mixtures thereof and is administered at a level of from 50 milligrams to 2500 milligrams, per day for up to 28 days.

5. The composition of any of Claims 1-4 wherein the non-steroidal anti-inflammatory drug is selected from the group consisting of ibuprofen, indomethacin, acetylsalicylic acid, and naproxen and wherein it is administered for up to 14 days, the one or more antimicrobials are administered for 1 to 21 days, and the one or more antisecretory agents are administered for up to 28 days.

6. The composition of any of Claims 1-5 wherein the one or more antimicrobials are selected from the group consisting of penicillin, erythromycin, metronidazole, doxycycline, tinidazole, amoxycillin, ampicillin, tetracycline, nitrofurantoin, and mixtures thereof.

7. The use of the compositions of any of Claims 1-6 for the manufacture of a composition for treatment of a human or lower animal subject having a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising administering to the subject from 50 milligrams to 5000 milligrams of bismuth, per day, for from 1 to 42 days; a gastropathic amount of a non-steroidal anti-inflammatory drugs for up to 14 days; and a therapeutically effective amount of each of one or more antimicrobials for from 1 to 21 days.

8. The use of the compositions of any of Claims 1-7 for the manufacture of a composition comprising a therapeutically effective amount of one or more antisecretory agents which are selected from the group consisting of H_2 receptor antagonists, proton pump inhibitors and mixtures thereof.

9. The use of the compositions of any of Claims 1-8 for the manufacture of a composition wherein the antisecretory agents are selected from the group consisting of cimetidine, ranitidine, famotidine, roxatidine, nizatidine, mifentidine, omeprazole, lansoprazole, pantoprazole, and mixtures thereof and wherein the antisecretory agents are administered for up to 28 days and the one or more antimicrobials are administered for 1 to 14 days.

10. The use of the compositions of any of Claims 1-9 for the manufacture of a composition wherein the bismuth is selected from the group consisting of bismuth aluminate, bismuth subcarbonate, bismuth subcitrate, bismuth citrate, tripotassium dicitrato bismuthate, bismuth subgallate, bismuth subsalicylate, bismuth tartrate, and mixtures thereof and wherein the bismuth is administered at a level of from 50 milligrams to 2500 milligrams, per day for up to 28 days.

11. The use of the compositions of any of Claims 1-10 for the manufacture of a composition wherein the non-steroidal anti-inflammatory drug is selected from the group consisting of ibuprofen, indomethacin, acetylsalicylic acid, and naproxen and wherein the one or more antimicrobials are selected from the group consisting of penicillin, erythromycin, metronidazole, doxycycline, tinidazole, amoxycillin, ampicillin, tetracycline, nitrofurantoin, and mixtures thereof.

INTERNATIONAL SEARCH REPORT

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	EP 0 437 294 A (GIST BROCADES NV) 17 July 1991 see page 3, line 18-24	1-11
A	TANAKA S ET AL: "Indomethacin increases mucus bismuth concentration in ranitidine bismuth citrate treated rats" GASTROENTEROLOGY, 108 (4 SUPPL.). 1995. A234., XP002059838 see the whole document	1-11

☐ Further documents are listed in the continuation of box C.

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Information on patent family members

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(57) Abstract

The present invention relates to methods and compositions for treating a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising bismuth, and a gastropathic amount of a non–steroidal anti–inflammatory drug. The inventions may further comprise therapeutically effective amounts of one or more anti–secretory drugs.

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COMPOSITIONS FOR THE TREATMENT OF GASTROINTESTINAL DISORDERS CONTAINING BISMUTH AND NSAID

BACKGROUND OF THE INVENTION

Upper abdominal pain and other gastrointestinal disorders are common and chronic problems for a vast number of the population. Of the individuals examined and diagnosed by their physicians, many can be shown to have diseases such as peptic or other ulcers, or non-ulcer dyspepsia. Until the mid 1980s, these conditions were thought to be caused by stress, diet or other environmental factors. Research now indicates that *Helicobacter pylori*, (hereinafter referred to as "*H. pylori*) a bacterium found exclusively in the gastric mucus of humans, plays a major role in the pathogenesis of these diseases and other gastrointestinal disorders.

Various methods and agents have been used to treat and/or eradicate gastrointestinal disorders caused by *H. pylori*. These include the administration of antacids, H_2 antagonists, and antimicrobials such as antibiotics. In addition, speculation on the benefits of other methods for treating *H. pylori* is also available in the art. An example of such is found in Tanaka, S., et al., "Gastroprotective Effect of Ranitidine Bismuth Citrate Is Associated With Increased Mucus Bismuth Concentration In Rats", Gut, 39:164-171 (1996). However, given the prevalence and incidence of infection with *H. pylori,* the difficulty in treating many patients suffering from such gastrointestinal disorders caused or mediated by *H. pylori*, and the potential for resistance with antibiotic-containing regimens, a continuing need exists for safe and effective treatments against *H. pylori*, preferably which would be effective as mass treatment therapies in large populations of *H. pylori* carriers.

Compositions and methods have been discovered by the present invention for the treatment of gastrointestinal disorders caused or mediated by *H. pylori* comprising the administration of bismuth salts, (other than salts formed between an H_2 receptor antagonist and a complex of bismuth with a carboxylic acid), and a non-steroidal anti-inflammatory drug. The present invention also comprises the optional administration of one or more antisecretory agents. It is believed that the administration of bismuth with a non-steroidal anti-inflammatory drug enhances gastric mucus bismuth concentrations.

Thus, an object of the present invention is to provide safe and effective compositions and methods of treating gastrointestinal disorders caused or mediated by *H. pylori*.

SUMMARY OF THE INVENTION

The present invention relates to a composition for treating a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising from about 50 milligrams to about 5000 milligrams, per day, of bismuth; a gastropathic amount of a non-steroidal anti-inflammatory drug; and pharmaceutically acceptable carriers.

The present invention also relates to a method for treatment of a human or lower animal subject having a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising administering to the subject from about 50 milligrams to about 5000 milligrams of bismuth, per day, for from about 1 to about 42 days, and a gastropathic amount of a non-steroidal anti-inflammatory drug.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods and compositions comprising bismuth and a non-steroidal anti-inflammatory drug for treating a gastrointestinal disorder caused or mediated by *Helicobacter pylori*. The inventions may optionally comprise therapeutically effective amounts of one or more antisecretory agents. The compositions also comprise pharmaceutically acceptable carriers. The present invention and the essential and optional components therein are described fully below.

Helicobacter pylori

H. pylori are spiral bacteria which reside in the stomach. When first identified in the early 1980s, *H. pylori* was referred to by the name *Campylobacter pyloridis*. In recent years, these bacteria have been implicated as a causative factor for gastritis, non-ulcerative dyspepsia, and various ulcers of the gastrointestinal tract. These organisms are described in detail in the following publications, all of which are incorporated herein by reference in their entireties: Korman, M.G., Tygat, G.N., "*Helicobacter pylori* and Peptic Ulcer", Scandinavian Journal of Gastroenterology, Suppl., 210:92-96 (1995); Marshall, B. J., "*Helicobacter pylori*", American Journal of Gastroenterology, 89(8 Suppl):S116-128 (Aug. 1994); Calam, J., "*Helicobacter pylori*", European Clinical Investigation, 24(8):501-510 (Aug. 1994); NIH Consensus Conference, "*Helicobacter pylori* in Peptic Ulcer Disease. NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease", JAMA, 272(1):65-69 (July 6, 1994); and Marshall, B. J., Warren, J. R., "Unidentified Curved Bacilli in the Stomach of Patients with Gastritis and Peptic Ulceration", The Lancet, 1311-1315 (1984).

Gastrointestinal Disorder

The term "gastrointestinal disorder", as used herein, encompasses any infection, disease or other disorder of the body, typically of the upper and/or lower gastrointestinal tract, caused or mediated by *H. pylori*. An individual having such a gastrointestinal disorder may be symptomatic or asymptomatic. Such disorders include, for example, *H. pylori* disorders not manifested by the presence of ulcerations in the gastric mucosa, including chronic active or atrophic gastritis, non-ulcer dyspepsia, esophageal reflux disease and gastric motility disorders; and peptic ulcer disease, i.e., *H. pylori*-mediated pre-pyloric, marginal, gastric, duodenal and/or jejunal ulcers.

In the present invention, the presence of a gastrointestinal disorder caused or mediated by *H. pylori* is preferably determined by any of the diagnostic methods recognized and utilized by the medical community. Details concerning such methods are described more fully in the following publications, all of which are incorporated herein by reference in their entireties: Megraud, F., "Diagnosis of *Helicobacter pylori* Infection", Scandinavian Journal of Gastroenterology, Supplement, 214: 44-46, 57-60 (1996); Cutler, A. F., "Testing for *Helicobacter pylori* In Clinical Practice", American Journal of Medicine, 100(5A): 35S-39S, 39S-41S (May 20, 1996); Megraud, F., "Diagnosis of *Helicobacter pylori*", Baillieres Clinical Gastroenterology, 9(3): 507-518 (Sept. 1995); and Feldman, R. A., et al., "Accuracy of Diagnostic Methods Used for Epidemiological Studies of *Helicobacter pylori*", Alimentary Pharmacology and Therapeutics, 9 Suppl. 2:21-31 (1995).

Bismuth

The present invention involves administration of bismuth. As used herein, the quantity of bismuth is by weight of elemental bismuth.

In the present inventions, bismuth may be in the form of a pharmaceutically-acceptable salt, or may be in the form of an organic complex which contains bismuth as an active ingredient. Such organic complexes include 2,2'-spirobi[1,3,2-benzodoxabismole]. Salts formed between an H_2 receptor antagonist and a complex of bismuth with a carboxylic acid are not included for use in the present inventions. Preferably, bismuth is administered in the present methods as a pharmaceutically-acceptable salt. Such bismuth salts include bismuth aluminate, bismuth subcarbonate, bismuth subcitrate, bismuth citrate, tripotassium dicitrato bismuthate, bismuth subgallate, bismuth subnitrate, bismuth tartrate, bismuth subsalicylate, and mixtures thereof. Bismuth citrate, bismuth subcitrate, tripotassium dicitrato bismuthate, bismuth tartrate, bismuth subsalicylate, and mixtures thereof are preferred bismuth salts for use in this invention.

The bismuth useful herein may be administered alone, or in combination with other pharmaceutically-acceptable components in a bismuth-containing composition. A

variety of such compositions containing bismuth salts are commercially available. Such compositions include DeNol, containing tripotassium dicitrato bismuthate (by Brocades); Bislumina, containing bismuth aluminate (by Mazuelos); Roter, containing bismuth subnitrate (by Roterpharma); Devrom®, containing bismuth subgallate (by The Parthenon Co., Inc.); and Pepto-Bismol®, containing bismuth subsalicylate (by The Procter & Gamble Company).

In general, bismuth may be administered in an amount of from about 50 milligrams to about 5000 milligrams per day, and preferably from about 50 milligrams to about 2500 milligrams, per day, for from about 1 to about 42 days, preferably for up to about 28 days, and most preferably for up to about 14 days.

Non-Steroidal Anti-Inflammatory Drugs

The present invention comprises the administration of bismuth with a non-steroidal anti-inflammatory drug ("NSAID"). The term "NSAID", as used herein, refers to any agent which has anti-inflammatory, antipyretic and analgesic properties. Examples of NSAIDs are described in U.S. Patent 4,985,459 to Sunshine et al., issued January 15, 1991, incorporated by reference herein in its entirety. For detailed disclosure of the chemical structure, synthesis, side effects, etc. of non-steroidal anti-inflammatory agents, references may be had to standard texts, including Anti-Inflammatory and Anti-Rheumatic Drugs, K. D. Rainsford, Vol. I-III, CRC Press, Boca Raton (1985), and Anti-Inflammatory Agents, Chemistry and Pharmacology, 1 R. A. Scherrer, et al., Academic Press, New York (1974), both of which are incorporated by reference herein.

Specific NSAIDs useful in the present invention include, but are not limited to: the oxicams, such as piroxicam, isoxicam, tenoxicam, sudoxicam, and CP-14,304; the salicylates, such as acetylsalicylic acid, disalcid, benorylate, trilisate, safapryn, solprin, diflunisal, and fendosal; the acetic acid derivatives, such as diclofenac, fenclofenac, indomethacin, sulindac, tolmetin, isoxepac, furofenac, tiopinac, zidometacin, acematacin, fentiazac, zomepiract, clidanac, oxepinac, and felbinac; the fenamates, such as mefenamic, meclofenamic, flufenamic, niflumic, and tolfenamic acids; the propionic acid derivatives, such as ibuprofen, naproxen, benoxaprofen, flurbiprofen, ketoprofen, fenoprofen, fenbufen, indoprofen, pirprofen, carprofen, oxaprozin, pranoprofen, miroprofen, tioxaprofen, suprofen, alminoprofen, and tiaprofenic; and the pyrazoles, such as phenybutazone, oxyphenbutazone, feprazone, azapropazone, and trimethazone. Mixtures of these NSAIDs may also be employed, as well as the pharmaceutically-acceptable salts and esters of these agents.

Another class of NSAIDs are disclosed in U.S. Patent No. 4,708,966, Loomans, et al., issued November 24, 1987. This patent discloses a class of non-steroidal anti-

inflammatory compounds which comprise specifically substituted phenyl compounds, especially substituted 2.6-di-tert-butyl phenol derivatives. For example, compounds selected from 4-(4'-pentyn-3'-one)-2,6-di-t-butylphenol; 4-(5'-hexynoyl)-2,6-di-t-butylphenol; 4-((S)-(-)-3'-methyl-5'-hexynoyl)-2,6-di-t-butyl-phenol; 4-((R)-(+)-3'-methyl-5'-hexynoyl)-2,6-di-t-butylphenol; and 4-(3',3'-dimethoxypropionyl)-2,6-di-t-butylphenol are useful in the present invention.

Examples of preferred NSAIDs useful in the present invention include, but are not limited to: acetylsalicylic acid, ibuprofen, fenbuprofen, fenoprofen, flurbiprofen, indomethacin, ketoprofen, naproxen, their pharmaceutically-acceptable salts, enantiomers thereof, and mixtures thereof. Ibuprofen, indomethacin, acetylsalicylic acid, and naproxen are especially preferred for use in the present invention.

NSAIDs are administered in a gastropathic amount. The term "gastropathic amount", as used herein, refers to a level and frequency of administration of NSAID which is sufficient to produce gastropathy, e.g. mucosal damage as judged by fiberoptic endoscopy, in normal subjects after a one week course of therapy. Such an amount will vary depending on the particular NSAID being administered, the size and/or condition of the subject receiving treatment and/or other medical factors determined by the administering physician. The gastropathic amounts for specific NSAIDs are known in the art. For example, acetylsalicylic acid administered at a levels of about 2.4 to 3.9 grams per day for one week will consistently produce mucosal injury without causing complications. Gastropathic amounts for other NSAIDs are levels which produce comparable gastropathy to the gastropathy produced by the acetylsalicylic acid levels disclosed herein.

The following publications provide greater detail on gastropathy and NSAIDs, and are incorporated herein by reference in their entireties: Heigh, R. I., "Use of NSAIDs. An Assault on the Upper Gastrointestinal Tract", Postgraduate Medicine, 96(6):63-68 (Nov. 1, 1996); Levi, S., et al., "Non-Steroidal Anti-Inflammatory Drugs: How Do They Damage the Gut?", British Journal of Rheumatology, 33(7):605-612 (July 1994); and Bower, P. R., "Non-Steroidal Anti-Inflammatory Drugs", British Journal of Rheumatology, 32 Suppl. 4:35-38 (June 1993).

In the present invention, the duration of NSAID administration is for up to about 14 days, and preferably for from 1 about to about 10 days. The duration of administration should be less than that associated with the development of complications. Therefore, the most preferred duration of administration of the NSAID is from about 1 to about 7 days. In addition to the publications mentioned in the preceding paragraph, complications associated with NSAID usage are discussed in Fenn, G. C., "Review Article: Controversies in NSAID-induced Gastroduodenal

Damage--Do They Matter?", Alimentary Pharmacology and Therapeutics, 8(1):15-26 (Feb. 1994), incorporated herein by reference in its entirety.

Antisecretory Agents

The present invention can optionally include one or more antisecretory agents. The term "antisecretory agent", as used herein, refers to agents selected from the group consisting of H_2 receptor antagonists, proton pump inhibitors, and mixtures thereof. These agents are administered in a therapeutically effective amount. The term "therapeutically effective amount", as used herein, refers to a level which is commonly known in the art and recognized and utilized by the medical community. Such an amount will vary depending on the particular agent(s) administered, the size and/or condition of the individual receiving treatment or other medical factors determined by the administering physician.

H_2 receptor antagonists are disclosed fully in U.S. Patent No. 5,294,433 to Singer et al., issued March 15, 1994, incorporated herein by reference in its entirety. Preferred H_2 receptor antagonists include cimetidine, etintidine, ranitidine, ICIA-5165, tiotidine, ORF-17578, luptidine, donetidine, famotidine, rozatidine, pifatidine, lamtidine, BL-6548, BMY-25271, zaltidine, nizatidine, mifentidine, BMY-52368,SKF-94482, BL-6341A, ICI-162846, ramixotidine, Wy-45727, SR-58042, BMY-25405, loxidine, DA-4634, bisfentidine, sufotidine, ebrotidine, HE-30-256,D-16637, FRG-8813, FRG-8701, impromidine, L-643728, HB-4-08, and mixtures thereof.

Preferred for use in the present invention are cimetidine, ranitidine, famotidine, roxatidine, nizatidine, mifentidine, and mixtures thereof. Most preferred are cimetidine and ranitidine.

Proton pump inhibitors are described in greater detail in the following publications, which are incorporated by reference herein in their entireties: U.S. Patent No. 4,786,505 to Lovgren, issued November 22, 1988; U. S. Patent No. 4,255,431 to Junggren, issued March 10, 1981; and U.S. Patent No. 4,853,230 to Lovgren, issued August 1, 1989. Preferred for use in the present invention are omeprazole, lansoprazole, pantoprazole, and mixtures thereof. Most preferred is omeprazole.

Antisecretory agents may be administered for from about 1 to about 42 days, preferably for up to about 28 days, and most preferably for up to about 14 days.

Pharmaceutically Acceptable Carriers

The compositions of the present invention may contain optional components which affect the physical and therapeutic characteristics of the present compositions. In particular, a variety of pharmaceutically-acceptable carriers and excipients may be included, depending upon the particular dosage form to be used. Various oral dosage forms can be used, including such solid forms as tablets, capsules, granules and bulk

powders. Tablets can be compressed, tablet triturates, enteric-coated, sugar coated, film-coated or multiple compressed, containing suitable binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents and melting agents. Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions, and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules, containing suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, melting agents, coloring, and flavoring agents.

Specific examples of pharmaceutically-acceptable carriers and excipients that may be used to formulate oral dosage forms of the present invention are described in U. S. Patent 3,903,297, Robert, issued September 2, 1975, incorporated by reference herein. Techniques and compositions for making dosage forms useful herein are described in the following references, all incorporated by reference herein: 7 Modern Pharmaceutics, Chapters 9 and 10 (Banker and Rhodes, editors, 1979); an Lieberman, et al., Pharmaceutical Dosage Forms: *Tablets* (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms (2d edition, 1976).

The compositions of this invention may be used according to the methods of this invention by administering the composition from 1 to 4 times per day, and preferably from 1 to 2 times per day; for from 1 to 28 days, preferably for from about 1 to about 21 days, and most preferably for from about 1 to about 14 days. The specific frequency of administration will depend upon such factors as the specific NSAID, bismuth compound or composition and antimicrobial(s) used, the levels at which the components are incorporated in the composition, the nature and severity of the condition to be treated, and the nature of any concurrent therapy, if any.

Method of Use

The methods of the present invention comprise the treatment of a human or lower animal subject having a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising administering to the subject bismuth and a non-steroidal anti-inflammatory drug (hereinafter referred to as "NSAID"). The present method may further comprise the administration of one or more antisecretory agents.

As used herein, the term "administering" refers to any method which, in sound medical practice delivers the compounds or compositions used in this invention to the subject to be treated in such a manner so as to be effective in the treatment of the gastrointestinal disorder. Preferably, the bismuth, NSAID, and antisecretory agent(s), if present, are administered orally.

The present invention encompasses methods wherein the administering of bismuth, the NSAID, and optionally the antisecretory agent(s) are performed

simultaneously (beginning and ending on the same day), concurrently (overlapping but not of the same duration of administration), or consecutively (sequential, but where the course of treatment is substantially continuous). Preferably, the bismuth and NSAID are administered concurrently and administration for both bismuth and the NSAID is commenced on the same day. Additionally, if one or more antisecretory agents are also present, it is preferred that the bismuth and the antisecretory agent(s) are administered simultaneously.

