Figure 31

Figure 31A

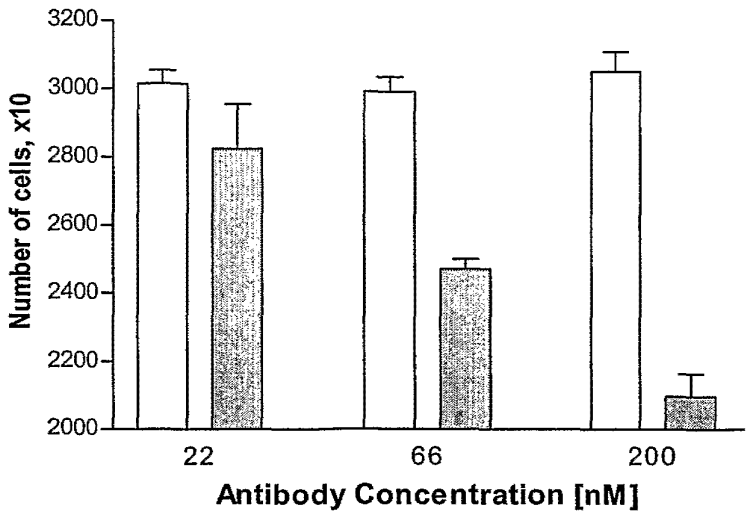

Figure 31B

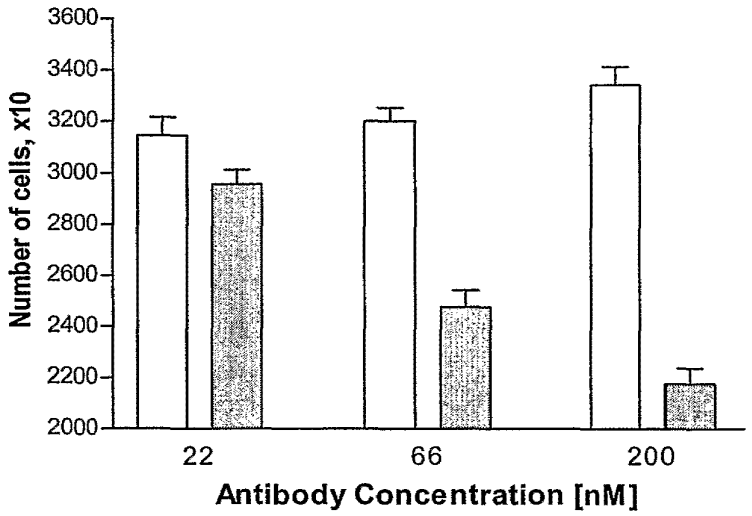

Figure 31A and **B:** Inhibition of Tumor Cell Proliferation. 2 x 10^4 DU4475 cells were serum starved overnight following treatment with desferrioxamine, then incubated with various amount of 18F1 (shadowed bar) or isotype control IgG (opened bar) in the presence of VEGF-A (A) or PlGF (B). After 2 days incubation, total cell number was determined using a Coulter cell counter. The results are shown as mean value with standard error.

Figure 32

Figure 32A

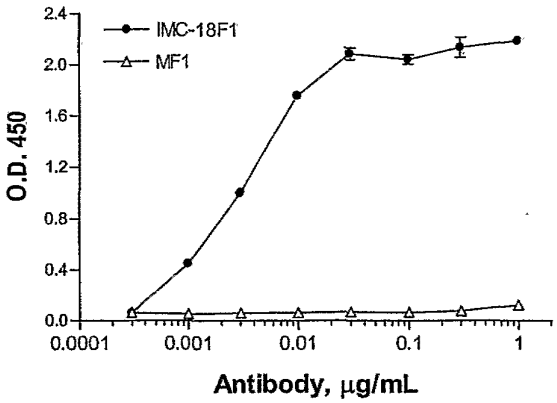

Figure 32B

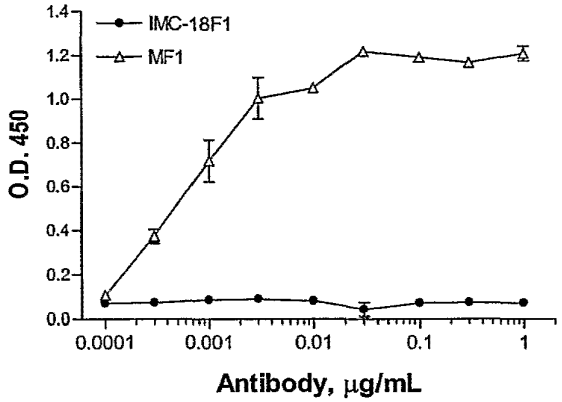

Figure 32C

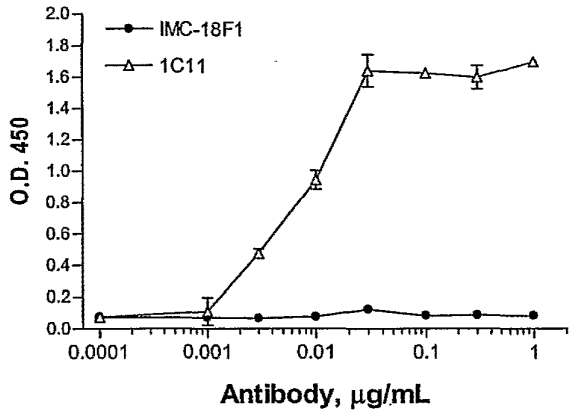

Figure 32A, B, and **C:** Specificity of 18F1 and Anti-mouse VEGFR-1 Antibody MF1. 18F1 has binding activity with immobilized recombinant human VEGFR-1 (A) but not with mouse VEGFR-1 (B) and human VEGFR-2 (C) as compared to positive control MF1 or VEGFR-2 specific antibody 1C11. Anti-mouse VEGFR-1 antibody MF1 did not bind to immobilized recombinant human VEGFR-1(A). 18F1 was used as a positive control in solid phase binding assay.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO: 13/149468
FILED: May 31, 2011
FOR: DOSAGE REGIMEN OF AN S1P RECEPTOR AGONIST

Art Unit: 1629
Examiner:

MS: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Sir:

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

Copies of the references are enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Please charge Deposit Account No. 19-0134 in the name of Novartis in the amount of $180 for payment of the fee pursuant to 37 CFR §1.17(p) for the submission of an Information Disclosure Statement under 37 CFR §1.97(c). The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 19-0134 in the name of Novartis.

Respectfully submitted,

Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
+1 862 7783785

Date: 7/19/12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO: 13/149468
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Respectfully submitted,

Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

Novartis Pharmaceuticals Corporation
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+1 862 7783785

Date: 7/19/12

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	1629
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
		US-			
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		US-			

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
		RU2199339C2	02-27-2003	TEKH; OOO BIO		☐
						☐
						☐
						☐
						☐
						☐

Examiner Signature		**Date Considered**	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw a line through citation if not in conformance and not considered. Include copy of this form with the next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kind Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Electronic Patent Application Fee Transmittal

Application Number:	13149468
Filing Date:	31-May-2011
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Filer:	Karen DeBenedictis/Denise Cooper
Attorney Docket Number:	PAT050279-US-CNT

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	12546461
Application Number:	13149468
International Application Number:	
Confirmation Number:	1536
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Customer Number:	1095
Filer:	Karen DeBenedictis/Denise Cooper
Filer Authorized By:	Karen DeBenedictis
Attorney Docket Number:	PAT050279-US-CNT
Receipt Date:	16-APR-2012
Filing Date:	31-MAY-2011
Time Stamp:	12:17:36
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$180
RAM confirmation Number	10438
Deposit Account	190134
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Foreign Reference	15-RU2199339-abstract.pdf	40981 6d172b70b289fcc3f73553558537f28780646834	no	1
Warnings:					
Information:					
2		50279-IDS.pdf	110493 dcf72d3d17fa0091a95bf23fbedea6f0f0465958	yes	2

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Transmittal Letter	1	1
	Information Disclosure Statement (IDS) Form (SB08)	2	2

Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30482 2e9d06a9df16f834b2bdaf514249e5fd6299bbbf	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	181956		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

With regard to other embodiments of the claimed invention, a medication for slowing progression of a demyelinating disease and for inhibiting or treating Primary-progressive multiple sclerosis, comprising an immunomodulator is known from the prior art (see RU 2199339 C2 27.02.2003) (D3).