The following non-limiting examples illustrate the composition and methods of use of the present invention.

EXAMPLE I

An asymptomatic young volunteer identified as having *H. pylori* infection through the results of a mass screening, is treated by a method of the present invention. The subject is orally administered approximately 2500 milligrams of bismuth in the form of bismuth subsalicylate daily, (sold by The Procter & Gamble Company under the name "Pepto-Bismol®"), in four equal doses, for 28 days; and approximately 100-200 milligrams of indomethacin daily, in four equal doses, for about 14 days. One to two months later, a diagnostic test performed on the volunteer shows no evidence of *H. pylori*.

In the above example, tripotassium dicitrato bismuthate, bismuth tartrate, bismuth citrate, and bismuth subnitrate are substituted, respectively, for bismuth subsalicylate, with substantially similar results.

EXAMPLE II

A human subject is suffering from chronic active gastritis. A diagnostic test reveals the presence of *H. pylori*. The individual is treated by orally administering approximately 500 milligrams of bismuth daily in the form of bismuth subcitrate ("DeNol", sold by Brocades), in two equal doses, for about 28 days; approximately 3.9 grams of acetylsalicylic acid daily, in three equal doses, for about 14 days; and approximately 20 milligrams of omeprazole daily, for 28 days. Administration of all three agents is commenced on the same day. One to two months later, the diagnostic test is repeated. The results show no evidence of *H. pylori*.

EXAMPLE III

A human subject is suffering from non-ulcer dyspepsia. A biopsy of the gastric mucosa is taken from the stomach of the subject. Analysis of the biopsy sample indicates the presence of urease in the sample and the presence of *H. pylori* in the stomach of the subject. The subject is given approximately 1200 milligrams of bismuth daily, (administered as bismuth subsalicylate in the composition Pepto-Bismol®, sold by The Procter & Gamble Company), in four equal doses, for about 21 days; 1200-3200

milligrams of ibuprofen daily, in three to four equal doses, for about 7 days; and 150 milligrams of ranitidine daily, in two equal doses, for about 21 days. Administration of all three agents is commenced on the same day. A biopsy sample taken and analyzed one to two months later shows no evidence of *H. pylori*.

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WHAT IS CLAIMED IS:

1. A composition for treating a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising:
   a) from 50 milligrams to 5000 milligrams, per day, of bismuth;
   b) a gastropathic amount of a non-steroidal anti-inflammatory drug; and
   c) pharmaceutically acceptable carriers.

2. The composition of Claim 1 further comprising a therapeutically effective amount of one or more antisecretory agents selected from the group consisting of H_2 receptor antagonists, proton pump inhibitors and mixtures thereof.

3. The composition of Claim 1 or 2 wherein the antisecretory agents are selected from the group consisting of cimetidine, ranitidine, famotidine, roxatidine, nizatidine, mifentidine, omeprazole, lansoprazole, pantoprazole, and mixtures thereof.

4. The composition of any of Claims 1-3 wherein the bismuth is selected from the group consisting of bismuth aluminate, bismuth subcarbonate, bismuth subcitrate, bismuth citrate, tripotassium dicitrato bismuthate, bismuth subgallate, bismuth subsalicylate, bismuth tartrate, and mixtures thereof and is administered at a level of from 50 milligrams to 2500 milligrams, per day for 7 to 28 days.

5. The composition of any of Claims 1-4 wherein the non-steroidal anti-inflammatory drug is selected from the group consisting of ibuprofen, indomethacin, acetylsalicylic acid, and naproxen and wherein it is administered for up to 14 days, and the one or more antisecretory agents are administered for 7 to 28 days.

6. The use of the compositions of any of Claims 1-5 for the manufacture of a composition for treatment of a human or lower animal subject having a gastrointestinal disorder caused or mediated by *Helicobacter pylori* comprising administering to the subject from 50 milligrams to 5000 milligrams of bismuth, per day, for from 1 to 42 days and a gastropathic amount of a non-steroidal anti-inflammatory drugs for up to 14 days.

7. The use of the compositions of any of Claims 1-6 for the manufacture of a composition comprising a therapeutically effective amount of one or more antisecretory agents which are selected from the group consisting of H_2 receptor antagonists, proton pump inhibitors and mixtures thereof.

8. The use of the compositions of any of Claims 1-7 for the manufacture of a composition wherein the bismuth is selected from the group consisting of bismuth aluminate, bismuth subcarbonate, bismuth subcitrate, bismuth citrate, tripotassium dicitrato bismuthate, bismuth subgallate, bismuth subsalicylate, bismuth tartrate, and mixtures thereof and is administered at a level of from 50 milligrams to 2500 milligrams, per day for 7 to 28 days.

9. The use of the compositions of any of Claims 1-8 for the manufacture of a composition wherein the non-steroidal anti-inflammatory drug is selected from the group consisting of ibuprofen, indomethacin, acetylsalicylic acid, and naproxen and the anisecretory agents are selected from the group consisting of cimetidine, ranitidine, famotidine, roxatidine, nizatidine, mifentidine, omeprazole, lansoprazole, pantoprazole, and mixtures thereof.

INTERNATIONAL SEARCH REPORT

In itional Application No
PCT/US 97/21462

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	TANAKA S ET AL: "Indomethacin increases mucus bismuth concentration in ranitidine bismuth citrate treated rats" GASTROENTEROLOGY, 108 (4 SUPPL.). 1995. A234., XP002059838 see the whole document	1,4,5
X	EP 0 437 294 A (GIST BROCADES NV) 17 July 1991 see page 3, line 18-24	1,4
X	WO 93 09784 A (PROCTER & GAMBLE) 27 May 1993 see page 5, line 27-31	1,4

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[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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(54) Title: NOVEL FORM OF *S*OMEPRAZOLE

(57) Abstract

The present invention relates to a novel form of the (-)-enantiomer of 5-methoxy-2- [[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole, i.e *S*-omeprazole. More specifically, it relates to a novel form of the magnesium salt of the *S*-enantiomer of omeprazole trihydrate. The present invention also relates to processes for preparing such a form of the magnesium salt of *S*-omeprazole and pharmaceutical compositions containing it. Furthermore, the present invention also relates to new intermediates used in the process.

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NOVEL FORM OF *S*-OMEPRAZOLE

Field of the Invention

The present invention relates to a novel form of the (-)-enantiomer of 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole, *i.e. S*-omeprazole. More specifically, it relates to a novel form of the magnesium salt of the *S*-enantiomer of omeprazole trihydrate. The present invention also relates to processes for preparing such a form of the magnesium salt of *S*-omeprazole and pharmaceutical compositions containing it. Furthermore, the present invention also relates to intermediates used in the process, and their preparation.

Background of the invention and prior art

The compound 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole, having the generic name omeprazole, and therapeutically acceptable salts thereof, are described in EP 5129. The specific alkaline salts of omeprazole are disclosed in EP 124 495. Omeprazole is a proton pump inhibitor, i.e. effective in inhibiting gastric acid secretion, and is useful as an antiulcer agent. In a more general sense, omeprazole may be used for prevention and treatment of gastric-acid related diseases in mammals and especially in man.

Omeprazole is a sulfoxide and a chiral compound, wherein the sulfur atom being the stereogenic center. Thus, omeprazole is a racemic mixture of its two single enantiomers, the *R* and *S*-enantiomer of omeprazole, herein referred to as *R*-omeprazole and *S*-omeprazole. The absolute configurations of the enantiomers of omeprazole have been determined by an X-ray study of an N-alkylated derivative of the (+)-enantiomer in non-salt form. The (+)-enantiomer of the non-salt form and the (-)-enantiomer of the non-salt form were found to have *R* and *S* configuration, respectively, and the (+)-enantiomer of the magnesium salt and the (-)-enantiomer of the magnesium salt were also found to have *R*

and *S* configuration, respectively. The conditions for the optical rotation measurement for each of these enantiomers are described in WO 94/27988.

Certain salts of single enantiomers of omeprazole and their preparation are disclosed in WO 94/27988. These compounds have improved pharmacokinetic and metabolic properties which will give an improved therapeutic profile such as a lower degree of interindividual variation.

WO 96/02535 discloses a process for the preparation of the single enantiomers of omeprazole and salts thereof, and WO 96/01623 discloses a suitable tableted dosage forms of for instance magnesium salts of *R*- and *S*-omeprazole.

Brief description of the drawings

Figure 1 shows a X-ray powder diffractogram of the magnesium salt of *S*-omeprazole trihydrate prepared according to the present invention.

Figure 2 shows a X-ray powder diffractogram of the potassium salt of *S*-omeprazole prepared and used in the present application (See examples 2 and 3)

Figure 3 shows a X-ray powder diffractogram of a magnesium salt of *S*-omeprazole dihydrate prepared and used in the present application (See example 5)

Figure 4 shows a X-ray powder diffractogram of a magnesium salt of *S*-omeprazole dihydrate which is a polymorph of the dihydrate shown in Figure 3 (See Example 6). This magnesium salt of *S*-omeprazole dihydrate has been prepared and can be used in the preparation of the magnesium salt of *S*-omeprazole trihydrate according to the present invention.

Figure 5 shows X-ray powder diffractogram of the magnesium salt of *S*-omeprazole prepared according to example A in WO 96/01623 .

Description of the Invention

It has surprisingly been found that the magnesium salt of *S*-omeprazole occurs in a number of structurally different forms. It is an object of the present invention to provide a substantially pure magnesium salt of *S*-omeprazole trihydrate, hereinafter referred to as the compound of the invention. This trihydrate can be obtained as a well defined compound. The present invention also provides a process to obtain and a method of differentiating the magnesium salt of *S*-omeprazole trihydrate from other forms of magnesium salts of *S*-omeprazole.

The compound of the invention is advantageous because it is more stable than the corresponding magnesium salt compounds in prior art and is therefore easier to handle and store. The compound of the invention is also easier to characterize because it exists in a well defined state. Additionally, the compound of the invention is easier to synthesize in a reproducible manner and thereby easier to handle in a full scale production.

The magnesium salt of *S*-omeprazole trihydrate obtained according to the present invention is substantially free from magnesium salts of *R*-omeprazole. The magnesium salt of *S*-omeprazole trihydrate obtained according to the present invention is also substantially free from other forms of magnesium salts of *S*-omeprazole, such as the corresponding magnesium salt compounds described in prior art, and dihydrates used in the preparation of the trihydrate compound according to the present invention.

The compound of the invention is characterized by the positions and intensities of the major peaks in the X-ray powder diffractogram , but may also be characterized by conventional FT-IR spectroscopy. These characteristics are not exhibited by any other form of magnesium salt of *S*-omeprazole and accordingly, the magnesium salt of *S*-omeprazole trihydrate is easily distinguishable from any other crystal form of the magnesium salt of *S*-omeprazole disclosed in prior art. The compound of the invention is characterized by being

highly crystalline, *i.e.* having a higher crystallinity than any other form of magnesium salt of *S*-omeprazole disclosed in the prior art. With the expression "any other form" is meant anhydrates, hydrates, solvates, and polymorphs or amorphous forms thereof disclosed in the prior art. Examples of any other forms of magnesium salt of *S*-omeprazole includes, but are not limited to, anhydrates, monohydrates, dihydrates, sesquihydrates, trihydrates, alcoholates, such as methanolates and ethanolates, and polymorphs or amorphous forms thereof.

The compound of the invention may also be characterized by its unit cell.

In a further aspect, the present invention provides processes for the preparation of the magnesium salt of *S*-omeprazole trihydrate which comprises;

a) treating a magnesium salt of *S*-omeprazole of any form, for example prepared according to procedures known in the art such as Example A in WO 96/01623 which is incorporated herein by reference, with water at a suitable temperature for a suitable time. By a suitable temperature is meant a temperature which induces the transformation of starting material to product without decomposing any of these compounds. Examples of such suitable temperatures include, but are not limited to, room temperature and above. By a suitable time is meant a time that results in high conversion of the starting material into product without causing any decomposition of either compounds, *i.e.* results in a good yield. This suitable time will vary depending on the temperature used in a way well known to people in the art. The higher the temperature, the shorter time is needed to give the desired conversion. The amount of water is not crucial and will depend on the process conditions used. The magnesium salt of *S*-omeprazole trihydrate is thereafter separated from the aqueous slurry, for example by filtration or centrifugation and thereafter dried to constant weight; or

b) oxidizing 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]thio]-1H-benzimidazole, with an oxidizing agent and a chiral titanium complex, optionally in the

presence of a base. The oxidation is carried out in an organic solvent, for example toluene or dichloromethane.

The crude product is converted to the corresponding potassium salt by treatment with a potassium source, such as methanolic potassium hydroxide or methanolic potassium methylate, followed by isolation of the formed salt.

The resulting potassium salt of *S*-omeprazole is thereafter converted to the corresponding magnesium salt by treatment with a magnesium source, such as magnesium sulfate in a lower alcohol, such as methanol. The solution is optionally filtered and the precipitation is initialized by addition of a non-solvent such as acetone. The product is filtered off and optionally washed with water and further processed as is described in a) above. Alternatively, the potassium salt may be treated with a magnesium source, such as magnesium sulfate in water, and isolation of the magnesium salt of *S*-omeprazole trihydrate, or any other conventional technique for transforming a potassium salt to the corresponding magnesium salt can be used and is within the scope of the present invention.

Yet a further aspect of the present invention is to provide a suitable intermediate used in the preparation of the compound of the invention, as well as a process for its preparation. The potassium salt of *S*-omeprazole is found to be such a suitable intermediate. The potassium salt of *S*-omeprazole may also be used as an active component of a pharmaceutical formulation to be used in the treatment of gastrointestinal diseases.

The compound of the invention, *i.e.* the magnesium salt of *S*-omeprazole trihydrate , prepared according to the present invention may be analyzed by XRPD, a technique which is known per se.

The amount of water in the magnesium salt of *S*-omeprazole trihydrate is determined by thermogravimetric analysis, a technique which is known per se.

The compound of the invention is effective as a gastric acid secretion inhibitor, and is useful as an antiulcer agent. In a more general sense, it can be used for prevention and treatment of gastric-acid related conditions in mammals and especially in man, including *e.g.* reflux esophagitis, gastritis, duodenitis, gastric ulcer and duodenal ulcer. Furthermore, it may be used for treatment of other gastrointestinal disorders where gastric acid inhibitory effect is desirable *e.g.* in patients on NSAID therapy, in patients with Non Ulcer Dyspepsia, in patients with symptomatic gastro-esophageal reflux disease, and in patients with gastrinomas. The compound of the invention may also be used in patients in intensive care situations, in patients with acute upper gastrointestinal bleeding, pre- and postoperatively to prevent aspiration of gastric acid and to prevent and treat stress ulceration. Further, the compound of the invention may be useful in the treatment of psoriasis as well as in the treatment of *Helicobacter* infections and diseases related to these. The compound of the invention may also be used for treatment of inflammatory conditions in mammals, including man.

Any suitable route of administration may be employed for providing the patient with an effective dosage of the magnesium salt of *S*-omeprazole trihydrate, according to the invention. For example, peroral or parental formulations and the like may be employed. Dosage forms include capsules, tablets, dispersions, suspensions and the like.

It is further provided a pharmaceutical composition comprising the magnesium salt of *S*-omeprazole trihydrate according to the invention, as active ingredient, in association with a pharmaceutically acceptable carrier, diluent or excipient and optionally other therapeutic ingredients. Compositions comprising other therapeutic ingredients are especially of interest in the treatment of *Helicobacter* infections. The invention also provides the use of the magnesium salt of *S*-omeprazole trihydrate of the invention in the manufacture of a medicament for use in the treatment of a gastric-acid related condition and a method of treating a gastric-acid related condition which method comprises administering to a subject suffering from said condition a therapeutically effective amount of the magnesium salt of *S*-omeprazole trihydrate according to the invention.

The compositions of the invention include compositions suitable for peroral or parental administration. The most preferred route is the oral route. The compositions may be conveniently presented in unit dosage forms, and prepared by any methods known in the art of pharmacy.

In the practice of the invention, the most suitable route of administration as well as the magnitude of a therapeutic dose of the magnesium salt of *S*-omeprazole trihydrate according to the invention in any given case will depend on the nature and severity of the disease to be treated. The dose, and dose frequency, may also vary according to the age, body weight, and response of the individual patient. Special requirements may be needed for patients having Zollinger-Ellison syndrome, such as a need for higher doses than the average patient. Children and patients with liver diseases generally will benefit from doses that are somewhat lower than the average. Thus, in some conditions it may be necessary to use doses outside the ranges stated below, for example long term treatments may request lower dosage. Such higher and lower doses are within the scope of the present invention. Such daily doses may vary between 5 mg to 300 mg.

In general, a suitable oral dosage form of the compound of the invention may cover a dose range from 5 mg to 300 mg total daily dose, administered in one single dose or equally divided doses. A preferred dosage range is from 10 mg to 80 mg.

The compound of the invention may be combined as the active component in intimate admixture with a pharmaceutical carrier according to conventional techniques, such as the oral formulations described in WO 96/01623 and EP 247 983, the disclosures of which are hereby incorporated as a whole by reference.

Combination preparations comprising the magnesium salt of *S*-omeprazole trihydrate and other active ingredients may also be used. Examples of such active ingredients include, but

are not limited to anti-bacterial compounds, non-steroidal anti-inflammatory agents, antacid agents, alginates and prokinetic agents.

The examples which follow will further illustrate the preparation of the compound of the invention, according to different process routes and including new intermediates. These examples are not intended to limit the scope of the invention as defined hereinabove or as claimed below.

Examples

Example 1

S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt trihydrate

Water (157 kg) was added to the wet crystals of the magnesium salt of *S*-omeprazole, prepared according to Example 4, below. The mixture was heated to 38°C with stirring and left for 3 hours. The crystals were filtered off and dried in vacuo. Yield: 31.6 kg

X-ray powder diffraction analysis was performed on a sample of the crystals prepared above according to standard methods, which can be found in *e.g.* Kitaigorodsky, A. I. (1973), Molecular Crystals and Molecules, Academic Press, New York; Bunn, C. W. (1948), Chemical Crystallography, Clarendon Press, London; or Klug, H.P. & Alexander, L. E. (1974), X-Ray Diffraction Procedures, John Wiley and Sons, New York. The analysis gave the diffractogram depicted in Figure 1. The main peaks, with positions and relative intensities, have been extracted from the diffractogram in Figure 1 and is given below in table 1. The relative intensities are less reliable and instead of numerical values the following definitions are used.

% Relative Intensity	Definition
25-100	vs (very strong)
10-25	s (strong)
3-10	m (medium)
1-3	w (weak)
<1	vw (very weak)

Some additional very weak peaks found in the diffractogram have been omitted from table 1.

Table 1. Positions and intensities of the major peaks in the XRP-diffractogram of the magnesium salt of *S*-omeprazole trihydrate.

d-value / Å	***Relative Intensity***
2.67	m
2.79	m
3.27	m
3.52	s
3.82	s
3.96	vs
4.14	m
5.2	m
5.6	m
6.7	vs
6.9	s
8.3	w
16.6	vs

Example 2

S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole potassium salt

A solution of 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]thio]-1H-benzimidazole (15.4 g, 46.8 mmol) in toluene (70 ml) was heated to 50°C and water (0.05 ml, 2,8 mmol) and D-(-)-diethyl tartrate (2.02 g, 9.82 mmol) were added. The reaction mixture was stirred for 20 minutes. Titanium(IV)isopropoxide (1.34 g, 4.68 mmol) was added and the reaction mixture was stirred for 45 minutes. The mixture was cooled to 30°C and diisopropylethylamine (0.91 g, 7.01 mmol) was added followed by cumene hydroperoxide (9.52 g, 51.89 mmol). The resultant mixture was stirred at 30°C for 3 hours. Methanol (40 ml) was added followed by potassium hydroxide (3.05 g, 46.8 mmol) in methanol (30 ml). Seed crystals were added and the reaction mixture was stirred at 35°C overnight. The precipitated product was filtered off, washed with methanol and toluene and dried in vacuo. Yield: 9.74 g (54%).

Example 3

S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole potassium salt

Water (157.6 µl) was added to a solution of 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]thio]-1H-benzimidazole in toluene (370 ml; 211.5 g/l) with a water content of 0.031% (w/w), followed by addition of D-(-)-diethyl tartrate (8.55 ml). The solution was heated to 50°C and stirred at this temperature for 20 minutes. Titanium(IV)isopropoxide (7.15 ml) was added and reaction was left at 50°C for 45 minutes. The temperature was lowered to 30°C and diisopropylethylamine (6.2 ml) was added. Cumene hydroperoxide was added at an appropriate speed to maintain the temperature from 28°C to 34°C. The temperature was raised to 35°C after 2 hours and

potassium methoxide (24.55 g) in methanol (222 ml) was added. The mixture was filtered after 14 hours and the crystals were washed with methanol:toluene (240 ml; 1:1) and methanol (120 ml) and dried. Yield: 79 g (74%), ee > 99.9%.