Bibliographic data: RU2199339 (C2) — 2003-02-27

METHOD FOR TREATING MULTIPLE SCLEROSIS

Page bookmark	RU2199339 (C2) - METHOD FOR TREATING MULTIPLE SCLEROSIS
Inventor(s):	BARBAS I M; TOTOLJAN N A; SKOROMETS A A; SMIRNOV M N; ZHIVOTOVSKAJA M L; JAKOVLEVA V S +
Applicant(s):	TEKH; OOO BIO +
Classification:	- **international:** *A61K38/20*; (IPC1-7): A61K38/20
	- **European:**
Application number:	RU20010104929 20010223
Priority number(s):	RU20010104929 20010223

Abstract of RU2199339 (C2)

FIELD: medicine, neurology. SUBSTANCE: during the stage of exacerbation patients with multiple sclerosis should be intravenously injected by drops with human interleukin-2 (h-IL-2) preparation, for example, roncoleukin, at the dosage of not less than 1 mln IU once per 7d. The method enables to considerably shorten therapy terms in patients with multiple sclerosis at the background of pronounced immune deficiency, decrease the number of relapses and prolong labor capacity period in patients with multiple sclerosis. EFFECT: higher efficiency of therapy. 1 cl, 2 ex, 2 tbl

Case PAT050279-US-CNT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO: 13/149468
FILED: May 31, 2011
FOR: DOSAGE REGIMEN OF AN S1P RECEPTOR AGONIST

Art Unit: 1629
Examiner:

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

This paper is being filed:

☒ supplemental to the Information Disclosure Statements filed May 31, 2011 and December 30, 2011.

Please charge Deposit Account No. 19-0134 in the name of Novartis in the amount of $180 for payment of the fee pursuant to 37 CFR §1.17(p) for the submission of an Information Disclosure Statement under 37 CFR §1.97(c). The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 19-0134 in the name of Novartis.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

☒ Copies of the references are enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Respectfully submitted,

Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
+1 862 7783785
Date: 4/11/12

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/149,468	05/31/2011	Peter C. Hiestand	PAT050279-US-CNT	1536

1095 7590 04/03/2012

NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

EXAMINER	
SPIVACK, PHYLLIS G	

ART UNIT	PAPER NUMBER
1629	

MAIL DATE	DELIVERY MODE
04/03/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	13/149,468	HIESTAND ET AL.	
	Examiner	Art Unit	
	PHYLLIS G. SPIVACK	1629	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *12-21* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *12-21* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *5/31/11; 9/29/11*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Applicants' Preliminary Amendment file May 31, 2011 is acknowledged. Claims 1-11 are canceled. New claims 12-21 are presented and represent all of the claims under consideration. A new Abstract and updated priority information are noted.

Information Disclosure Statements filed May 31, 2011 and September 29, 2011 are further acknowledged and have been reviewed. The reference to Dr. Maschkowskij cited on the Information Disclosure Statement filed September 29, 2011 is not present in the instant file or in the parent application.

The abstract of the disclosure is objected to because the subject matter under consideration is limited to administration of the compound 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-propane-1,3-diol, or a pharmaceutically acceptable salt thereof. The subject matter under consideration excludes prevention of neo-angiogenesis associated with a demyelinating disease. The terms depicted in formula X, i.e., Z, R_{1x}, R_{2Z} and R_{3Z} are not defined. Correction is required. See MPEP § 608.01(b).

Claims 18 and 19 are objected to under 37 CFR 1.75 as being substantial duplicates. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

Applicants are advised that should claim 18 be found allowable, claim 19 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof.

The disclosure is objected to for the following informality:

On page 12 of the specification, line 22, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-propane-1,3-diol is misspelled.

Appropriate correction is required.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims12-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Virley, D.J., Journal of the American Society for Experimental NeuroTherapeutics, in view of LaMontagne. et al., Cancer Research, and further in view of Kovarik et al., WO 06/058316.

Virley teaches the administration of 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-propane-1,3-diol, also known as FTY720, which is a sphingosine-1-phosphate receptor modulator, for the treatment of multiple sclerosis (MS). Virley distinguishes between the categories of relapsing-remitting MS and primary progressive MS. See the introduction on page 638, as well as the discussion of experimental models for MS on

page 640. In order to provide predictive indices for clinical application, experimental autoimmune encephalomyelitis (EAE) models are discussed.

Due to the majority of MS patients presenting relapsing-remitting symptoms before progressing onto a chronic phase, a number of animal models of EAE have been designed to simulate the more dynamic clinical and pathological features of relapsing-remitting MS. One such model, using the Biozzi AB/H mouse, involves the inoculation of homologous spinal cord homogenate (or more specifically MOG peptide) in adjuvant without the additional use of *Bordetella pertussis* toxin, and reproducibly induces a chronic relapsing-remitting demyelinating disease. The dynamic chronicity of symptoms is expressed as an acute induction of disease (loss of tail tone and hindlimb paralysis), followed by reduced severity (remission) and then a relapse disease episode. The development of clinical signs in this model are preceded by a loss in weight, whereas remission periods are associated with an increase in body weight, implicating changes in weight as surrogate markers of disease status. Reductions in the degree of inflammation and evidence for remyelination are thought to reflect the remission period in this EAE model, whereas relapses are thought to be indicative of an amplified inflammatory response, gliosis and demyelination within the CNS.

In particular, Virley teaches the oral administration of FTY720 in a Lewis rat EAE model demonstrates a dramatic reduction in clinical severity, mortality and the infiltration of leukocytes into the CNS. Proinflammatory cytokines such as IL-2, IL-6, and IFN's were markedly suppressed in the CNS following prophylactic FTY720 treatment relative to saline treatment. The mechanistic effect of FTY720 on reducing lymphocyte trafficking and CNS inflammation within EAE, was further studied with contrast-enhanced MRI, using superparamagnetic iron-oxide nanoparticles to track macrophage infiltration. The oral administration of FTY720 was shown to significantly reduce the magnitude and extent of cellular infiltration into the CNS of EAE-sensitized rats. These effects with FTY720 treatment corresponded to reductions in lesion burden and blood brain barrier disruption assessed by MRI signatures and a marked reduction in neurological disability during the acute and relapsing phase of the model. Therapeutic administration of FTY720 at the point of relapse also significantly suppressed further progression of clinical signs, providing compelling evidence for this

agent as a potential therapeutic agent for MS. Data from a phase II clinical trial with FTY720, confirmed a relapse reduction rate of more than 50% in 281 relapsing-remitting MS patients for 6 months of treatment, relative to placebo. Inflammatory disease activity, as visualized by gadolinium-enhanced T1-weighted MR imaging was shown to be dramatically reduced by up to 80%, after 6 months of oral, once a day, treatment. Progressive disease activity in the form of new T2 MRI lesions was also demonstrated to be reduced by more than 60% after FTY720 treatment. The onset of effect was demonstrated as early as 2 months following treatment, and MS patients showed no significant adverse events over the 6-month trial duration relative to placebo. See page 644.

Virley fails to teach the required dosage of 0.5 mg, as well as methods of inhibiting or treating a neo-angiogenesis association with MS.

However, LaMontagne teaches FTY720 to be an anti-angiogenic agent. The compound becomes phosphorylated *in vivo* and interacts with spingosine-1-phosphate (S1P) receptors. The effect is on vascular permeability, an important aspect of angiogenesis. See the Abstract.

Kovarik teaches dosage regimens involving S1P receptor agonists, of which FTY720 is clearly encompassed. Maintenance dosages are disclosed on page 15, line 9, as required by all of the present claims. Specifically, on line 16, page 17, a daily dose of 0.5 mg is taught for the treatment of autoimmune diseases, of which MS is recited as an example, on lines 7-8 on page 14.

Therefore, in view of the teachings of Virley, LaMontagne and Kovarik, one skilled in the neurology art would have been motivated to administer FTY720 with a reasonable expectation of success in inhibiting neo-angiogenesis associated multiple sclerosis, in alleviating relapses in MS and slowing the progression of MS. Such would have been obvious because FTY720 is a known anti-angiogenic agent that has been taught for use in the treatment of autoimmune diseases such as multiple sclerosis, at a dosage of 0.5 mg. According to Virley, FTY720 significantly reduced - by more than 60% - progressive disease activity after treatment. FTY720 administration resulted in a relapse reduction rate of more than 50% in 281 relapsing-remitting MS patients.

No claim is allowed.

Foster et al., US 2006/0046979, is cited to show further the state of the art.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Phyllis G. Spivack whose telephone number is 571-272-0585. The Examiner can normally be reached from 10:30 to 7 PM.