$[\alpha]_D^{20}$ = +28.7° (c = 1%, water); Assay: 89% is S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole potassium salt (11% is methanol).

1H-NMR (200 MHz, DMSO-d6, δ ppm): 2.23 (s, 3H), 2.24 (s, 3H), 3.71 (s, 3H), 3.75 (s, 3H), 4.40 (d, 1H), 4.78 (d, 1H), 6.58 (dd, 1H), 7.00 (d, 1H), 7.35 (d, 1H), 8.25 (s, 1H).

The products from Examples 2 and 3 were analysed using X-ray powder diffraction as described in Example 1 and gave the diffractogram depicted in Figure 2 and given below in Table 2. Some additional very weak peaks found in the diffractogram have been omitted from Table 2.

Table 2. Positions and intensities of the major peaks in the XRP-diffractogram of the potassium salt of *S*-omeprazole.

d-value/Å	Relative intensity	d-value/ (Å)	Relative intensity
13.6	vs	3.52	m
10.6	vw	3.42	w
7.8	m	3.38	w
6.8	m	3.34	m
6.5	m	3.28	w
6.2	w	3.20	m
6.1	m	3.12	w
5.8	s	3.06	w
5.4	m	3.03	w
5.3	w	2.97	w
5.2	w	2.93	vw
5.0	vw	2.89	w
4.75	m	2.85	m
4.71	w	2.76	w
4.52	w	2.71	vw
4.42	w	2.66	vw
4.32	w	2.58	w
4.27	m	2.57	w
3.98	vw	2.56	w
3.92	w	2.52	vw
3.89	w	2.47	vw
3.87	w	2.45	vw
3.81	w	2.43	vw
3.74	m	2.40	vw
3.60	m	2.38	vw
3.55	m	2.31	vw

$\alpha 1 = 1.54060$ Å

Example 4

S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt

Methanol (148 kg) was added to *S*-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole potassium salt (71 kg, methanol content = 13%). $MgSO_4$ x 7 H_2O (40 kg) was added to the mixture while stirring. After 70 minutes the mixture was filtered and the filtrate was washed with methanol (46 kg). The solution was concentrated to a volume of 100 liter, acetone (253 kg) was added and the resulting mixture was left for 4 hours. The precipitated product was filtered off, washed with acetone and water. The wet crystals were immediately used as is described in Example 1.

Example 5

S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt dihydrate

5.0 g of the moist product from Example 4 with an approximate dry content of 74%, was dried in vacuum at 35 °C over night to yield 3.58 g (2.68 mmol) of S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt dihydrate, named Form B.

The product was analyzed using X-ray powder diffraction as described in Example 1, and the analyze gave the diffractogram depicted in Figure 3 and given below in Table 3. Some additional peaks with low intensities found in the diffractogram have been omitted from Table 3.

Table 3. Positions and intensities of the major peaks in the XRP-diffractogram of the magnesium salt of *S*-omeprazole dihydrate, Form B.

d-value / Å	Relative Intensity
4.19	m
4.45	m
4.68	m
4.79	s
4.91	s
4.98	s
5.1	m
5.4	s
5.5	m
5.6	m
5.8	m
6.3	m
6.7	s
7.9	m
8.1	s
11.0	m
11.8	m
14.9	vs

Convertion of magnesium salt of S-omeprazole dihydrate to trihydrate

This material was subsequently processed to S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt trihydrate according to the procedure described for the moist substance in Example 1.

Example 6

S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt dihydrate

A methanolic solution of S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt was prepared as is described in Example 4. Such a solution of S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt (1.86g) in 5 ml methanol was concentrated by evaporation until 1.58 ml methanol remained. Then, a mixture of 1.6 ml water and 6.32 ml aceton was added. The solution was allowed to crystallize during 26 h at room temperature. The resulting crystals were filtered off and dried at 40 °C under reduced pressure giving 1.17 g of S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt dihydrate, named form A.

The product was analyzed using X-ray powder diffration as described in Example 1 and gave the diffractogram depicted in Figure 4 and given below in Table 4. Some additional peaks with low intensities found in the diffractogram have been omitted from Table 4.

Table 4. Positions and intensities of the major peaks in the XRP-diffractogram of the magnesium salt of *S*-omeprazole dihydrate, Form A.

d-value / Å	Relative Intensity
3.04	s
3.14	s
3.18	m
4.05	s
4.19	s
4.32	m
4.54	s
4.69	vs
5.2	s
5.3	s
5.8	s
6.2	vs
6.6	s
15.5	vs

Example 7

S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt trihydrate

22,0 g (29,1 mmol) of S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulfinyl]-1H-benzimidazole potassium salt was dissolved in 40 mL of water. The solution was seeded with 0,11 g (0,1 mmol) S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt trihydrate. 22 mL (69,6 mmol) of $MgSO_4$ (aq) was added under a 3 h period. The slurry was filtered off and the precipitate was elutriated in water for approximately 30 minutes and the crystals were filtered off and dried (35 °C, vacuum).

Yield: 9,15 g (11,6 mmol; 80%). The substance had a purity (HPLC):99,8 area%, Mg content: 3,40 % (w/w) and ee: 99,8%.

The product was analyzed using X-ray powder diffraction and the result complies with Figure 1 and Table 1.

Reference Example A

S-5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole magnesium salt

(The method used is in accordance with the method described in Example A in WO 96/01623)

Magnesium (0.11g, 4.5 mmol) was dissolved and reacted with methanol (50 ml) at 40°C with a catalytic amount of methylene chloride. The reaction was run under nitrogen and was finished after five hours. At room temperature a mixture of the two enantiomers [90%(-)-isomer and 10%(+)-isomer] of 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole (2.84 g, 8.2 mmol) was added to the magnesium methoxide solution. The mixture was stirred for 12 hours whereupon a small amount of water (0.1 ml) was added in order to precipitate inorganic magnesium salts. After 30 minutes stirring, these inorganic salts were filtered off and the solution was concentrated on a rotavapor. The residue was now a concentrated methanolic solution of the enantiomeric mixture (i.e. the title compound contaminated with the (+)-isomer), with an optical purity (enantiomeric excess, e.e.) of 80%. This mixture was diluted with acetone (100 ml) and after stirring at room temperature for 15 minutes, a white precipitate was obtained. Additional stirring for 15 minutes and thereafter filtration afforded 1.3 g (50%) of the title compound as white crystals. Chiral analyses of the crystals and mother liquor were performed by chromatography on an analytical chiral column. The optical purity of the crystals and mother liquor was found to be 98.4 e.e. and 64.4% e.e., respectively. Thus, the

optical purity (e.e.) has been enhanced from 80% to 98.4% simply by crystallizing the Mg-salt from a mixture of acetone and methanol. The product was crystalline as shown by powder X-ray diffraction and the magnesium content was 3.44% as shown by atomic absorption spectroscopy. $[\alpha]_D^{20}=-131.5°$ (c=0.5%, methanol).

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The product was analyzed using X-ray powder diffraction as described in Example 1 and gave the diffractogram depicted in Figure 5 and given below in Table 5. Some additional very weak peaks found in the diffractograms have been omitted from Table 5.

Table 5. Positions and intensities of the major peaks in the XRP-diffractogram shown in Figure 5.

d-value / Å	*Relative Intensity*
2.90	s
3.41	s
3.90	s
4.13	s
4.79	vs
5.00	vs
5.4	vs
5.7	s
6.3	s
6.8	s
7.8	s
8.4	vs
10.8	s
12.2	s
15.1	vs

5

Claims.

1. The magnesium salt of *S*-omeprazole trihydrate.

2. The magnesium salt of *S*-omeprazole trihydrate according to claim 1, characterized by being highly crystalline.

3. The magnesium salt of *S*-omeprazole trihydrate according to claim 1, characterized by the following major peaks in its X-ray powder diffractogram.

d-value / Å	*Relative Intensity*
2.67	m
2.79	m
3.27	m
3.52	s
3.82	s
3.96	vs
4.14	m
5.2	m
5.6	m
6.7	vs
6.9	s
8.3	w
16.6	vs

4. A process for the preparation of the magnesium salt of *S*-omeprazole trihydrate according to any of claims 1-3 which comprises treating a magnesium salt of *S*-omeprazole of any other form with water.

5. A process for the preparation of the magnesium salt of *S*-omeprazole trihydrate according to any of claims 1-3 which comprises the following steps;

a) mixing a potassium salt of *S*-omeprazole with an organic solvent;

b) converting the potassium salt of *S*-omeprazole into a corresponding magnesium salt of *S*-omeprazole by treating the said potassium salt with a magnesium source;

c) precipitating the magnesium salt of *S*-omeprazole by addition of a non-solvent;

d) isolating the obtained magnesium salt of *S*-omeprazole;

e) treating the obtained magnesium salt of *S*-omeprazole with water; and

f) isolating and drying the magnesium salt of *S*-omeprazole trihydrate thus obtained.

6. A process according to claim 5 wherein said organic solvent used in step a) is methanol.

7. A process according to any of claims 5-6, wherein the said non-solvent used in step c) is acetone.

8. A process according to claim 5 wherein steps a) to e) are replaced by the single step;

i) converting the potassium salt of *S*-omeprazole into a corresponding magnesium salt of *S*-omeprazole by treating said potassium salt with a magnesium source in water.

9. A process according to any of claims 5-8, wherein the said magnesium source used in step b) of claims 5-7 or step i) of claim 8 is magnesium sulfate.

10. A process for the preparation of a potassium salt of *S*-omeprazole to be used in any of claims 5-9, which process comprises the following steps;

a) oxidizing 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]thio]-1H-benzimidazole into *S*-omeprazole in an organic solvent;

b) converting the *S*-omeprazole into the corresponding potassium salt of *S*-omeprazole by treating said *S*-omeprazole with a potassium source ;

c) isolating the potassium salt of *S*-omeprazole thus obtained.

11. A process according to claim 10, wherein said organic solvent used in step a) is toluene.

12. A process according to any of claims 10-11, wherein said potassium source used in step b) is methanolic potassium methoxide or methanolic potassium hydroxide.

13. Potassium salt of *S*-omeprazole prepared according to claim 10 characterized by the following peaks in its X-ray powder diffractogram.

d-value/Å	Relative intensity	d-value/ (Å)	Relative intensity
13.6	vs	3.52	m
10.6	vw	3.42	w
7.8	m	3.38	w
6.8	m	3.34	m
6.5	m	3.28	w
6.2	w	3.20	m
6.1	m	3.12	w
5.8	s	3.06	w
5.4	m	3.03	w
5.3	w	2.97	w
5.2	w	2.93	vw
5.0	vw	2.89	w
4.75	m	2.85	m
4.71	w	2.76	w
4.52	w	2.71	vw
4.42	w	2.66	vw
4.32	w	2.58	w
4.27	m	2.57	w
3.98	vw	2.56	w
3.92	w	2.52	vw
3.89	w	2.47	vw
3.87	w	2.45	vw
3.81	w	2.43	vw
3.74	m	2.40	vw
3.60	m	2.38	vw
3.55	m	2.31	vw

$\alpha 1 = 1.54060$ Å

14. A pharmaceutical composition comprising the magnesium salt of *S*-omeprazole trihydrate according to any of claims 1-3 as active ingredient in association with a pharmaceutically acceptable carrier and optionally other therapeutic ingredients.

15. Use of the magnesium salt of *S*-omeprazole trihydrate defined in any of claims 1-3 in the manufacture of a medicament for use in the treatment of a gastric acid related condition.

16. A method of treating a gastric acid related condition which method comprises administering to a subject suffering form said condition a therapeutically effective amount of the magnesium salt of *S*-omeprazole trihydrate defined in any of claims 1-3.

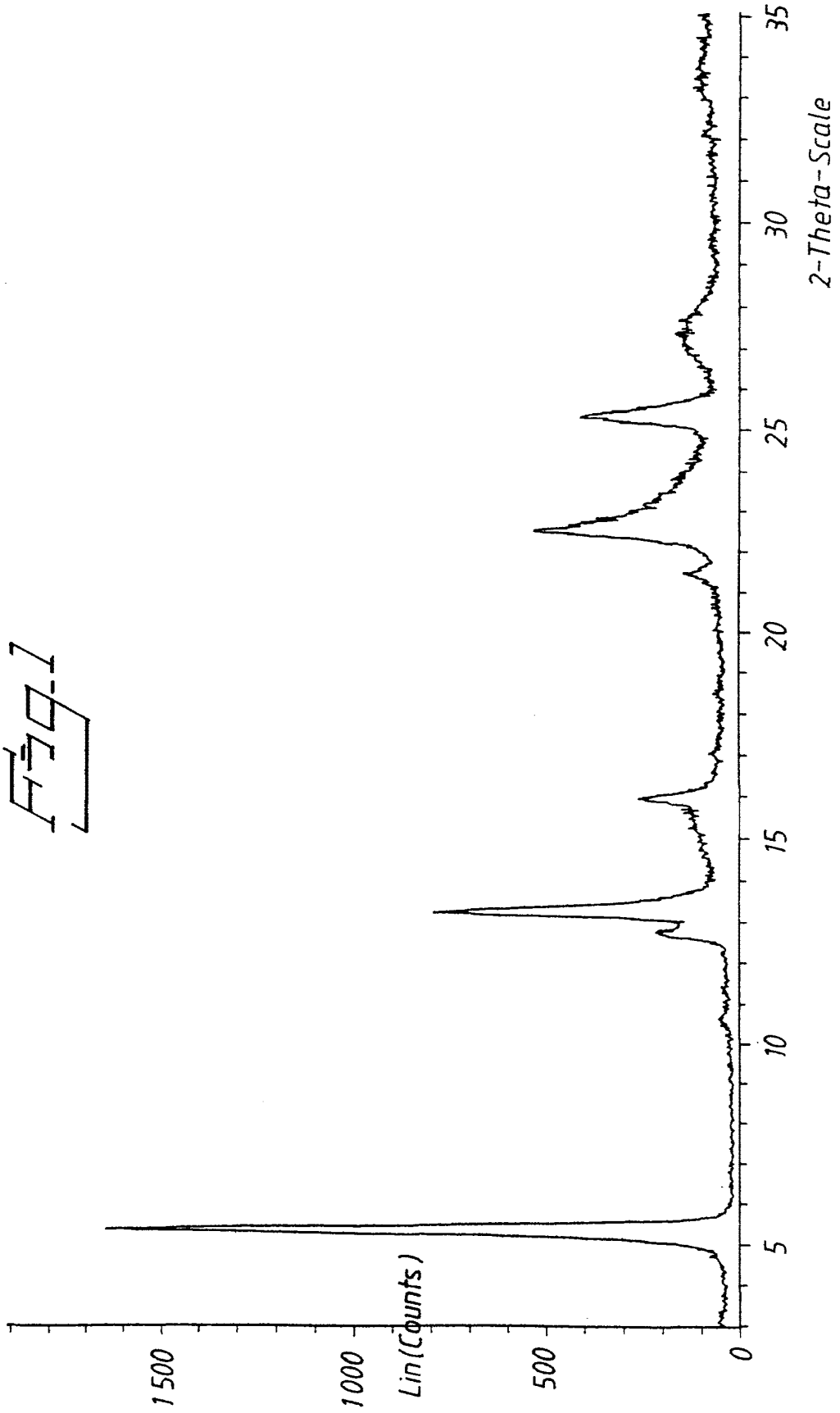

Fig 1

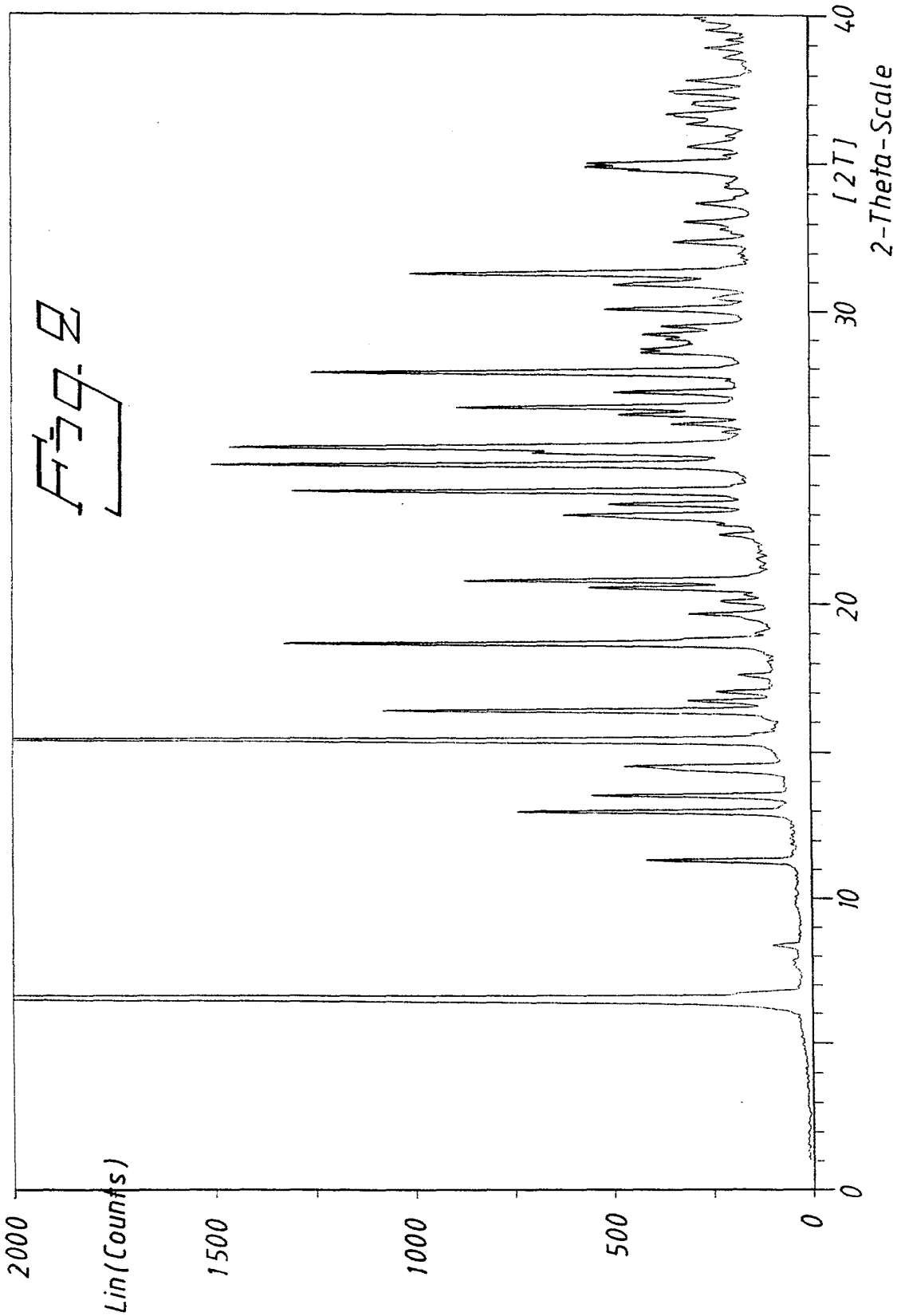
Fig. 2
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1000
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0
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20
30
40
[2T]
2-Theta-Scale