If attempts to reach the Examiner by telephone are unsuccessful after one business day, the Examiner's supervisor, Jeff Lundgren, can be reached 571-272-5541. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

March 24, 2012

/Phyllis G. Spivack/
Primary Examiner, Art Unit 1629

SEARCH HISTORY

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     (FILE 'HOME' ENTERED AT 16:29:46 ON 21 MAR 2012)

     FILE 'HCAPLUS' ENTERED AT 16:30:00 ON 21 MAR 2012
                E HIESTAND PETER C/AU
L1           71 SEA ABB=ON  ("HIESTAND P C"/AU OR "HIESTAND PETER"/AU OR
                "HIESTAND PETER C"/AU)
                E SCHNELL CHRISTIAN/AU
L2           50 SEA ABB=ON  ("SCHNELL CHRISTIAN"/AU OR "SCHNELL
CHRISTIAN
                R"/AU OR "SCHNELL CHRISTIAN RENE"/AU)
L3            2 SEA ABB=ON  L1 AND L2
                SELECT RN L3 1-2

     FILE 'REGISTRY' ENTERED AT 16:30:28 ON 21 MAR 2012
L4            9 SEA ABB=ON  (162359-55-9/BI OR 507468-43-1/BI OR
800379-64-0/BI
                 OR 127464-60-2/BI OR 162359-56-0/BI OR 216974-75-3/BI
OR
                284461-73-0/BI OR 557795-19-4/BI OR 890128-81-1/BI)

     FILE 'HCAPLUS' ENTERED AT 16:30:33 ON 21 MAR 2012
L5            2 SEA ABB=ON  L4 AND L3

     FILE 'REGISTRY' ENTERED AT 16:34:13 ON 21 MAR 2012
L6              STRUCTURE  162359-55-9
L7            9 SEA SSS SAM L6
L8          178 SEA SSS FUL L6
L9          142 SEA ABB=ON  L8 AND NR=1 AND NRS=1
L10          60 SEA ABB=ON  L9 AND  N=1 AND O=2
L11           0 SEA ABB=ON  L10 AND C=33
L12           1 SEA ABB=ON  162359-55-9/RN
L13          38 SEA ABB=ON  L10 AND C=19
L14          35 SEA ABB=ON  L13 AND H=33
L15          20 SEA ABB=ON  L14 NOT D/ELS

     FILE 'HCAPLUS' ENTERED AT 16:39:56 ON 21 MAR 2012
L16        1026 SEA ABB=ON  L15
L17           1 SEA ABB=ON  L16 AND (NEO(W)ANGIOGENESIS OR
NEOANGIOGENESIS )
L18         258 SEA ABB=ON  L16 AND MULTIPLE SCLEROSIS
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L20            6 SEA ABB=ON  L19 AND ?ANGIOGENESIS?
L21           82 SEA ABB=ON  L19 AND S1P
L22          258 SEA ABB=ON  L19 OR L20 OR L21
L23           68 SEA ABB=ON  L22 AND (PRD<20070625 OR PD<20070625)
L24           68 SEA ABB=ON  L23 AND (?MULTIPLE?(W)?SCLEROSIS? OR
?ANGIOGENESIS?
                 )
L25            0 SEA ABB=ON  L24 AND (?DOSAGE? OR ?DOSE?)(4A)(0.5)
L26           11 SEA ABB=ON  L24 AND (?DOSAGE? OR ?DOSE?)
L27           68 SEA ABB=ON  L24 OR L26
L28           68 SEA ABB=ON  L27 AND (?TREAT? OR ?THERAPY? OR ?INHIBIT?
OR
                 ?RELAPS? OR ?ALLEVIAT?(4A)?RELAPS? OR ?PROGRES? OR
?SLOW? OR
                 ?RETARD? OR ?CONTROL? OR ?LIMIT?)
L29           16 SEA ABB=ON  L28 AND ?RECEPT?(6A)?MODULAT?
L30           68 SEA ABB=ON  L28 OR L29
                 SAV L30 SPI468L30/A

     FILE 'MEDLINE, BIOSIS, EMBASE, DRUGU' ENTERED AT 17:22:17 ON 21 MAR
2012
L31           61 SEA ABB=ON  L26

     FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE' ENTERED AT 17:23:05 ON 21
MAR 2012
L32           55 DUP REMOV L26 L31 (17 DUPLICATES REMOVED)
L33            0 SEA ABB=ON  L32 AND (?DOSAGE? OR ?DOSE?)(5A)(0.5 OR
0.5(W) MG)



     FILE HOME

     FILE HCAPLUS

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	Spivack
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
/P.S./		WO 2006/058316	06-01-2006			☐
/P.S./		WO 2004/113330	12-29-2004			☐
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						☐

Examiner Signature	/Phyllis Spivack/	Date Considered	03/24/2012

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Substitute for form 1449/PTO				Complete if Known	
				Application Number	
				Filing Date	
				First Named Inventor	Hiestand, Peter C. et al.
				Art unit	
				Examiner Name	
Sheet	2	of	2	Attorney Docket Number	PAT050279-US-CNT

INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Use as many sheets as necessary)

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
/P.S./		Brinkmann, Volker et al., "The Immune Modulator FTY720 Targets Sphingosine 1-Phosphate Receptors", The Journal of Biological Chemistry, Vol. 277, No. 24, Issue of June 14, pp. 21453-21457, (2002).	☐
/P.S./		Miller et al., Neurol, & Neurosci. Reports, (September, 2010), 1095), pp. 397-406	☐
/P.S./		Hla, T., FASEB Journal, (March 6, 2006), 20(4), part 1, A20.	☐
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Examiner Signature	/Phyllis Spivack/	Date Considered	03/24/2012

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BIB DATA SHEET

CONFIRMATION NO. 1536

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/149,468	05/31/2011	514	1629	PAT050279-US-CNT

APPLICANTS

Peter C. Hiestand, Allschwil, SWITZERLAND;
Christian Schnell, Hesingue, FRANCE;

**** CONTINUING DATA ****************************

This application is a CON of 12/303,765 12/08/2008 ABN
which is a 371 of PCT/EP2007/005597 06/25/2007

**** FOREIGN APPLICATIONS ***************************

UNITED KINGDOM 0612721.1 06/27/2006

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

06/15/2011

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
☑ Yes ☐ No	☑ Yes ☐ No	/PHYLLIS G SPIVACK/ Examiner's Signature	☐ Initials	SWITZERLAND	0	10	3

ADDRESS

NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080
UNITED STATES

TITLE

S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

FILING FEE RECEIVED	FEES: Authority has been given in Paper	
1480	No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 1 of 2

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	Spivack
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
/P.S./		US-2006/0046979	03-02-2006	Carolyn Ann Foster et al.	
		US-			
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FOREIGN PATENT DOCUMENTS

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/P.S./		WO 2004/028521	04-08-2008	Novartis AG		☐
/P.S./		WO 2003/099192	12-04-2003	Novartis AG		☐
/P.S./		WO 2003/097028	11-27-203	Novartis AG		☐
/P.S./		WO 2004/050073	06-17-2004	Doosan Corporation		☐
/P.S./		WO 2005/123104				☐
						☐

Examiner Signature	/Phyllis Spivack/	Date Considered	03/24/2012

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

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Sheet 2 of 2

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Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		~~Maochkowski M.D. Pharmaceuticals, Moscow, Nowaja Wolna, 2001, in 2 volumes, vol. 1 p. 11~~	☐
/P.S./		LaMontagne K. „Antagonism of Sphingosine-1-Phosphate Receptors by FTY720 Inhibits Angiogenesis.." Cancer Research, Jan 2006, 66, 221-231. Found on:	☐
/P.S./		Hla. T. „Physiological and pathological actions of sphingosine 1-phosphate" Seminars in Cell & Developmental Biology, Oct 2004, 15(5), 513-520.	☐
/P.S./		Kappos L et al. „FTY720 in relapsing MS...." 23.06.2005 online (found 02.06.2011) URL:http://www.ms-in-europe.com/printversion/index.php?anr=105&cnr=4/>	☐
/P.S./		Ho J.W et al. „ Effects of a novel immunomodulating agent.." Molecular cancer theraputics, 2005 Set, 4(9), 1430-1438.Found:	☐
/P.S./		Virely D.J. "Developing therapeutics for the treatment of multiple sclerosis." Journal of American Society for Experimental Neuro Therapeutics. Oct 2005, 2, 638-649. Found on: http://pubget.com/paper/16489371	☐
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Search Notes	Application/Control No.	Applicant(s)/Patent under Reexamination	
	13/149,468	HIESTAND ET AL.	
	Examiner	Art Unit	
	PHYLLIS G. SPIVACK	1629	

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
STN & inventors search	3/21/12	PS
PALM inventors search	3/24/12	PS