Fig 3

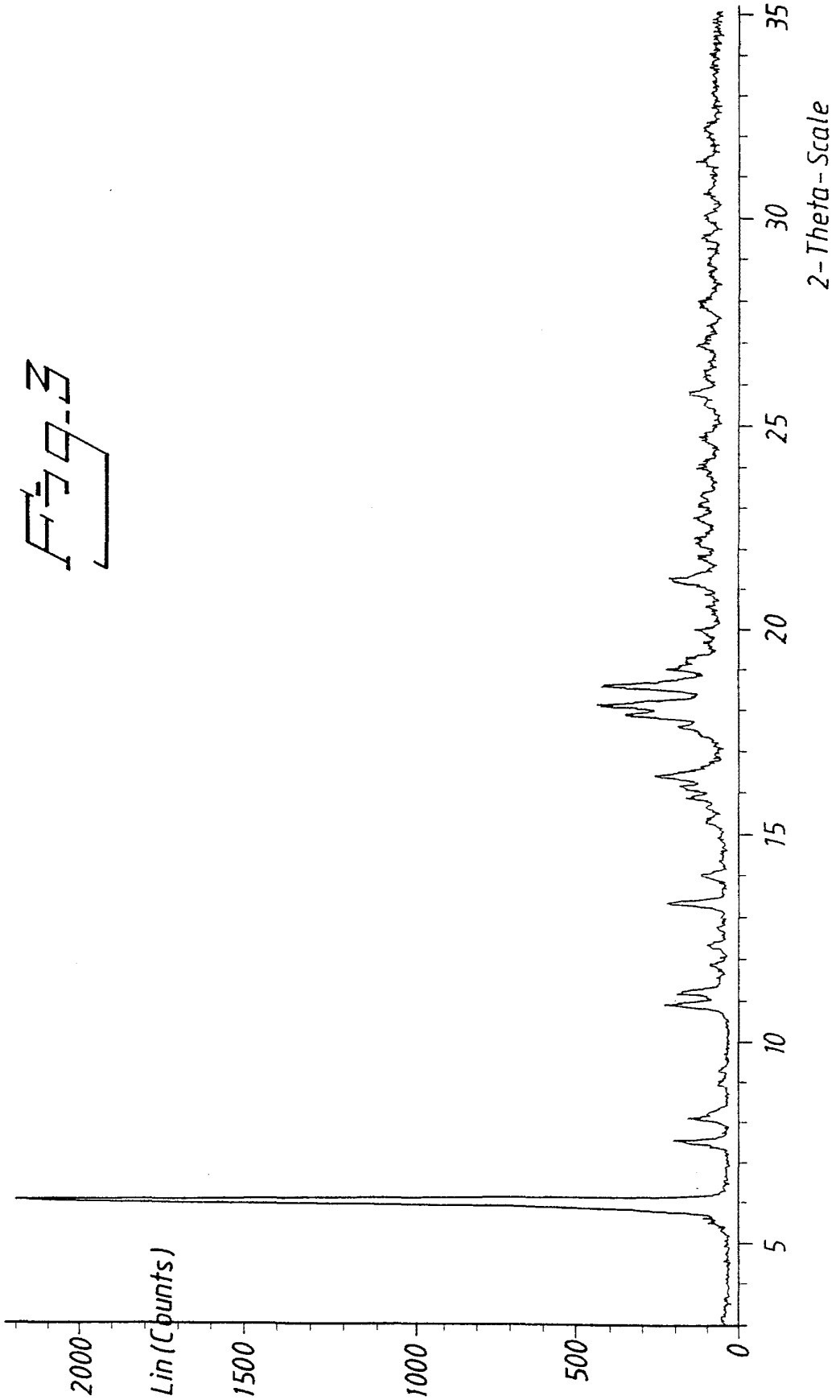


Fig 4

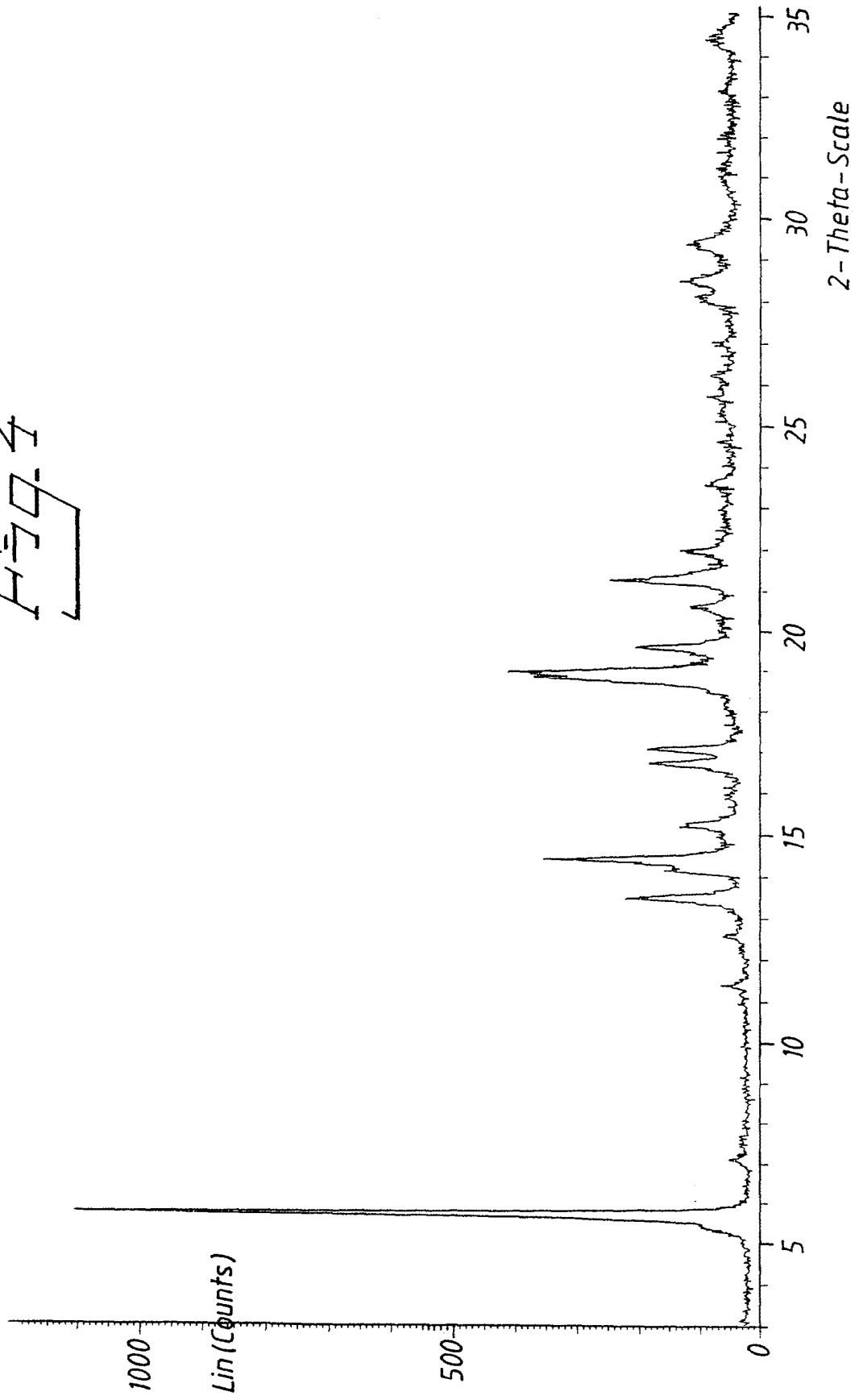
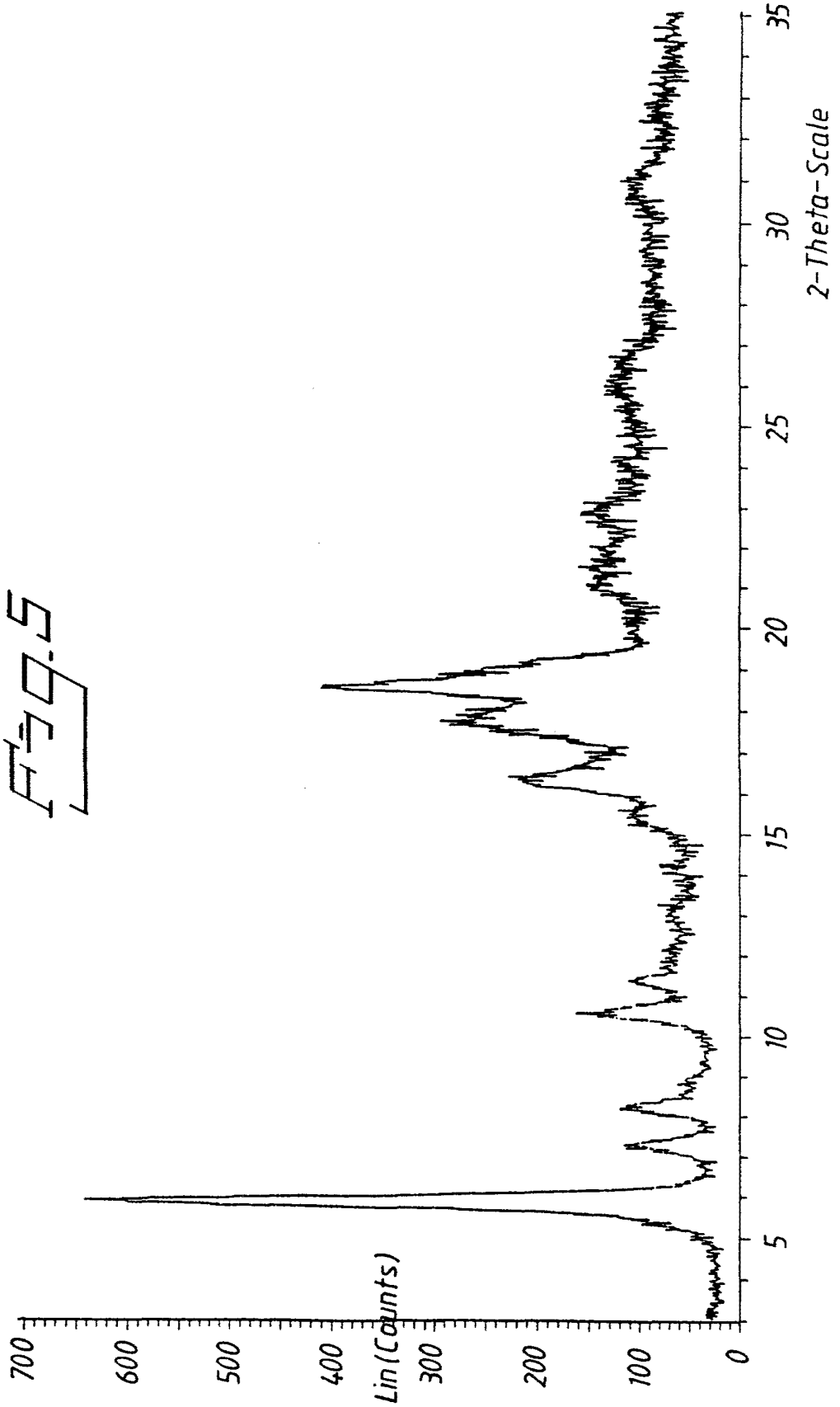


Fig 5


700
600
500
400
300
200
100
0
Lin (Counts)
5
10
15
20
25
30
35
2-Theta-Scale

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00974

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07D 401/12, A61K 31/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9601623 A1 (ASTRA AKTIEBOLAG), 25 January 1996 (25.01.96) --	1-15
A	WO 9427988 A1 (ASTRA AKTIEBOLAG), 8 December 1994 (08.12.94) --	1-15

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 1998	07 -08- 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Göran Karlsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00974

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 16
because they relate to subject matter not required to be searched by this Authority, namely:

A method for treatment of the human or animal body by therapy, see rule 39.1.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No.
PCT/SE 98/00974

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9601623 A1	25/01/96	AU 2993795 A	09/02/96
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		CA 2170647 A	25/01/96
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		CZ 9600732 A	17/07/96
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		EP 0723436 A	31/07/96
		ES 2100142 T	16/06/97
		FI 961057 A	29/03/96
		GR 97300014 T	31/05/97
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		HU 75775 A	28/05/97
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		EP 0652872 A	17/05/95
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		HU 71888 A	28/02/96
		HU 9500247 D	00/00/00
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CORRECTED VERSION*

PCT

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(74) Agent: ASTRA AKTIEBOLAG; Patent Dept., S-151 85 Södertälje (SE).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: NOVEL FORM OF *S*-OMEPRAZOLE

(57) Abstract

The present invention relates to a novel form of the (-)-enantiomer of 5-methoxy-2- [[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole, i.e *S*-omeprazole. More specifically, it relates to a novel form of the magnesium salt of the *S*-enantiomer of omeprazole trihydrate. The present invention also relates to processes for preparing such a form of the magnesium salt of *S*-omeprazole and pharmaceutical compositions containing it. Furthermore, the present invention also relates to new intermediates used in the process.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A1

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(71) Applicant *(for all designated States except US)*: ASTRA AKTIEBOLAG [SE/SE]; S-151 85 Södertälje (SE).

(72) Inventors; and
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(74) Agent: ASTRA AKTIEBOLAG; Patent Dept., S-151 85 Södertälje (SE).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: OMEPRAZOLE SODIUM SALT

(57) Abstract

This invention relates to a novel form of the sodium salt of 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl]sulfinyl]-1H-benzimidazole, known under the generic name of omeprazole sodium salt. This invention also relates to processes for its preparation of omeprazole sodium form B which is thermodynamically stable, as well as pharmaceutical compositions containing it and its use in the treatment of gastrointestinal disorders.

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OMEPRAZOLE SODIUM SALT

Field of the invention

This invention relates to a novel form of 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole, known under the generic name omeprazole. More specifically, it relates to a novel form of the sodium salt of omeprazole, namely a well-defined omeprazole sodium monohydrate salt, hereinafter referred to as omeprazole sodium form B, and its use in the treatment of gastrointestinal disorders, pharmaceutical compositions containing it and preparation thereof.

Background of the invention and prior art

The compound 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole having the generic name omeprazole, as well as therapeutically acceptable salts thereof, are described in EP 5129. The specific alkaline salts of omeprazole, such as the sodium salt, are disclosed in EP 124 495. The omeprazole sodium salt produced according to examples 1 and 2 of EP 124 495 is a mixture of crystal forms and amorphous material. One of the crystal forms present in this mixture, hereinafter referred to as omeprazole sodium form A, is a hydrate with one to two water molecules, of which one water molecule is strongly bound in the crystal structure while the other is easily removed by drying. The resulting dried substance containing one strongly bound water molecule is very hygroscopic and absorbs water rapidly under normal conditions.

Omeprazole is a proton pump inhibitor, *i.e.* effective in inhibiting gastric acid secretion, and is useful as an antiulcer agent. In a more general sense, omeprazole may be used for treatment of gastric-acid related diseases in mammals and especially in man.

Brief description of the drawings

Figure 1 is an X-ray powder diffractogram of omeprazole sodium form B.
Figure 2 is an X-ray powder diffractogram of omeprazole sodium form A.
Figure 3 is an X-ray powder diffractogram of omeprazole sodium prepared according to prior art.

Description of the invention

It has surprisingly been found that the sodium salt of omeprazole exists in a number of different crystal forms. It is an object of the present invention to provide a well-defined, thermodynamically stable at ambient temperature, and industrially useful form of omeprazole sodium, namely omeprazole sodium form B. Another object of the present invention is to provide a process for the preparation of omeprazole sodium form B, substantially free from other forms of the sodium salt of omeprazole. X-ray powder diffraction (XRPD) is used as a method of differentiating omeprazole sodium form B from other forms of the sodium salt of omeprazole.

It has been found that the sodium salt of omeprazole may crystallize in at least two different crystal forms, of which omeprazole sodium form B is one. One other form is omeprazole sodium form A with one to two moles of water. Omeprazole sodium form A is one of the crystal forms present in the mixture of crystal forms and amorphous material obtained in example 1 and example 2 in EP 124 495. However, there is no omeprazole sodium form B present in the mixture of forms obtained when preparing omeprazole sodium salt as described in either example 1 or example 2 in EP 124 495.

Omeprazole sodium form B is a crystalline form exhibiting advantageous properties, such as being well-defined, stable, and being a true monohydrate crystal form. Omeprazole sodium form B is thermodynamically more stable than omeprazole sodium form A. Omeprazole sodium form B is essentially non-hygroscopic and can therefore in industrial

processes, such as pharmaceutical manufacturing processes, be charged in a fixed amount in contrast to omeprazole sodium form A which must be charged in amounts calculated from a recent assay of omeprazole or indirectly from a recent assay of its water content. Other advantages include easier preparation and higher reproducibility between batches. This is especially important in production scale and leads to a higher production capacity.

Omeprazole sodium form A, which is thermodynamically unstable, can under certain storing conditions be completely or partly converted to omeprazole sodium form B. Omeprazole sodium form B is thereby characterized in being thermodynamically more stable than omeprazole sodium form A and any other form of omeprazole sodium prepared according to prior art. Omeprazole sodium form B is further characterized as being essentially non-hygroscopic.

With the expression "any other form" is meant anhydrates, hydrates, solvates and amorphous materials, including polymorphs disclosed in the prior art. Examples of any other forms of sodium salts of omeprazole includes, but are not limited to, anhydrates, monohydrates, dihydrates, sesquihydrates, trihydrates, alcoholates and polymorphs or amorphous forms thereof.

Omeprazole sodium form B is characterized by the positions and intensities of the peaks in the X-ray powder diffractogram, as well as by the unit cell parameters which have been calculated from the peak positions. The corresponding data for omeprazole sodium form A are totally different, whereas form B is easily distinguishable from form A.

Omeprazole sodium form B according to the present invention is characterized in providing an X-ray powder diffraction pattern exhibiting substantially the following d-values;

d-value/Å	relative intensity	d-value/Å	relative intensity
9.8	vs	3.37	w
7.8	vw	3.25	vw
6.7	s	3.17	vw
6.5	s	3.14	w
6.2	vw	3.12	m
5.9	m	3.05	w
5.8	vw	2.99	w
5.4	w	2.98	m
5.1	w	2.91	m
4.6	m	2.89	m
4.5	m	2.79	vw
4.3	s	2.62	vw
4.1	m	2.59	vw
3.96	m	2.50	vw
3.92	m	2.45	vw
3.71	s	2.40	vw
3.60	w	2.37	vw
3.43	vw	2.28	vw

Omeprazole sodium form B according to the present invention is characterized by having a monoclinic unit cell with parameters

a = 15.09 Å, b = 12.83 Å, c = 9.82 Å, β = 94.4°.

According to the invention there is further provided a process for the preparation of omeprazole sodium form B as well as a process for the preparation of omeprazole sodium form A.

Omeprazole sodium form B can also be characterized by FT-IR.

Omeprazole sodium form B is prepared by treating omeprazole with an aqueous base, $Na^+ B^-$, wherein Na denotes sodium and B denotes hydroxide or alkoxide, in an appropriate solvent, such as isopropanol optionally containing some water, at ambient temperature. Once the mixing has taken place the total mixture may be agitated, for example stirred, for a further period of time, *e.g.* about 0-2 hours, at ambient temperature. The crude mixture may optionally be filtered at this stage. Seeds of omeprazole sodium form B may be added to the crystallization solution in order to induce the crystallization. The slurry is thereafter further agitated for a time period of about 10-24 h to ensure as complete crystallization as possible. It is also possible to cool the mixture in order to complete the crystallization and thereby improving the yield. The omeprazole sodium form B is thereafter separated, for example by filtration or centrifugation, followed by washing with an appropriate solvent, preferably the same solvent as used above, and thereafter dried to constant weight.

Omeprazole sodium form B may also be prepared by recrystallizing the sodium salt of omeprazole of any form, or mixtures thereof, in an appropriate solvent such as ethanol or isopropanol, optionally containing some water.

The omeprazole sodium form B obtained according to the present invention is substantially free from other forms of sodium salts of omeprazole, such as omeprazole sodium form A.

The compound of the invention, *i.e.* omeprazole sodium form B, prepared according to the present invention is analyzed, characterized and differentiated from omeprazole sodium form A by X-ray powder diffraction, a technique which is known per se. Another suitable

technique to analyze, characterize and differentiate omeprazole sodium form B from omeprazole sodium form A is by conventional FT-IR.

The amount of water in omeprazole sodium form B and omeprazole sodium form A is determined by thermogravimetric analysis, a technique which is known per se. The water content can also be determined by Karl Fischer.

Omeprazole sodium form B is effective as a gastric acid secretion inhibitor, and is useful as an antiulcer agent. In a more general sense, it can be used for treatment of gastric-acid related conditions in mammals and especially in man, including *e.g.* reflux esophagitis, gastritis, duodenitis, gastric ulcer and duodenal ulcer. Furthermore, it may be used for treatment of other gastrointestinal disorders where gastric acid inhibitory effect is desirable *e.g.* in patients on NSAID therapy, in patients with Non Ulcer Dyspepsia, in patients with symptomatic gastro-esophageal reflux disease, and in patients with gastrinomas. The compound of the invention may also be used in patients in intensive care situations, in patients with acute upper gastrointestinal bleeding, pre- and postoperatively to prevent aspiration of gastric acid and to treat stress ulceration. Further, the compound of the invention may be useful in the treatment of psoriasis as well as in the treatment of *Helicobacter* infections and diseases related to these. The compound of the invention may also be used for treatment of inflammatory conditions in mammals, including man.

Any suitable route of administration may be employed for providing the patient with an effective dosage of omeprazole sodium form B according to the invention. For example, peroral or parenteral formulations and the like may be employed. Dosage forms include capsules, tablets, dispersions, solutions, suspensions and the like. Omeprazole sodium form B is, because of it being highly soluble in water, especially suitable for parenteral formulations, such as for intravenous administration.

According to the invention there is further provided a pharmaceutical composition comprising omeprazole sodium form B, as active ingredient, in association with a

pharmaceutically acceptable carrier, diluent or excipient and optionally other therapeutic ingredients. Compositions comprising other therapeutic ingredients are especially of interest in the treatment of *Helicobacter* infections. The invention also provides the use of omeprazole sodium form B in the manufacture of a medicament for use in the treatment of a gastric-acid related condition and a method of treating a gastric-acid related condition which method comprises administering to a subject suffering from said condition a therapeutically effective amount of omeprazole sodium form B.

The compositions of the invention include compositions suitable for peroral or parenteral administration. The compositions may be conveniently presented in unit dosage forms, and prepared by any methods known in the art of pharmacy.

Combination therapies comprising omeprazole sodium form B and other active ingredients in separate dosage forms may also be used. Examples of such active ingredients include anti-bacterial compounds, non-steroidal anti-inflammatory agents, antacid agents, alginates and prokinetic agents.

In the practice of the invention, the most suitable route of administration as well as the magnitude of a therapeutic dose of omeprazole sodium form B in any given case will depend on the nature and severity of the disease to be treated. The dose, and dose frequency, may also vary according to the age, body weight, and response of the individual patient. Special requirements may be needed for patients having Zollinger-Ellison syndrome, such as a need for higher doses than the average patient. Children and patients with liver diseases as well as patients under long term treatment will generally benefit from doses that are somewhat lower than the average. Thus, in some conditions it may be necessary to use doses outside the ranges stated below. Such higher and lower doses are within the scope of the present invention.

In general, a suitable dose range for parental administration is from 10 mg to 300 mg, and preferably from 20 mg to 80 mg.

A suitable oral dosage form may cover a dose range from 5 mg to 300 mg total daily dose, administered in one single dose or equally divided doses. A preferred dosage range is from 10 mg to 80 mg.

The compound of the invention may be combined as the active component in intimate admixture with a pharmaceutical carrier according to conventional techniques, such as the oral formulations described in WO 96/01623 and EP 247 983, the disclosures of which are hereby incorporated as a whole by reference.

The examples which follow will further illustrate the preparation of the compound of the invention, i.e. omeprazole sodium form B, but are not intended to limit the scope of the invention as defined hereinabove or as claimed below.

Examples

Example 1

Preparation of omeprazole sodium form B from omeprazole

120 gram of omeprazole, 480 ml of isopropanol and 13.2 gram of NaOH(s) dissolved in 26.7 gram of water, was added to a 3-necked glass vessel. The slurry was stirred for an additional 40 minutes at ambient room temperature. The obtained solution was filtered through a clarifying filter and the filter was washed with 20 ml of isopropanol. The isopropanol wash was combined with the previous isopropanol solution containing the product. The solution was seeded with 6 gram of omeprazole sodium form B in 25 ml of isopropanol. The slurry was stirred for an additional 25 hours and the product was filtered and dried at 40°C.

Yield 84.5 %.

Example 2

Preparation of omeprazole sodium form B from omeprazole sodium form A

30 gram of omeprazole sodium form A, prepared according to example 3 below, and 25 ml of ethanol was added to a 3-necked glass vessel. The slurry was seeded with omeprazole sodium form B and then stirred for an additional 24 hours at room temperature. The product was then filtered and dried at 50°C.
Yield: 80%

Example 3

Preparation of omeprazole sodium form A from omeprazole

14.8 kg sodium hydroxide was dissolved in 42 l water in a separate vessel.

120 kg omeprazole was added to 927 l isopropanol in a 4000 l glass lined reactor. The aqueous sodium hydroxide was charged to the slurry. Omeprazole was dissolved and the clear solution was filtered in a closed pressure filter to a 1200 l glass lined reactor. The solution was heated and 228 l methanol was charged at 50 °C to initiate the crystallization. The batch was seeded with a slurry of 1.2 kg omeprazole sodium methanol wet in isopropanol. The solution was cooled from 51 °C to - 8 °C. The formed slurry was kept at - 8 to - 9 °C for 4 hours with moderate stirring. Centrifuged substance was flushed with a cool mixture of isopropanol and methanol, 76 l and 20 l respectively, and then dried in a rotary dryer at approximately 35 mbar with a jacket temperature of 65 °C. Dried substance was de-lumped in a mill.

Yield: 126.0 kg omeprazole sodium methanol wet.

A sample of the omeprazole sodium methanol wet (32.3 kg) was charged into a rotary dryer. An equilibration process with steam in order to remove methanol was performed at 39 - 87 mbar and with a jacket temperature of 50 °C. The equilibration process took 3 days. Equilibrated substance was de-lumped in a mill.

Yield: 25.7 kg

Example 4

Characterization of omeprazole sodium form B and omeprazole sodium form A using X-ray powder diffraction (XRPD)

X-ray powder diffraction analysis was performed according to standard methods which can be found in *e.g.* Bunn, C. W. (1948), Chemical Crystallography, Clarendon Press, London; or Klug, H.P. & Alexander, L. E. (1974), X-Ray Diffraction Procedures, John Wiley and Sons, New York. The unit cell parameters for form A and B have been calculated from the X-ray powder diffractograms using the program "TREOR" by Werner,P.-E., Eriksson,L. And Westdahl,M., J. Appl. Crystallogr. 18 (1985) 367 - 370. The fact that the positions of all peaks in the diffractograms for form A and form B may be calculated using the respective unit cell parameters, proves that the unit cells are correct and that the diffractograms are indicative of the pure forms. The diffractogram of omeprazole sodium form B, prepared according to Example 1 in the present application, is shown in Figure 1 and the diffractogram of omeprazole sodium form A, prepared according to Example 3, is shown in Figure 2.

The peaks, identified with d-values calculated from the Bragg formula and intensities, have been extracted from the diffractograms for form A, form B and from the diffractogram obtained from material produced according to prior art, and are given in Table 1. In this table the unit cell parameters for forms A and B are also given. The relative intensities are less reliable and instead of numerical values the following definitions are used;

% Relative Intensity	Definition
25-100	vs (very strong)
10-25	s (strong)
3-10	m (medium)
1-3	w (weak)
<1	vw (very weak)

Some additional very weak peaks found in the diffractograms have been omitted from table 1.

Reference Example A

Preparation of omeprazole sodium according to prior art in accordance with the method described in Example 2 in EP 124 495

Omeprazole (1300 g; 3.77 mol) was added under vigorous mechanical stirring to a mixture of tetrahydrofurane (13 L) and 50% aqueous NaOH (296 g, 3.7 mol) and stirring was continued for 45 min. Trichloroethylene (5.7 L) was added and stirring was continued over night at room temperature. The mixture was cooled to +5°C and then stirred for 3 h. The precipitate was filtered off and the filter cake was washed with trichloroethylene (5 L) and dried under reduced pressure at 50°C giving omeprazole sodium salt (1314 g, 95%), m.p. 208-210 °C.

The product was analyzed using X-ray powder diffraction and gave the diffractogram depicted in Figure 3 and given above in Table 1. Some additional very weak peaks found in the diffractograms have been omitted from Table 1.

Table 1. X-ray powder diffraction data for omeprazole sodium form A, form B and according to prior art. Only peaks below 2θ = 40° have been included.

All peaks noted for form A and form B can be indexed with the unit cells given below.

Unit cell form A: a = 15.757 (3) Å, b= 8.126 (1) Å, c= 15.671 (6) Å, β= 94.21 (2) °

Unit cell form B: a= 15.086 (6) Å, b= 12.835 (4) Å, c= 9.815 (3) Å, β= 94.41 (3)°

Omeprazole sodium form A		Omeprazole sodium form B		Omeprazole sodium according to prior art	
d-value/Å	Relative intensity	d-value/Å	relative intensity	d-value/Å	Relative intensity
				17.8	vw
15.6	vs	9.8	vs	15.5	vs
				13.9	vw
				10.2	vw
				8.9	m
7.9	m	7.8	vw	8.0	m
7.2	m	6.7	s	7.2	m
				6.9	w
6.8	w	6.5	s	6.8	w
6.6	vw	6.2	vw		
6.5	w	5.90	m	6.5	vw
				6.4	vw
				6.2	vw
				5.91	vw
				5.83	w
				5.52	vw

Table 1, continued

Omeprazole sodium form A		Omeprazole sodium form B		Omeprazole sodium according to prior art	
d-value/Å	Relative intensity	d-value/Å	relative intensity	d-value/Å	Relative intensity
5.35	vw	5.76	vw	5.37	w
5.20	s	5.36	w	5.21	w
				5.15	m
				4.81	vw
4.70	vw	5.12	w	4.70	vw
				4.63	vw
4.40	vw	4.57	m	4.40	vw
4.29	vw	4.46	m		
				4.27	vw
4.17	vw	4.29	s	4.17	vw
3.935	s	4.11	m	3.938	w
				3.846	vw
3.831	w	3.963	m		
3.744	w	3.920	m	3.748	vw
				3.711	vw
3.611	w	3.713	s	3.610	vw
3.543	w	3.601	w	3.545	w
3.522	w	3.431	vw	3.519	vw
3.488	w	3.375	w		
				3.464	vw
3.411	vw	3.254	vw	3.410	vw
				3.304	vw
				3.256	vw
				3.151	vw

Table 1, continued

Omeprazole sodium form A		Omeprazole sodium form B		Omeprazole sodium according to prior art	
d-value/Å	Relative intensity	d-value/Å	relative intensity	d-value/Å	Relative intensity
3.125	m	3.173	vw	3.125	vw
				3.079	vw
3.021	vw	3.137	w	3.026	vw
2.919	w	3.119	m	2.911	vw
				2.854	vw
2.833	w	3.050	w		
				2.775	vw
2.676	vw	2.993	w		
2.626	vw	2.980	m		
2.606	vw	2.906	m	2.601	vw
				2.553	vw
2.534	vw	2.892	m		
2.425	vw	2.793	vw	2.430	vw
		2.624	vw		
		2.589	vw		
		2.499	vw		
		2.447	vw		
		2.402	vw		
		2.372	vw		
		2.283	vw		

CLAIMS

1. Omeprazole sodium form B, characterized in being thermodynamically stable.

2. Omeprazole sodium form B, characterized in being essentially non-hygroscopic.

3. Omeprazole sodium form B according to claim 1 or claim 2, characterized in providing an X-ray powder diffraction pattern exhibiting substantially the following d-values and intensities;

d-value/Å	relative intensity	d-value/Å	relative intensity
9.8	vs	3.37	w
7.8	vw	3.25	vw
6.7	s	3.17	vw
6.5	s	3.14	w
6.2	vw	3.12	m
5.9	m	3.05	w
5.8	vw	2.99	w
5.4	w	2.98	m
5.1	w	2.91	m
4.6	m	2.89	m
4.5	m	2.79	vw
4.3	s	2.62	vw
4.1	m	2.59	vw
3.96	m	2.50	vw
3.92	m	2.45	vw
3.71	s	2.40	vw
3.60	w	2.37	vw
3.43	vw	2.28	vw

4. Omeprazole sodium form B according to any of claims 1-3, characterized by having a monoclinic unit cell with parameters

a = 15.09 Å, b = 12.83 Å, c = 9.82 Å, $\beta = 94.4°$.

5. A process for the preparation of omeprazole sodium form B as defined in any of claims 1-4, which includes the step of;

a) preparing the sodium salt of omeprazole by addition of an aqueous base to omeprazole in a solvent mixture comprising an alcohol and water,

b) allowing the solution to crystallize, optionally using omeprazole sodium form B to induce crystallization;, and

c) isolating the omeprazole sodium form B thus obtained.

6. A process according to claim 5, wherein said aqueous base used in step a) is sodium hydroxide.

7. A process according to any of claims 5-6, wherein said alcohol used in step a) is isopropanol.

8. A process for the preparation of omeprazole sodium form B as defined in any of claims 1-4, comprising the steps of;

a) dissolving omeprazole sodium of any form, or a mixture of any forms, in a solvent mixture comprising alcohol and water;

b) allowing the solution to crystallize, optionally using omeprazole sodium form B to induce crystallization, and

c) isolating the omeprazole sodium form B thus obtained.

9. A pharmaceutical formulation comprising omeprazole sodium form B as defined in any of claims 1-4 in admixture with a pharmaceutically acceptable excipient.

10. A pharmaceutical formulation suitable for i.v. administration comprising omeprazole sodium form B as defined in any of claims 1-4 in admixture with a pharmaceutically acceptable excipient.

11. The use of omeprazole sodium form B as defined in any of claims 1-4, as active ingredient in the manufacture of medicament for use in treatment of gastrointestinal disorders.

12. The use of omeprazole sodium form B as defined in any of claims 1-4 in the manufacture of a pharmaceutical formulation for i.v. administration.

13 A method of treatment of gastrointestinal disorders which comprises administration of a therapeutically effective amount of omeprazole sodium form B as defined in any of claims 1-4, to a patient suffering from gastrointestinal disorders.

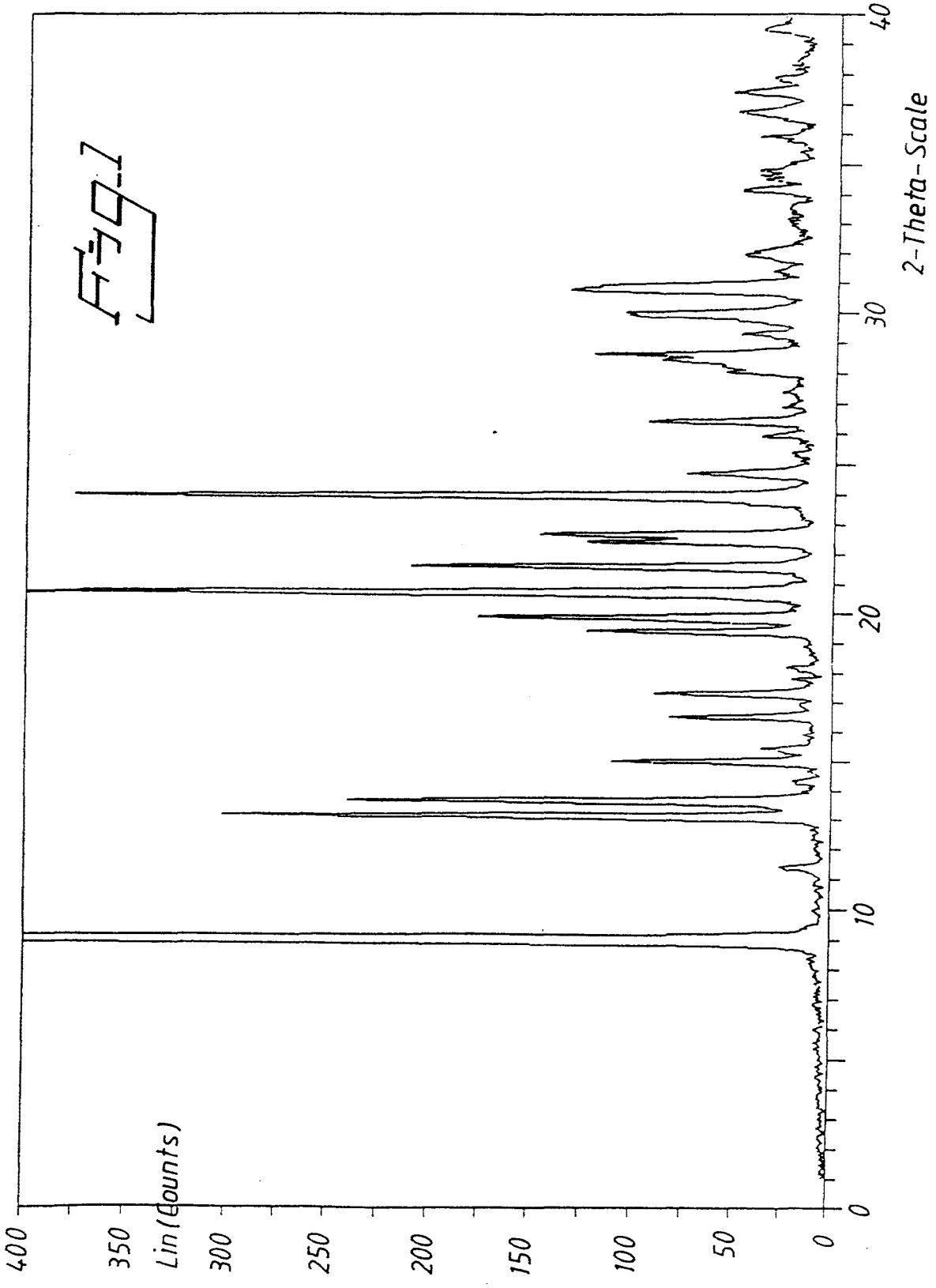
Fig 1
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100
150
200
250
300
350
400
0
10
20
30
40
2-Theta-Scale

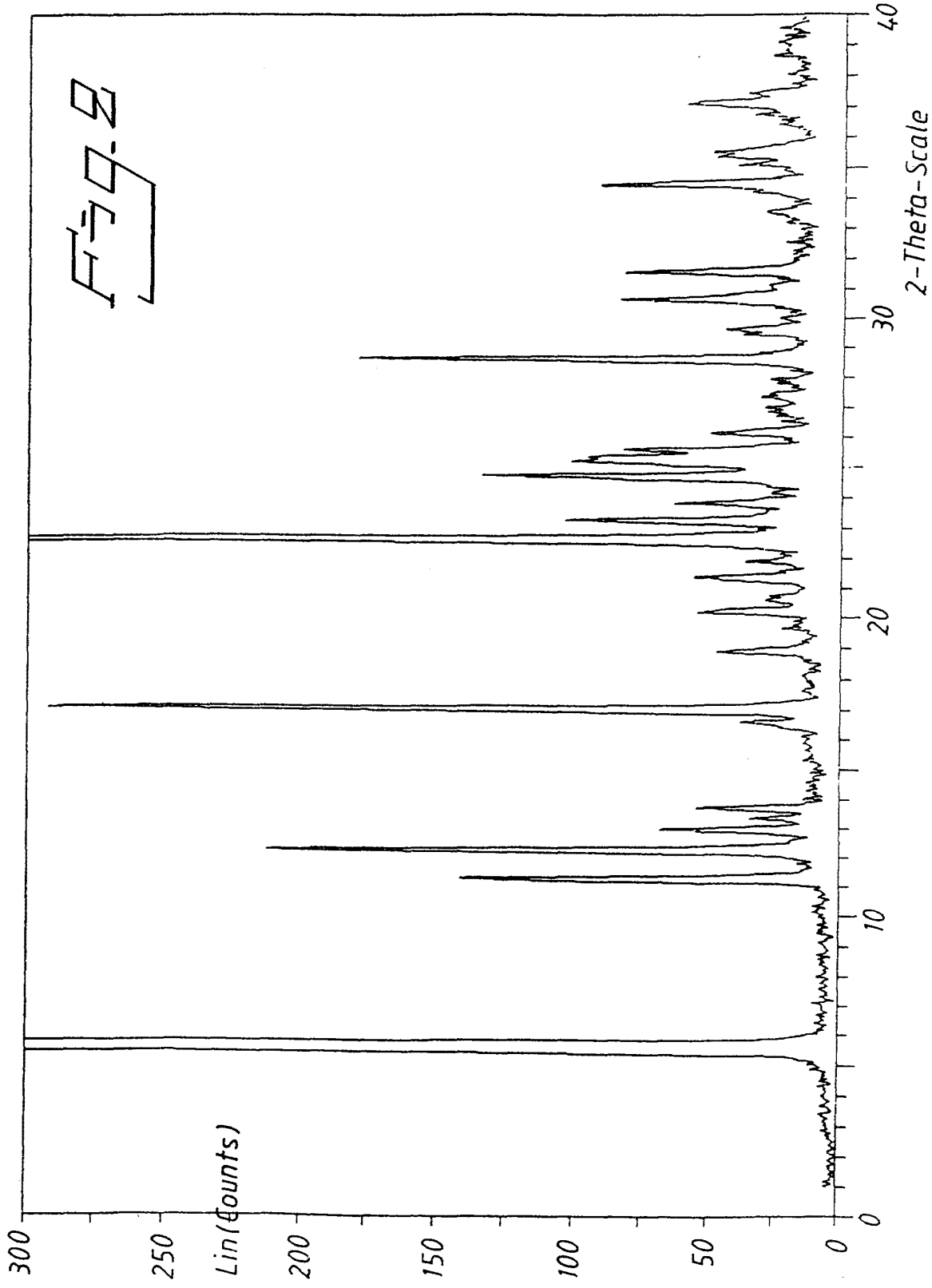
Fig 2
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2-Theta-Scale