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/149,468	05/31/2011	Peter C. Hiestand	PAT050279-US-CNT

CONFIRMATION NO. 1536

1095
NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

PUBLICATION NOTICE

OC000000050106203

Title:S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

Publication No.US-2011-0237682-A1
Publication Date:09/29/2011

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(Use as many sheets as necessary)

Sheet	1	of	2

Complete if Known	
Application Number	13/149468
Filing Date	May 31, 2011
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

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		US-2006/0046979	03-02-2006	Carolyn Ann Foster et al.	
		US-			
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		WO 2004/028521	04-08-2008	Novartis AG		☐
		WO 2003/099192	12-04-2003	Novartis AG		☐
		WO 2003/097028	11-27-203	Novartis AG		☐
		WO 2004/050073	06-17-2004	Doosan Corporation		☐
		WO 2005/123104				☐
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Examiner Signature		**Date Considered**	

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Attorney Docket Number	PAT050279-US-CNT

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Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		Maschkowskij M.D. Pharmaceuticals, Moscow, Nowaja Wolna, 2001, in 2 volumes, vol.1 p.11	☐
		LaMontagne K. „Antagonism of Sphingosine-1-Phosphate Receptors by FTY720 Inhibits Angiogenesis.." Cancer Research, Jan 2006, 66, 221-231. Found on:	☐
		Hla. T. „Physiological and pathological actions of sphingosine 1-phosphate" Seminars in Cell & Developmental Biology, Oct 2004, 15(5), 513-520.	☐
		Kappos L et al. „FTY720 in relapsing MS...." 23.06.2005 online (found 02.06.2011) URL:http://www.ms-in-europe.com/printversion/index.php?anr=105&cnr=4/>	☐
		Ho J.W et al. „ Effects of a novel immunomodulating agent.." Molecular cancer theraputics, 2005 Set, 4(9), 1430-1438.Found:	☐
		Virely D.J. "Developing therapeutics for the treatment of multiple sclerosis." Journal of American Society for Experimental Neuro Therapeutics. Oct 2005, 2, 638-649. Found on: http://pubget.com/paper/16489371	☐
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(54) Title: BIS-AROMATIC ALKANOLS

(57) Abstract: The present invention relates to biphenylyl derivatives, processes for their production, their uses and pharmaceutical compositions containing them.

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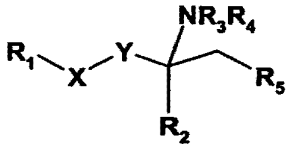
Bis-aromatic Alkanols

The present invention relates to biphenylyl derivatives, processes for their production, their uses and pharmaceutical compositions containing them.

More particularly the present invention provides a compound of formula I



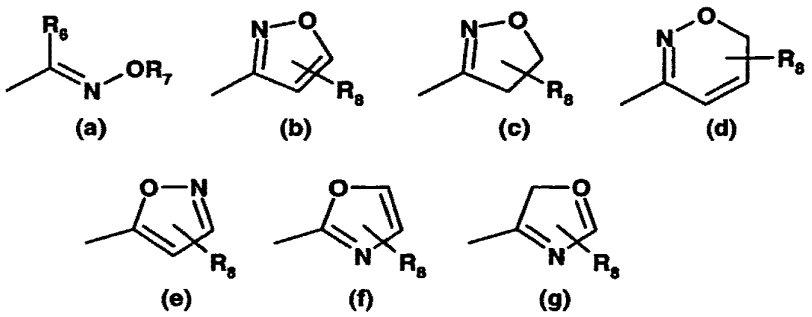
I

wherein

Y is $-CH_2CH_2-$, $-CH_2CH(OH)-$, $-CH(OH)CH_2-$, $-C(O)CH_2-$, $-CH_2C(O)-$, $-CH=CH-$; or 1,2-cyclopropylene;

X is arylene or C_{5-6}heteroarylene optionally substituted by one to three substituents selected from halogen, nitro, C_{1-10}alkyl and halogen-substituted C_{1-6}alkyl;