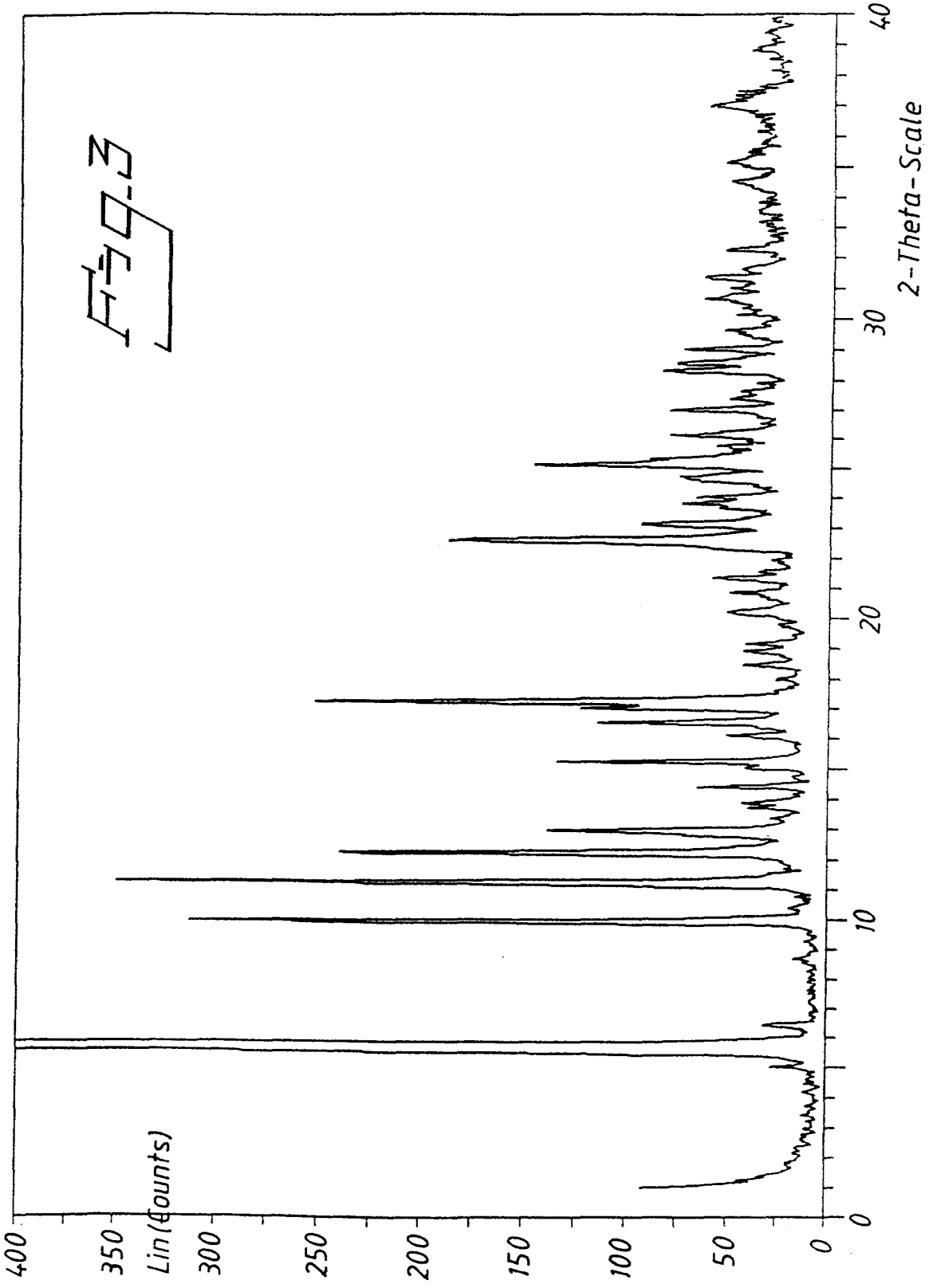
Fig. 3
Lin (Counts)
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200
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300
350
400
0
10
20
30
40
2-Theta-Scale

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01124

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07D 401/12, A61K 31/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0124495 A2 (AKTIEBOLAGET HÄSSLE), 7 November 1984 (07.11.84) -- --------	1-12

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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Date of the actual completion of the international search	Date of mailing of the international search report
23 Sept 1998	12 -10- 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Göran Karlsson Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01124

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13
because they relate to subject matter not required to be searched by this Authority, namely:

A method for treatment of the human or animal body by therapy, see rule 39.1.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No.
PCT/SE 98/01124

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0124495 A2	07/11/84	SE 0124495 T3	
		AU 563842 B	23/07/87
		AU 2525784 A	06/09/84
		BG 44538 A	15/12/88
		BG 60837 B	30/04/96
		CA 1264751 A	23/01/90
		CS 241150 B	13/03/86
		CS 8401515 A	13/06/85
		DK 99584 A	05/09/84
		DK 160044 B,C	21/01/91
		FI 83649 B	30/04/91
		FI 840851 A	05/09/84
		GB 2137616 A,B	10/10/84
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		US 4738974 A	19/04/88

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(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING A SALT OF ACETAMINOPHEN AND AT LEAST ONE OTHER ACTIVE INGREDIENT

(57) Abstract

Pharmaceutical composition comprising an alkali or alkaline–earth metal salt of acetaminophen and at least one other active ingredient selected from the group consisting of analgesics, decongestants, expectorants, antitussives, antihistamines, gastrointestinal agents, diurectics, bronchodilators and mixtures thereof.

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PHARMACEUTICAL COMPOSITION CONTAINING A SALT OF ACETAMINOPHEN AND AT LEAST ONE OTHER ACTIVE INGREDIENT

This is a continuation-in-part of application Serial No. 08/987,210, filed December 9, 1997, which is a continuation-in-part of application Serial No. 08/771,176, filed December 20, 1996, both of which are hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to salts of acetaminophen and, more particularly, to alkali metal and alkaline-earth metal salts of acetaminophen.

BACKGROUND OF THE INVENTION

Acetaminophen (APAP) is a well established therapeutic agent having both analgesic and antipyretic activity. Acetaminophen's relatively poor solubility in water and its bitter taste, however, make it difficult to formulate into to consumer acceptable oral dosage forms. Most commercially available acetaminophen oral dosage forms incorporate a taste masking coating on the acetaminophen particles or employ flavors and sweeteners to mask the bitter taste of the drug.

Other approaches for dealing with the solubility and taste of acetaminophen include the formation of amino acid esters of acetaminophen. I. M. Kovach in *Diss. Abstr. Int. B* 1975, 36(2), 734-5 describes the synthesis of p-acetamidophenyl glycinate (APG), α-p-acetamidophenyl aspartate (AAPA) and β-p-acetamidophenyl aspartate (BAPA). These esters are reported to have a less bitter taste than acetaminophen. APG-HBr was five times more water soluble than acetaminophen, whereas BAPA-HCl was four times less water soluble than APAP.

It is also known that the formation of an appropriate salt of a hydrophobic compound, such as a lipophilic carboxylic acid, will usually improve the aqueous solubility of the compound. Sodium ibuprofen and sodium naproxen are examples of pharmaceutically active lipophilic carboxylic acids which have improved aqueous solubility in their salt form. These salts are typically formed by reacting the carboxylic acid with a strong base, such as sodium hydroxide or potassium hydroxide.

USSR Inventor's Certificate No. 629,209, published September 11, 1978, describes a method of preparing bis-[β-(4-acetylaminophenyloxy)ethyl] ether by reacting 4-acetylaminophenol with an alkaline agent, such as potassium hydroxide, in a solution of an organic solvent, such as dimethylformamide, followed by reacting the resulting solution of potassium phenolate with chlorex at the boiling point of the reaction mixture. The resulting ether is reported as being useful for the treatment of animals with helminthic diseases.

USSR Inventor's Certificate 1,803,833, published March 23, 1993, describes a method of preparing acetaminophen for fluorescence intensity measurements. The acetaminophen sample was prepared by first dissolving in isopropyl alcohol and then treating with an 8% solution of potassium hydroxide solution and chloroform at a KOH:chloroform volume ratio of 3-4. Heating was then carried out for 15-20 minutes at 70-80°C before the measurement of the sample's fluorescence intensity.

While both of the of thc above-identified USSR Inventor's Certificates report the treatment of acetaminophen with potassium hydroxide, neither document reports the isolation of any potassium salt of acetaminophen.

M.S. Yu et al. in US Patent No. 5,360,615 discusses a pharmaceutical carrier system for enhancing the solubility of acidic, basic or amphoteric pharmaceuticals by partial ionization to produce a highly concentrated primarily non-aqueous

solution suitable for filling softgels or for two-piece encapsulation or tablet formation. The acetaminophen solution comprised 25-40% (wt.) of acetaminophen, 0.4-1.0 moles of hydroxide ion per mole of acetaminophen and 1-20% (wt.) water in polyethylene glycol. An exemplary concentrated solution of acetaminophen suitable for use as a softgel fill contained 1 equivalent APAP (35% by wt.), 1 equivalent potassium hydroxide, and the balance polyethylene glycol 600.

US Patent No. 5,273,759 to D.L. Simmons describes the addition of $Mg(OH)_2$ in solid form to tablets containing APAP.

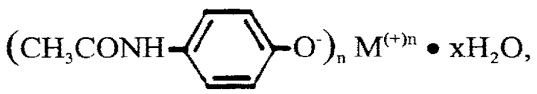
Both Yu et al. and Simmons fail to report the isolation of any discrete salts of acetaminophen.

A need exists for isolated salts of acetaminophen with improved aqueous solubility and taste when compared to the conventional form of acetaminophen.

SUMMARY OF THE INVENTION

The present invention provides isolated salts of acetaminophen. The isolated salts are preferably the alkali metal and alkaline-earth metal salts of acetaminophen.

In a further aspect of the invention the isolated salts have the formula:



wherein n is 1 or 2, M is alkali metal when n is 1 and M is alkaline-earth metal when n is 2 and x is from 0 to about 10. These salts have been shown to have both improved aqueous solubility and a less bitter taste than the free acid form of acetaminophen. The invention also includes methods of making such salts.

The present invention also provides compositions comprising the isolated salts of acetaminophen and at least one other active ingredient selected from the group consisting of analgesics, decongestants, expectorants, antitussives, antihistamines, diuretics, gastrointestinal agents, diuretics, bronchodilators, sleep-inducing agents, and mixtures thereof.

Another aspect of the invention relates to the method of administering such salts, alone or in combination with other active ingredients, to mammals in the need of an analgesic and/or antipyretic therapeutic agent. The present invention further relates to orally adminsterable dosage forms containing salts of acetaminophen, alone or in combination with such other active ingredients.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a plot the results of dissolution tests for tablets containing acetaminophen free acid and the isolated salts of acetaminophen.

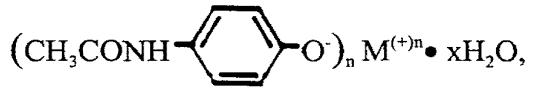
Figure 2 is a plot of acetaminophen plasma concentrations versus time for the bioequivelency study in dogs described in Example VII.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Prior to the present invention there has been no reported isolation of any discreet salts (phenolates) of APAP. Furthermore, *in situ* solution characterization of any deprotonated APAP species has not been reported either. As used in the present invention, the "free acid" of acetaminophen means the protonated phenolic form of APAP.

The lack of discussion on APAP salts in the scientific literature may be due in part to the fact that the anionic form of APAP is stable in aqueous solution (pH > 11) for only a short period of time (< 24h). If the salt is not quickly isolated after formation, p-aminophenolate (PAP) can form and result in discoloration of the resulting product.

As used in the present invention, isolated salts of acetaminophen refers to salts of p-hydroxyacetanilide which are formed by the deprotonation of the phenolic proton of acetaminophen. The isolated salts are preferably the alkali metal and alkaline-earth metal salts of acetaminophen. In a further aspect of the invention the isolated salts have the formula:



wherein n is 1 or 2, M is alkali metal when n is 1 and M is alkaline-earth metal when n is 2 and x is from 0 to about 10.

The salts of APAP are prepared via a one step aqueous reaction of APAP with the desired mono or divalent metal hydroxide. Suitable mono or divalent metal hydroxides include sodium hydroxide, calcium hydroxide, lithium hydroxide, potassium hydroxide, magnesium hydroxide and cesium hydroxide. The molar ratio of hydroxide to acetaminophen is about 1:2 to about 10:1, preferably about 1:2 to about 1:1. The APAP and metal hydroxide are dissolved in water or a mixture of water and a water-miscible organic solvent, such as acetonitrile, methanol, isopropanol, ethanol or tetrahydrofuran. The crude reaction products are then recovered or isolated by precipitation upon the addition of a less polar water miscible solvent such as acetonitrile, ethanol or tetrahydrofuran. Alternatively, the crude product can be recovered or crystallized by cooling (0°C) or lyophilization of the reaction mixture. The recovery or isolation should generally be carried out as soon as the reaction product is formed so as to reduce the likelihood of product

discoloration due to the formation of PAP. The final product may be dried under vacuum.

The APAP salts of the present invention are also amenable to cation exchange reactions. For example, an aqueous slurry or solution of a monovalent metal salt of acetaminophen is contacted with a divalent metal cation whereby the anhydrous, divalent metal salt of acetaminophen is formed via a cation exchange reaction. The salt is then immediately recovered. Specifically, $C_{16}H_{16}N_2O_4Ca$ may be prepared by reacting an aqueous solution of $C_8H_8NO_2Na$ with 0.5 equivalent of calcium chloride ($CaCl_2$). After drying, the resulting $C_{16}H_{16}N_2O_4Ca$ was found to be anhydrous.

In addition to the anhydrous form, various hydration states of APAP salts can be prepared depending on the reaction conditions. These hydrated salts preferably have less than 10 moles of water per mole of APAP salt, and includes, for example, acetaminophen sodium pentahydrate, acetaminophen sodium hexahydrate, acetaminophen sodium heptahydrate, acetaminophen calcium dihydrate and acetaminophen lithium hexahydrate.

The aqueous solubility at 22°C of the APAP salts of the present invention is 490-540, 450-470 and 13 mg/mL for sodium, lithium and calcium, respectively. Accordingly, the sodium, lithium and calcium salts have solubilities equivalent to approximately 260-280, 250-270, and 10 mg/mL, respectively, of APAP free acid.

The APAP salts have significantly increased dissolution rates compared to the conventional free acid form of acetaminophen. In 0.1N hydrochloric acid using USP Dissolution Apparatus 2 (paddle speed: 50 rpm) at 37°C, the concentration of acetaminophen at 30 seconds was as follows:

APAP Form (Powder)	Mg/mL of APAP
Sodium Salt	0.30
Lithium Salt	0.32
Calcium Salt	0.20
Free Acid (control)	0.02

Figure 1 illustrates the tablet dissolution rates of the salts of the present invention. The sodium, lithium and calcium salts of APAP and the conventional form of APAP were each compressed into tablets and the dissolution rates were evaluated using the conditions described above. The dissolution media was assayed for acetaminophen in the free acid form. Figure 1 shows that the salts of the present invention have significantly higher acetaminophen dissolution rates that the conventional free acid.

The calcium and sodium salts of acetaminophen have been observed not to have the bitter properties of the conventional free acid form of acetaminophen. The calcium salt was almost tasteless, while the sodium salt was observed to be somewhat salty. The improved taste properties of the salts of the present invention will allow for acetaminophen oral dosage forms with improved taste to be formulated.

The onset of action of acetaminophen is believed to be hastened, relative to the free acid form, with the isolated salts of the present invention. The increase solubility of the salts of the present invention, results in faster peak acetaminophen plasma concentration. This property will potentially provide faster onset of action of the analgesic and/or antipyretic activity of acetaminophen.

The acetaminophen salts of the present invention may be administered to a mammal in a therapeutically effective amount, which is an amount that produces the desired therapeutic response upon oral administration, and can be readily determined by one skilled in the art. In determining such amounts, the particular compound being

administered, the bioavailability characteristics of the compound, the dose regime, the age and weight of the patient, and other factors must be considered. A typical unit dose orally administered to a human would range from about 80-1000 mg (APAP free acid basis) .

The compositions and methods of the present invention may also preferably include at least one other active ingredient selected from the group consisting of analgesics, decongestants, expectorants, antitussives, antihistamines, gastrointestinal agents, diuretics, bronchodilators, sleep-inducing agents and mixtures thereof. When the other active ingredient is selected from the group consisting of decongestants, expectorants, antitussives, antihistamines and mixtures thereof, the compositions are particularly useful for the treatment of cough, cold, cold-like and/or flu symptoms in mammals, such as humans. As used in the present invention, cold-like symptoms include coryza, nasal congestion, upper respiratory infections, allergic rhinitis, otitis, and sinusitis.

The analgesics useful in combination with the acetaminophen salts of this invention include acetyl salicylic acid, indomethacin, optically active isomers or racemates of ibuprofen, naproxen, flurbiprofen, carprofen, tiaprofenic acid, cicloprofen, ketoprofen, ketorolac, etodolac, indomethacin, sulindac, fenoprofen, diclofenac, piroxicam, benzydomine, nabumetone, tramadol, codeine, oxycodone, hydrocodone, pharmaceutically acceptable salts thereof and mixtures thereof. Cyclooxygenase-2 (COX-2) inhibitors, such as flosulide, nimesulide, celecoxib, 5-(4-aminosulfonyl-3-fluorophenyl)-4-cyclohexyl-2-methyloxazole, meloxicam, nabumetone, etodolac, pharmaceutically acceptable salts thereof and mixtures thereof, may be used as an analgesic in the present invention..

The decongestants (sympathomimetics) suitable for use in the compositions of the present invention include pseudoephedrine, phenylpropanolamine,

phenylephrine, ephedrine, pharmaceutically acceptable salts thereof and mixtures thereof.

The expectorants (also known as mucolytic agents) preferred for use in the present invention include guaifenesin, glyceryl guaiacolate, terpin hydrate, ammonium chloride, N acetylcysteine, bromhexine, ambroxol, domiodol, 3-iodo-1,2-propanediol, pharmaceutically acceptable salts thereof and mixtures thereof.

The antitussives preferred for use in the present invention include those such as dextromethorphan, chlophedianol, carbetapentane, caramiphen, noscapine, diphenhydramine, codeine, hydrocodone, hydromorphone, fominoben, benzonatate, pharmaceutically acceptable salts thereof and mixtures thereof.

The antihistamines which may be employed include chlorpheniramine, brompheniramine, dexchlorpheniramne, dexbrompheniramine, triprolidine, doxylamine, tripelennamine, cyproheptadine, hydroxtzine, pyrilamine, azatadine, promethazine, acrivastine, astemizole, cetirizine, ketotifen, loratidine, temelastine, terfenadine, norastemizole, fexofenadine, pharmaceutically acceptable salts thereof and mixtures thereof.

Examples of gastrointestinal agents preferred for use in the present invention include anticholinergics, including: atropine, clidinium and dicyclomine; antacids, including: aluminum hydroxide, bismuth subsalicylate, bismuth subcitrate, calcium carbonate and magaldrate; anti-gas agents, including simethicone; H2-receptor antagonists, including: cimetidine, famotidine, nizatidine and ranitidine; laxatives, including: phenolphthalein and casanthrol; gastroprotectants, including: sucralfate and sucralfate humid gel; gastrokinetic agents, including: metoclopramide and eisaprode; proton pump inhibitors, including omeprazole and antidiarrheals, including: diphenoxylate and loperamide; pharmaceutically acceptable salts thereof and mixtures thereof.

The diuretics useful in the invention include caffeine and pamabrom. Also useful are bronchodilators such as terbutaline, aminophylline, pinephrine, isoprenaline, metaproterenol, bitoterol, theophylline, albuterol, pharmaceutically acceptable salts thereof and mixtures thereof.

Sleep-inducing agents suitable for use in the invention include melatonin, estazolam, zolpidem, promethacine, pharmaceutically acceptable salts thereof and mixtures thereof.

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases including inorganic bases and organic bases. Salts derived from nonorganic bases include sodium, potassium, lithium, ammonia, calcium, magnesium, ferrous, zinc, manganous, aluminum, ferric, manganic salts and the like. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, tertiary and quaternary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as triethylamine, tripropylamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, lysine, arginine, histidine, caffeine, procaine, N-ethylpiperidine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglycamine, theobromine, purines, piperazine, piperidine, polyamine resins and the like.

As with the acetaminophen salts of the present invention, these other active ingredients are administered to a mammal in a therapeutically effective amount, which is an amount that produces the desired therapeutic response upon oral administration, and can be readily determined by one skilled in the art. In determining such amounts, the particular compound being administered, the bioavailability characteristics of the compound, the dose regime, the age and weight of the patient, and other factors must be considered. Many of these other active ingredients, as well as their acceptable dosage ranges are described in the following: U.S. Pat. No. 4,552,899 to Sunshine et

al., issued Nov. 12, 1985; U.S. Pat. No. 4,783,465 to Sunshine et al., issued Nov. 8, 1988; and U.S. Pat. No. 4,619,934 to Sunshine et al., issued Oct. 28, 1986, which are all incorporated by reference herein. Other antitussives, expectorants, antihistamines, decongestants, and gastrointestinal agents suitable for use in the invention are described in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa., 18th ed. Chapters 39, 42, 43, 58 and 59 (1990), which is hereby incorporated by reference. These other active ingredients may be administered concomitantly as a combination product with the acetaminophen salt or they may be administered as separate products prior to or after the administration of the APAP salt.

The acetaminophen salts of the present invention, alone or in combination with the other active ingredients, are generally administered orally in a solid dosage form. Suitable solid preparations include as swallowable, chewable or fast dissolving tablets, pills, capsules, caplets, powders, wafers, sachets, gelatin coated tablets, softgels and granules. In preparing solid dosage forms, the salt of acetaminophen, alone in combination with such other active ingredients, can be mixed with conventional solid fillers or carriers, such as corn starch, talc, calcium phosphate, calcium sulphate, calcium stearate, magnesium stearate, stearic acid, sorbitol, microcrystalline cellulose, mannitol, gelatin, natural or synthetic gums, such as carboxymethylcellulose, methylcellulose, alginate, dextran, acacia gum, karaya gum, locust bean gum and other conventional carriers. Additionally, other excipients such as diluents, binders, lubricants, disintegrants, colors and flavoring agents may be employed. The dosage form can also be film coated. It may also be desirable to coat the acetaminophen salt and/or other active ingredients with a conventional, pharmaceutically acceptable polymeric film prior to the preparation of the dosage form.

Conventional methods can be used for preparing the solid dosage forms of the present invention. Suitable techniques are described in *Remington's Pharmaceutical Sciences*, 18th Ed., Chapter 89 (1990) which is hereby incorporated by reference.

The following example illustrates a specific embodiment of the present invention. This invention, however, is not confined to the specific limitations set forth in this example but rather to the scope of the appended claims. Unless otherwise stated, the percentages and ratios given below are by weight.

EXAMPLE I

This Example discloses the preparation of acetaminophen sodium ($C_8H_8NO_2Na•6H_2O$).

30 mL 1N NaOH solution (0.030 mol) were added to a stirred suspension of 4.53 g (0.033 mol) acetaminophen in 25 mL water. After all solids dissolved, 200 mL acetonitrile was added while the solution was rapidly stirred. The resulting white precipitate (9.15 g, 99% yield as the 6-hydrate) was collected on a frit, washed with tetrahydrofuran (THF) and dried at room temperature. ^1H NMR (DMF d_7) δ 9.4 (s, 1H, NH), 7.1 (m, 2H, Ar-H), 6.3 (m, 2H, Ar-H),1.96 (s, 3H, CO-CH_3); IR (cm^{-1}, KBr) 3421 (broad, OH), 1635 (sharp, CO), 1594 (sharp), 1534 (sharp), 1500 (sharp), 1279 (sharp) ; Combustion analysis calculated for $C_8H_8NO_2Na•6H_2O$: C 34.16, H 7.12, N 4.98; found C 34.05, H 6.96, N 5.00; Water content calculated for $C_8H_8NO_2Na•6H_2O$: 38%, Found: 38% (Karl Fischer); FAB mass spectral analysis m/e calculated for $C_8H_8NO_2Na•6H_2O$:173, found 174 (M + 1). The aqueous solubility at 22°C was 493 mg/mL.

EXAMPLE II

This Example discloses the preparation of acetaminophen sodium ($C_8H_8NO_2Na•7H_2O$).

80g (2.00 mol) NaOH was dissolved in 400 mL water and added dropwise to a flask charged with 302g (2.00 mol) APAP dissolved in 2100 mL *i*-propanol, at 50°C with stirring. The solution was cooled to room temperature, whereupon an off-

white precipitate formed. The solids were filtered, washed with three 200 mL portions of *i*-propanol, and dried under a vacuum (500g, 84 % as the 7-hydrate). The ^{1}H NMR and IR spectra were identical to that of $C_8H_8NO_2Na•6H_2O$. Combustion analysis calculated for $C_8H_8NO_2Na•7H_2O$: C 32.11 H 7.41 N 4.68; Found: C 31.99, H 7.38, N 4.31; Water content calculated for $C_8H_8NO_2Na•7H_2O$: 42.1%; Found 42.7% (Karl Fischer). The aqueous solubility at 22°C was 541 mg/mL.

EXAMPLE III

This Example discloses the preparation of acetaminophen calcium ($C_{16}H_{16}N_2O_4Ca•2H_2O$).

5g (0.033 mol) APAP and 1.22g (0.016 mol) $Ca(OH)_2$ were suspended in 200 mL water and the mixture was stirred for 4h, whereupon all solids went into solution. The solution was frozen in a bath of liquid nitrogen and lyophilized, leaving a light microcrystalline off-white solid (5.44g, 100% crude yield based on the hydrate X 2). ^{1}H NMR (DMF d_7) δ 9.39 (s, 2H, NH), 7.15 (m, 4H, Ar), 6.80 (m, 4H, Ar), 2.10 (s, 6H, CO-CH_3). IR 3287 (broad), 1648 (sharp, C=O), 1594, 1541, 1500, 1279 (sharp) Combustion analysis calculated for $C_{16}H_{16}N_2O_4Ca•2H_2O$: C 51.05, H 5.36, N 7.45; 9.6, Found: C 51.21, H 5.21, N 7.63. Water content calculated (Karl Fischer) for $C_{16}H_{16}N_2O_4Ca•2H_2O$: 9.6%, Found: 9.8%. The aqueous solubility at 22°C was 13 mg/mL.

EXAMPLE IV

This Example discloses the preparation of acetaminophen lithium ($C_8H_8NO_2Li•6H_2O$).

5g (0.033 mol) APAP was dissolved in 30 mL i-propanol/THF (1:3, degassed with argon). This solution was added rapidly to a flask charged with 1.38g of (0.033 mol) $LiOH•H_2O$ dissolved in 20 mL water (argon degassed). The colorless solution was stored at 0° C for 16 h, whereupon white crystals formed. The crystals were filtered under argon, washed with THF and dried under a vacuum for 16 h (4.25g, 6 hydrate). ^1H NMR (DMF-d^7) δ 9.39 (s, 1H, NH), 7.15 (m, 2H, Ar-H), 6.80 (m, 2H, Ar-H), 2.10 (s, 3H, CO-CH_3); IR 3568 (sharp), 3402, 3243 (broad), 1672, 1618 (sharp), 1533, 1501, 1407, 1267, 1174 (sharp). Combustion analysis calculated for $C_8H_8NO_2Li•6H_2O$: C 36.23, H 7.60, N 5.28; Found: C 36.67, H 7.68, N 5.23; Water content calculated (Karl Fischer) for $C_8H_8NO_2Li•6H_2O$: 40.1%, Found: 38.4%. The aqueous solubility at 22°C was 455 mg/mL.

EXAMPLE V

This Example discloses an alternative preparation of acetaminophen lithium ($C_8H_8NO_2Li•6H_2O$).

Acetaminophen (15.1g; 0.1 mol), water, 90 mL and lithium hydroxide 1 N (100 mL, 0.1 mol) were placed in a 2 L beaker. After the solution became clear, acetonitrile (1500 mL) was added. The resulting white solids were filtered, washed with THF (ca. 500 mL) and dried at ambient leaving a dry white solid (23.0 g, 87% based on $C_8H_8NO_2Li•6H_2O$). ^1H NMR (DMF-d^7) δ 2.0 (s,3H, CO-CH3), 6.5 (m, 2H, Ar-H), 7.2 (m, 2H, Ar-H), 9.3 (s,1H, Ac-NH-Ar); IR 3568 (sharp), 3402, 3243 (broad), 1672, 1618 (sharp), 1533, 1501, 1407, 1267, 1174 (sharp). Combustion analysis calculated for $C_8H_8NO_2Li•6H_2O$: C 36.23, H 7.60, N 5.28; Found: C 36.56, H 7.56, N 5.05. Water content calculated (Karl Fischer) for $C_8H_8NO_2Li•6H_2O$: 40.1%, Found: 40.0%. The aqueous solubility at 22°C was 472 mg/mL.

EXAMPLE VI

This Example discloses the preparation of an anhydrous acetaminophen calcium ($C_{16}H_{16}N_2O_4Ca$).

Acetaminophen (90.6g, 0.60 mol) was suspended in 135 mL water and a solution containing sodium hydroxide (24.0g, 0.6 mol) and 36mL water was added at 18-26°C over 30 min. To the resulting NaAPAP-slurry, a solution containing calcium chloride ($CaCl_2$) (44.1g, 0.3 mol) and 54 mL water was added at 20-25°C over 30 min. at room temperature. The reaction mixture was then heated to 60°C within 60 min. Immediately after reaching 60°C, the slurry was cooled to 20°C within 60 min. and stirred at 20°C for 30 min. The resulting $C_{16}H_{16}N_2O_4Ca$ (79g, 78%) was filtered off, washed with *i*-propyl alcohol (75 mL) and dried overnight at 80°C under vacuum. ^1H NMR (D_2O) δ 7.01 (d,8,4H), 6.57 (d,8,4H), 2.06 (s, 6H, CO-CH_3). IR (cm^{-1}): 1651 (sharp, C=O), 1506, 1276, 854 (sharp). Combustion analysis calculated for $C_{16}H_{16}N_2O_4Ca$: C 55.65, H 4.7, N 8.23; Found: C 55.80, H 4.53, N 8.13.

EXAMPLE VII

A study was conducted in dogs to determine the bioavailability of acetaminophen sodium. The free acid form of acetaminophen was used as the control. Compressed cylindrical pellets having the following composition were prepared:

Acetaminophen Sodium - compressed neat (no excipients).

Control - 150 mg APAP, 30 mg microcrystalline cellulose, and 30 mg dextrates.

Eight male purebred beagles having a body weight at initial dosing of approximately 9 to 14 kg were used in the study. The dogs were fed PMI® Certified Canine Diet Meal No. 5007 and water, both *ab libitum*. The dogs were fasted overnight for approximately 12 hours prior to dosing and food was returned 4 hours after dosing.

The dogs were divided into two groups and each group was dosed with either acetaminophen sodium or the control (free acid APAP) pellets. A single dose equivalent to 300 mg of acetaminophen free acid was administered via an oral gavage using a stomach tube. Each dose was followed by 20 mL of water. After a period of one week, the each group was dosed again, but with the other form of acetaminophen. Twelve blood samples were collected form each dog on each dosing day (1 prior to dosing and 11 thereafter). The plasma was separated and tested for acetaminophen.

The following summarizes the pharmacokinetic measurements for acetaminophen:

Parameter	APAP Sodium	Control
AUC (ug-hr/mL)	31.4 ± 5.7	27.4 ± 6.1
C_{max} (ug/mL)	23.6 ± 4.2	19.4 ± 6.9
T_{max} (hr)	0.27 ± 0.1	0.60 ± 0.3

AUC = areas under the plasma concentration-time curve to the last quantifiable concentration.
C_{max} = peak plasma concentration.
T_{max} = peak time.

Figure 2 is a plot of the acetaminophen plasma concentration-time curve. This Figure demonstrates that the acetaminophen salt of the present invention is absorbed faster than the free acid acetaminophen control. The faster T_{max} for the acetaminophen salt suggests faster onset of action of the analgesic and antipyretic activities relative to the free acid control.

EXAMPLE VIII

This Example discloses the preparation and testing of tablets containing anhydrous calcium acetaminophen (CaAPAP) and one other active ingredient selected from the group of chlorpheniramine maleate (CPM), dextromethorphan hydrobromide (DEX), diphenhydramine hydrochloride (DPH) and pseudoephedrine hydrochloride (PE). The target weight of the tablet (free APAP basis) was 325 mg. The following ingredients were used to make the tablets:

Ingredient	Formulation 1 (mg/Tab)	Formulation 2 (mg/Tab)	Formulation 3 (mg/Tab)	Formulation 4 (mg/Tab)
CaAPAP	368.23	368.23	368.23	368.23
CPM	2.00	-	-	-
DEX	-	15.00	-	-
DPH	-	-	25.00	-
PE	-	-	-	30.00
Microcrystalline Cellulose (Avicel PH 200)	520.77	507.77	497.77	492.77
SiO_2 (Cab-O-Sil M5)	4.50	4.50	4.50	4.50
Mg Stearate NF	4.50	4.50	4.50	4.50

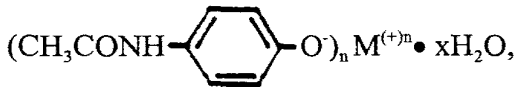
Appropriate amounts of these ingredients were weighed to make a 180 g batch. After sieving, the ingredients were combined and mixed using a PK Blender. The ingredients were then tableted using a single-punch Korsh tablet press. The weight, hardness, thickness and disintegration times were evaluated and are reported below. The dissolution of the CaAPAP was measured using USP Method II apparatus by monitoring the APAP concentration in gastric fluid(GF). The percent dissolution of APAP from the tablet formulations at 2 min. is also reported.

	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Weight Range (mg)	917±6	900±4	907±9	913±4
Thickness range (mm)	5.72±0.02	5.65±0.03	5.72±0.02	5.56±0.02
Hardness range (kP)	7.9±0.1	9.1±0.3	7.1±1.1	8.8±0.5
Disintegration time (sec)	10 to 15	10 to 15	20	15 to 20
% dissolution of CaAPAP at 2 minutes in GF	100%	-	100%	100%

Various modifications can be made from the above-described embodiments without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising the isolated compound



wherein n is 1 or 2, M is alkali metal when n is 1 and M is alkaline-earth metal when n is 2 and x is from 0 to about 10, and at least one other active ingredient selected from the group consisting of analgesics, decongestants, expectorants, antitussives, antihistamines, gastrointestinal agents, diuretics, bronchodilators and mixtures thereof.

2. The composition of claim 2 wherein the alkali metal is selected from the group consisting of sodium, potassium, cesium and lithium.

3. The composition of claim 2 wherein the alkaline-earth metal is selected from the group consisting of calcium and magnesium.

4. The composition of claim 2 wherein the isolated compound is in a hydrated form.

5. The composition of claim 2 wherein the isolated compound is in an anhydrous form.

6. The composition of claim 2 wherein the analgesic is selected from the group consisting of acetyl salicylic acid, indomethacin, optically active isomers or racemates of ibuprofen, naproxen, flurbiprofen, carprofen, tiaprofenic acid, cicloprofen, ketoprofen, ketorolac, etodolac, indomethacin, sulindac, fenoprofen, diclofenac, piroxicam, benzydomine, nabumetone, tramadol, codeine, oxycodone, hydrocodone, flosulide, nimesulide, celecoxib, 5-(4-aminosulfonyl-3-fluorophenyl)-

4-cyclohexyl-2-methyloxazole, meloxicam, nabumetone, etodolac, pharmaceutically acceptable salts thereof and mixtures thereof.

7. The composition of claim 2 wherein the decongestant is selected from the group consisting of pseudoephedrine, phenylpropanolamine, phenylephrine and ephedrine, pharmaceutically acceptable salts thereof and mixtures thereof.

8. The composition of claim 2 wherein the expectorant is selected from the group consisting of guaifenesin, glyceryl guaiacolate, terpin hydrate, ammonium chloride, N acetylcysteine and bromhexine, ambroxol, domiodol, 3-iodo-1,2-propanediol, pharmaceutically acceptable salts thereof and mixtures thereof.

9. The composition of claim 2 wherein the antitussive is selected from the group consisting of dextromethorphan, chlophedianol, carbetapentane, caramiphen, noscapine, diphenhydramine, codeine, hydrocodone, hydromorphone, fominoben, benzonatate, pharmaceutically acceptable salts thereof and mixtures thereof.

10. The composition of claim 2 wherein the antihistamine is selected from the group consisting of chlorpheniramine, brompheniramine, dexchlorpheniramne, dexbrompheniramine, triprolidine, doxylamine, tripelennamine, cyproheptadine, hydroxtzine, pyrilamine, azatadine, promethazine, acrivastine, astemizole, cetirizine, ketotifen, loratidine, temelastine, terfenadine, norastemizole, fexofenadine, pharmaceutically acceptable salts thereof and mixtures thereof.

11. The composition of claim 2 wherein the gastrointestinal agent is selected from the group consisting of atropine, clidinium, dicyclomine, aluminum hydroxide, bismuth subsalicylate, bismuth subcitrate, simethicone, calcium carbonate, magaldrate, cimetidine, famotidine, nizatidine, ranitidine, phenolphthalein, casanthrol, sucralfate, sucralfate humid gel, metoclopramide, eisaprode, omeprazole, diphenoxylate, loperamide, pharmaceutically acceptable salts thereof and mixtures thereof.

12. The composition of claim 2 wherein the diuretic is selected from the group consisting of caffeine and pamabrom.

13. The composition of claim 2 wherein the bronchodilator is selected from the group consisting of terbutaline, aminophylline, pinephrine, isoprenaline, metaproterenol, bitoterol, theophylline, albuterol, pharmaceutically acceptable salts thereof and mixtures thereof.

14. The composition of claim 2 wherein the sleep-inducing agent is selected from the group consisting of melatonin, estazolam, zolpidem, promethacine, pharmaceutically acceptable salts thereof and mixtures thereof.

15. A method of eliciting an onset hastened analgesic or antipyretic response in a mammal, comprising the oral administration of the composition of claim 1.

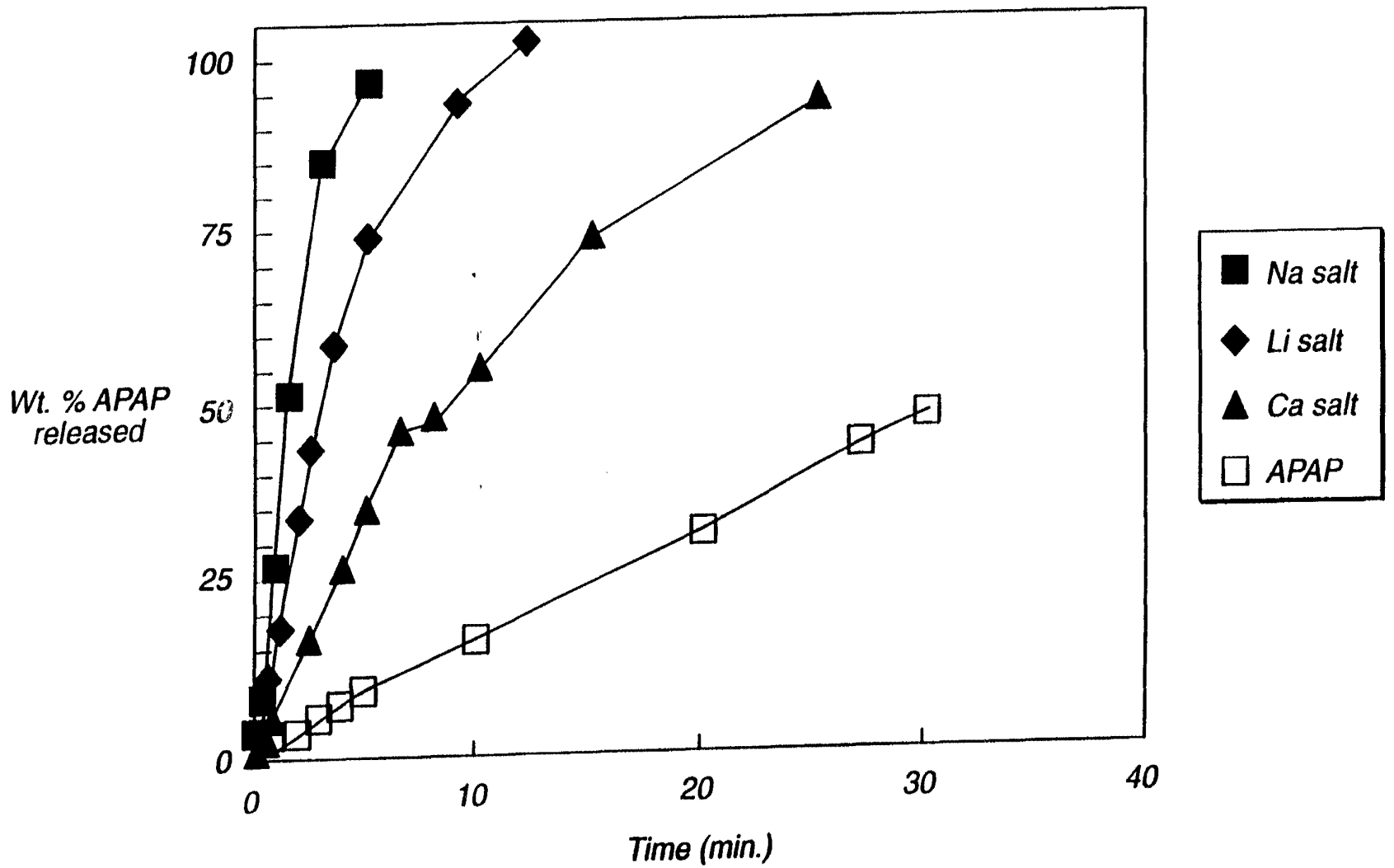
16. The method of claim 15 wherein the alkali metal is selected from the group consisting of lithium, sodium, cesium and potassium.

17. The method of claim 15 wherein the alkaline-earth metal is selected from the group consisting of calcium and magnesium.

18. The method of claim 15 wherein the salt is in a hydrated form.

19. The method of claim 15 wherein the salt is in an anhydrous form.

FIG. 1


100
75
50
25
0
Wt. % APAP released
0
10
20
30
40
Time (min.)
Na salt
Li salt
Ca salt
APAP

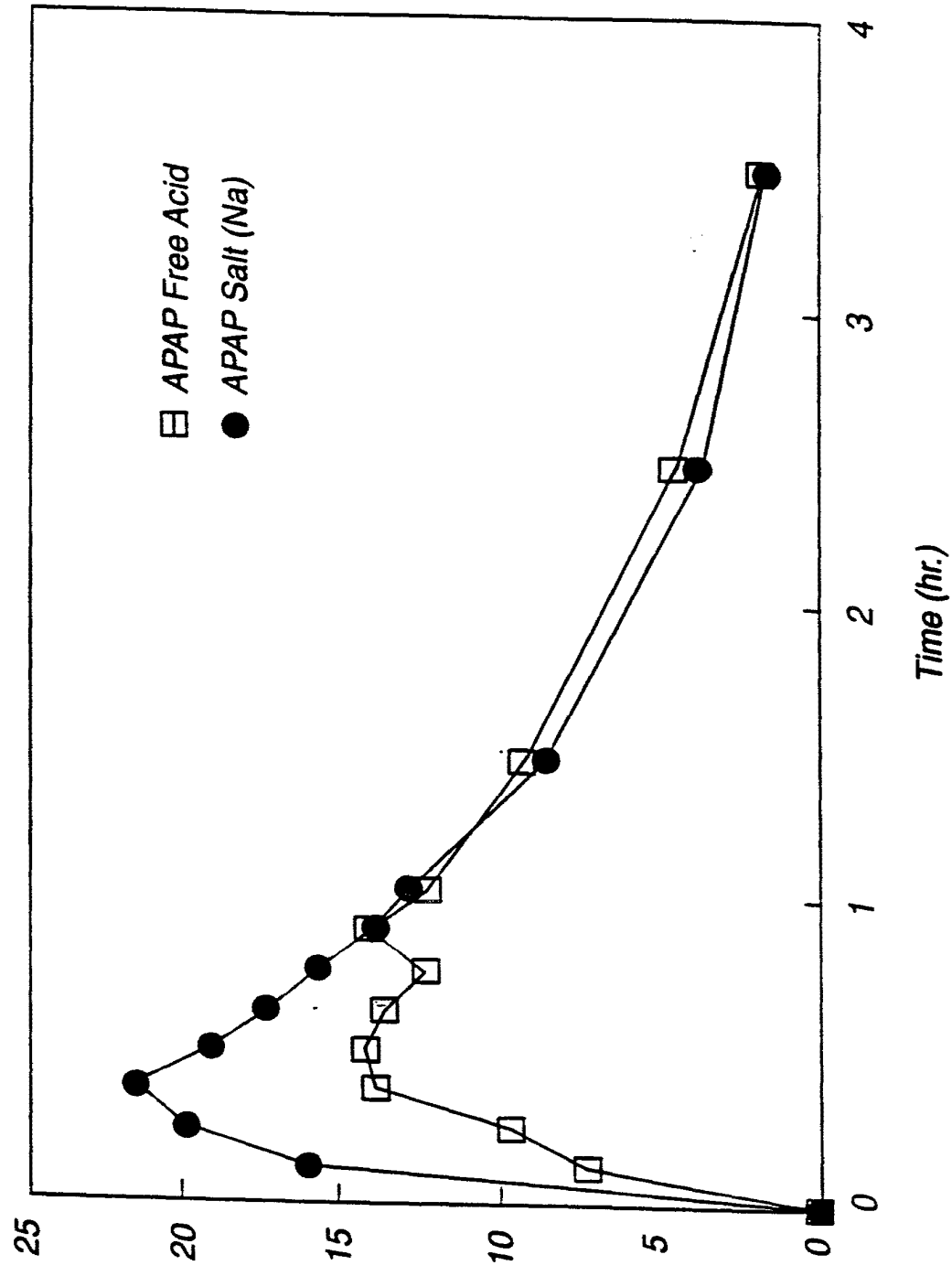

APAP Free Acid
APAP Salt (Na)
Plasma Concentration (ug/mL)
Time (hr.)
0
5
10
15
20
25
0
1
2
3
4

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No
PC., US 99/13064

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/165

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 23595 A (PROCTER & GAMBLE) 8 September 1995 (1995-09-08) page 6, line 23 -page 7, line 11; claims 1,7	1-14
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-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
5 November 1999	12/11/1999

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PC./US 99/13064

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

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International Application No
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INTERNATIONAL SEARCH REPORT

rnational application No.
PCT/US 99/ 13064

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 15-19
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 15-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

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(72) Inventors; and
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(74) Agent: PLOUGMANN, VINGTOFT & PARTNERS A/S; Sankt Annæ Plads 11, Postboks 3007, DK–1021 København (DK).

(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: MODIFIED RELEASE MULTIPLE–UNITS COMPOSITIONS OF NON–STEROID ANTI–INFLAMMATORY DRUG SUBSTANCES (NSAIDs)

(57) Abstract

An oral pharmaceutical modified release multiple–units composition for the administration of a therapeutically and/or prophylactically effective amount of a non–steroid anti–inflammatory drug substance (in the following abbreviated "an NSAID substance") to obtain both a relatively fast or quick onset of the therapeutic effect and the maintenance of a therapeutically active plasma concentration for a relatively long period of time. The modified release multiple–units composition comprises at least two fractions of multiple units such as a first and a second fraction. The first fraction comprises individual units which are designed to quickly release the drug substance and the second fraction comprises individual units which are designed to slowly release the drug substance to enable a delayed and extended release of the drug substance. Typically, the second fraction comprises multiple units which are coated with a sustained release coating designed to release the drug substance in such a manner that the maintenance of a therapeutically active plasma concentration for a relatively long period of time are obtained. By suitable adjustment of the release pattern of the at least first and second fraction a composition is obtained which is adapted to once– or twice–a–day administration.

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MODIFIED RELEASE MULTIPLE-UNITS COMPOSITIONS OF NON-STEROID ANTI-INFLAMMATORY DRUG SUBSTANCES (NSAIDs)

The present invention relates to an oral pharmaceutical modified release multiple-units composition for the administration of a therapeutically and/or prophylactically effective amount of a non-steroid anti-inflammatory drug substance (in the following abbreviated "an NSAID substance") to obtain both a relatively fast or quick onset of the therapeutic effect and the maintenance of a therapeutically active plasma concentration for a relatively long period of time. The modified release multiple-units composition comprises at least two fractions of multiple units such as a first and a second fraction. The first fraction comprises individual units which are designed to quickly release the drug substance and the second fraction comprises individual units which are designed to slowly release the drug substance to enable a delayed and extended release of the drug substance. Typically, the second fraction comprises multiple units which are coated with a sustained release coating designed to release the drug substance in such a manner that the maintenance of a therapeutically active plasma concentration for a relatively long period of time are obtained. By suitable adjustment of the release pattern of the at least first and second fraction a composition is obtained which is adapted to once- or twice-a-day administration.

TECHNICAL BACKGROUND

Drug levels can be maintained above the lower level of the therapeutic plasma concentration for longer periods of time by giving larger doses of conventionally formulated dosage forms. However, it is not a suitable approach to increase dosage as such doses may produce toxic and undesired high drug levels. Alternatively, another approach is to administer a drug at certain intervals of time, resulting in oscillating drug levels, the so-called peak and valley effect. This approach is generally associated with several potential problems, such as a large peak (toxic effect) and valley (non-active drug level) effect, and a lack of patient compliance leading to drug therapy inefficiency or failure. If, however, the plasma concentration is kept constant over the therapeutic level using conventional tablets, an unacceptably high daily dosage is required if the active substance is not administered very frequently.

Controlled release compositions are known which are designed to rapidly release a fraction of a total drug dose. This loading dose is an amount of a drug which will provide a desired pharmacological response as fast as possible according to the biopharmaceutical properties of the drug substance. Generally, such compositions in some more or less sophisticated manner are composed of a sustained release part and a part which either contains a free amount of the drug substance or it releases the drug substance in the same manner as if the drug substance had been formulated as a plain formulation (e.g. in the form of normal tablets or granulates). Such compositions which initially release a burst of a therapeutic agent and then release the agent at an essentially constant rate are described, e.g., in WO 95/14460 (Euroceltique S.A.) published on 1 June 1995. The composition described therein relates to a sustained release opioid formulation comprising a plurality of substrates comprising the active ingredient in a sustained release matrix or coated with a sustained release coating comprising a retardant material. The sustained release beads are then coated with an opioid in immediate release form or, in the case the composition is in the form of a gelatine capsule, an amount of free opioid (i.e. the opioid is included as such and has not been processed into a specific formulation e.g. by means of pharmaceutically acceptable excipients) is incorporated into the gelatin capsule via inclusion of a sufficient amount of opioid within the capsule. In a further alternative, the gelatine capsule itself is coated with an immediate release layer of the opioid.

Generally, the rationale which lies behind the kind of compositions which have been described to enable an immediate release of a drug substance as well as a sustained release of the drug substance is to combine a traditional formulation approach (such as, e.g., i) plain tablets which have a disintegration time in water of at the most about 15 min for uncoated tablets, cf. Ph. Eur. (the requirements for coated tablets or capsules are at the most 30 min), ii) a traditionally formulated granulate or iii) loose powder of the drug substance itself) with a controlled release approach. By doing so the immediate release part of the composition is intended to release the drug substance in a manner which corresponds to a plain tablet formulation or the like and the term "immediate" is in such a context intended to denote that the release of the drug substance is faster than the release from a sustained release composition. The immediate release is in no way intended to be faster than that of a traditional or plain composition.

Especially in those cases where the drug substance has a low solubility in an acidic medium having a pH of from about 1 to about 3, i.e. a pH corresponding to the pH in the stomach, the traditional formulation approach will lead to a pharmaceutical composition which has a suitable fast disintegration time but not necessarily a suitable dissolution rate of the drug substance under acidic conditions, i.e. a plain tablet will rapidly disintegrate into granules but the dissolution of the drug substance from the composition and/or the disintegrated composition under acidic conditions may be unsuitable low due to the solubility properties of the drug substance itself. The availability of a drug substance with respect to absorption, i.e. entrance into the circulatory system, is dependant on the presence of the drug substance on dissolved form as it is generally accepted that only dissolved substances are capable of passing the mucous membranes in the gastro-intestinal tract. Therefore, it is important that the dissolution of the drug substance is suitably fast even under acidic conditions in order to enable an initial absorption already from the stomach so that a true fast or immediate therapeutic response is obtainable. Furthermore, if a drug substance – dependant on pH – can exist on un-ionized as well as ionized form (e.g. acetyl salicylic acid which at an acid pH below its pK_a value predominantly is present on an unloaded, i.e. un-ionized form, whereas at a pH above its pK_a value predominantly is present on ionized form). For drug substances which are weak acids it is very important to ensure a proper bioavailability of the drug substance already under acidic conditions in order to achieve a true rapid therapeutic effect. However, the various approaches disclosed with respect to achievement of a combination of a rapid and a sustained effect (e.g. in the publications mentioned above) do not seem to take the above-mentioned factors into account and, hence, there is a need for developing compositions which enable a true rapid onset of the therapeutic effect as well as a sustained effect. To this end, we have especially focused on compositions comprising a drug substance suitable for use in situations where a rapid effect is needed but also in situations where an extended effect is desirable in order to develop compositions suitable for administration less frequent than compositions on the market today, more specifically to enable administration on a once or twice daily basis. Examples of suitable drug substances are, e.g., substances which have a pain relief effect. More specifically, interesting drug substances are those belonging to the class of drug substances normally denoted NSAIDs or NSAID substances.

In EP-A-0 438 249A1 (ELAN Corporation P.L.C.) is given another example of a composition which has been designed to release naproxen immediately and sustained. However, as shown in Example 18 herein, the so-called immediate release of naproxen does not take place under acidic conditions, i.e. conditions prevailing in the stomach. Accordingly, such a composition is not within the scope of the present application.

As will be apparent from the following the present inventors have developed a composition in multiple-units form for a quick release as well and an delayed and extended release.

Multiple-units formulation techniques according to the invention aim at a modified release of a drug substance in a predetermined pattern to control the peak plasma concentration without affecting the bioavailability, i.e. the extent of drug availability. The release of an NSAID substance from a composition according to the present invention is controlled in a very flexible manner as described below. Many advantages are obtained, e.g., the frequency of undesirable side effects may be reduced, and due to the control of the time it takes to obtain the peak plasma concentration and the prolongation of the time at the therapeutically active plasma concentration, the frequency of the administration may be reduced to a dosage taken only twice or once a day. This also serves to improve patient compliance. A further advantage of the modified release multiple-units dosage form is that high local concentrations of the active substance in the gastro-intestinal system are avoided, due to the units being distributed freely throughout the gastrointestinal tract, independent of gastric emptying.

Moreover, patients suffering from pain and/or inflammatory conditions and/or related conditions very often require high daily dosages of NSAID substances. If such high dosage of an NSAID substance should be given only once a day, the release from the dosage form must be safe, predictable and reliable. The composition should also be very storage stable because an immediate release due to accidental damaging of e.g. the coating or capsule of a high dosage form may result in undesired high plasma concentrations, so-called dose dumping, which could cause undesired side effects. Furthermore, from a technical point of view, the release rate and the release pattern of the active drug substance from the composition should not significantly change during the shelf-life of the composition. Even a minor change in the release rate and/or release pattern may have a significant impact on the *in vivo* performance of the composition.

By use of a coated multiple unit dosage form, the risk of dose dumping due to e.g. rupturing of a coating is reduced because the amount of active ingredient in each coated unit is negligible.

The compositions according to the present invention are intended to reduce or essentially eliminate problems identified with other kind of compositions intended for administration once daily. Thus, a major disadvantage of the once-a-day treatment in the art may be a low plasma concentration at the end of the dosing period and thereby the lack of therapeutic response. As the treatment of pain and/or inflammatory conditions and/or related conditions, is a balance of therapeutic effect on the one hand and the risk of side effects on the other hand, e.g. due to accumulation of drug, the dosage interval is generally calculated so that the drug concentration is substantially decreased at the time of intake of the next dosage. Accordingly, the patient will very often suffer from disease symptoms before the drug concentration subsequent to the next dosage has reached the therapeutic level. In addition, it should be noted that in the treatment of pain and/or inflammatory conditions and/or related conditions, relatively higher dosages, corresponding to a relatively higher peak concentration, are often needed in case the symptoms break through. Accordingly, a relatively higher initial plasma concentration of an NSAID substance may be necessary compared to the plasma concentration at steady state.

However, to the best of our knowledge no oral non-steroid anti-inflammatory modified release pharmaceutical composition has been disclosed which at the same time can be produced in an easy, cheap and reliable manner and which provides a suitable profile for release of active substance (under acidic, neutral and basic conditions) resulting in an extended period of action so that the inflammatory condition is both rapidly alleviated after administration and avoided for a period of about 12 to 24 hours.

Therefore, there is a need for developing a composition comprising a non-steroid anti-inflammatory drug substance permitting the administration of dosages only once or twice a day in a safe and reliable manner, and which is easy to produce, preferably involving conventional production methods and as few production steps as possible. It is also important that an NSAID composition for daily administration comprises the active ingredient in such a way that the composition has a reliable dissolution rate since a

change in the dissolution pattern of the NSAID substance could be disadvantageous for the patient.

BRIEF DISCLOSURE OF THE INVENTION

The purpose of the present invention is to provide an oral modified release multiple-units composition for administration of a daily dosage of an NSAID substance in a dosage form which only requires administration at the most twice daily, preferably once daily, and which overcomes the drawbacks of hitherto suggested formulations of modified release compositions containing an NSAID substance in that the dosage form both provides a substantially fast release from a first fraction comprising multiple units and a delayed and extended release from a second fraction of multiple units of the NSAID substance whereby alleviation of symptoms is achieved shortly after administration and is maintained for at least 12 hours, preferably 24 hours after administration.

A further aspect of the invention is to provide a process for the preparation of a composition of an oral pharmaceutical modified release multiple-units composition containing an NSAID substance, and in addition, a method for treating patients with a composition according to the invention whereby the interval between each administration is increased to about 12-24 hours.

Accordingly, the present invention relates to an oral pharmaceutical modified release multiple-units composition in unit dosage form for administration of a therapeutically and/or prophylactically effective amount of a non-steroid anti-inflammatory drug substance (an NSAID substance), a unit dosage form comprising two NSAID-containing fractions,

i) a first NSAID-containing fraction of multiple-units for quick release of the NSAID substance, and

ii) a second NSAID-containing fraction of multiple-units for extended release of the NSAID substance,

the first fraction which – when subjected to dissolution method II as defined herein employing 0.07 N HCl as dissolution medium – releases at least 50% w/w of the NSAID substance present in the fraction within the first 20 min of the test,

the second fraction being in the form of coated delayed release multiple-units for extended release of the NSAID substance.

The present invention also relates to a composition for the administration of a therapeutically and/or prophylactically effective amount of an NSAID substance to obtain both a relatively fast onset of the therapeutic effect and the maintenance of a therapeutically active plasma concentration for a relatively long period of time, a unit dosage of the composition comprising at least two fractions as follows:

a first fraction of quick release multiple-units for relatively quick release *in vivo* of an NSAID substance to obtain a therapeutically and/or prophylactically active plasma concentration within a relatively short period of time, and

a second fraction of coated modified release multiple-units for extended release *in vivo* of an NSAID substance to maintain a therapeutically and/or prophylactically active plasma concentration in order to enable dosing once or twice daily,

the formulation of the first and the second fractions, with respect to release therefrom and with respect to the ratio between the first and the second fraction in the unit dosage, being adapted so as to obtain:

a relative fast *in vitro* release of the NSAID substance from the first fraction of quick release multiple-units, as measured by the dissolution method II as defined herein,

an extended *in vitro* release of the NSAID substance from the second fraction of extended release multiple-units relative to the *in vitro* release of the first fraction of the NSAID substance, as measured by the dissolution method III as defined herein,

the quick release and the extended *in vitro* release being adapted so that the first fraction is substantially released when the release from the second fraction is initiated corresponding to at least 50% w/w release of the NSAID substance contained in the

first fraction at the time when at the most about 15% w/w such as, e.g., at the most about 10% w/w or at the most about 5% w/w of the NSAID substance contained in the second fraction is released as measured by the dissolution method III as defined herein.

It should be noted that the dissolution methods mentioned above and throughout the specification of course may be adjusted to specific drug substances and in some cases replaced with other dissolution methods. However, the requirements claimed herein should still be fulfilled.

The modified release multiple-units dosage forms of the present invention achieve and maintain therapeutic plasma concentrations for a prolonged period of time. In order to achieve the relatively fast absorption for the first fraction it requires that NSAID substances dissolve in the stomach (cf. the discussion above). Since the solubility of an NSAID substance such as, e.g., lornoxicam is < 1 mg /100 ml in 0.1 N HCl (aqueous solution of 0.1 N hydrochloric acid) the present inventors have found that incorporation such an NSAID substance in free form or in the form of a traditional formulation does not give the desired quick release under acidic conditions to enable a fast onset of the therapeutic effect *in vivo*. However, and as it will be discussed in detail below, a quick release of an NSAID substance (which is a weak acid or has a very low solubility under acidic conditions) takes place under acidic conditions provided that the drug substance is presented in a formulation wherein specific means has been used in order to manipulate the release rate so that the release becomes much faster than from a traditional composition. Thus, in contrast to the prior art composition in which focus only has been on the extended release rate part of the compositions and on the possibility of changing the release from this part, the present inventors have found it necessary to adjust the release rate from both the fast and the slow release part of a composition when the NSAID substance either has a very low solubility in 0.1 N hydrochloric acid or has a pK_a below about 5.5 such as, e.g., about 4-5. Thus, both the fast release fraction and the delayed release fraction must be manipulated with respect to release in order to achieve a suitable overall release rate.

The first fraction of the composition constitutes the quick releasing part of the composition whereas the second fraction of the composition constitutes the delayed and extended release part of the composition. In the first fraction, the release rate is primarily governed by the formulation of the fraction, i.e. the ingredients employed and