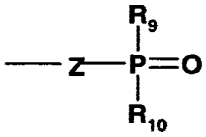
R_1 is aryl, aryl-C_{2-4}alkenyl, heteroaryl, or heteroaryl-C_{2-4}alkenyl each being substituted by (i) one to three substituents selected from hydrogen, halogen, amino, phenyl, heteroaryl, heteroaryl-C_{1-4}alkyl, C_{1-10}alkyl, cycloalkyl-C_{1-4}alkyl, cycloalkyl-C_{1-4}alkoxy, C_{1-10}alkoxy, C_{2-10}alkenyl, C_{2-10}alkynyl, C_{1-10}alkylthio, C_{1-10}alkylsulfonyl, C_{1-10}alkyl-sulfinyl, C_{1-4}alkyl-$S(O)_2NH$-, phenylC_{1-6}alkyl, or phenylC_{1-6}alkoxy, in each of which any aliphatic part of the group may be straight or branched chain and optionally substituted by up to three substituents selected from halogen, amino, hydroxy, cyano, or cycloalkyl groups and optionally interrupted by a double or triple bond or one or more C(O), NR_{12}, S, S(O), $S(O)_2$ or O groups, wherein R_{12} is hydrogen or C_{1-6}alkyl; and any aromatic group may be optionally substituted by one to three substituents selected from halogen, cyano, amino, C_{1-4}alkyl halogen-substituted-C_{1-4}alkyl and C_{1-8}alkoxy; and/or (ii) a group of formula (a), (b), (c), (d), (e), (f) or (g):



in which each of

R_6, R_7 and R_8 independently, is hydrogen; phenyl, C_{1-10}alkyl, cycloalkyl, heteroaryl, heteroaryl-C_{1-4}alkyl, C_{1-10}alkoxy, C_{2-10}alkenyl, C_{2-10}alkynyl, C_{1-10}alkylthio, C_{1-10}alkylsulfonyl, C_{1-10}alkylsulfinyl, phenylC_{1-8}alkyl, or phenylC_{1-6}alkoxy, in each of which any aliphatic part of the group may be straight chain or branched and may be optionally substituted by up to three halogen, hydroxy, cycloalkyl, or C_{1-4}alkoxy groups and optionally interrupted by a double or triple bond or one or more C(O), NR_{12}, S, S(O), $S(O)_2$ or O groups, and any aromatic group may be optionally substituted by one to three substituents selected from halogen, CF_3, C_{1-8}alkyl and C_{1-8}alkoxy;

R_2 is hydrogen; halogen; C_{1-4}alkyl optionally substituted with one or more halogens; C_{2-6} alkenyl; C_{2-6}alkynyl; cycloalkyl optionally substituted by halogen; aryl optionally substituted with hydroxy; or C_{1-4}alkyl optionally substituted on the terminal C atom by OH or a residue of formula (h):



(h)

in which Z is a direct bond, O, S, $(CH_2)_{1-2}$, CF_2, or NR_{11} where R_{11} is H, (C_{1-4})alkyl or halogen-substituted (C_{1-4})alkyl; and each of R_9 and R_{10}, independently, is H, OH, (C_{1-4})alkyl optionally substituted by one to three halogens, or (C_{1-4})alkoxy optionally substituted by halogen; with the proviso that R_9 and R_{10} are not both hydrogen;

each of R_3 and R_4, independently, is H or C_{1-4}alkyl optionally substituted by halogen or acyl; and R_5 is H, –OH, -Oacyl, –NHacyl, or a residue of formula (h) as defined above;

provided that at least either R_2 comprises a terminal OH or a residue of formula (h) or R_5 is OH or a residue of formula (h),

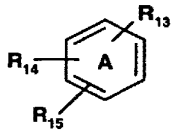
or a salt thereof.

Alkyl as a group and as a structural element of other groups, e.g. halogen-substituted-alkyl, alkoxy, acyl, alkylthio, alkylsulfonyl and alkylsulfinyl, may be straight or branched chain, e.g. methyl, ethyl, propyl, iso-propyl or butyl. Alkenyl as a group and as a structural element of other groups contains one or more carbon-carbon double bonds and may be e.g. vinyl. Any double bonds may be in the cis- or trans- configuration. Alkynyl as a group and as a structural element of other groups and compounds contains at least one carbon – carbon triple bond and may also contain one or more C=C double bonds, and may be e.g. propyn-2-yl. Alkyl, alkenyl, alkynyl or cycloalkyl substituted by halogen, e.g. as R_2, may be alkyl, alkenyl, alkynyl or cycloalkyl wherein one or more H are replaced by halogen, e.g. Cl or F, e.g. $CHCl\text{-}CH_3$ or CF_3; halogen-substituted alkyl, alkenyl, alkynyl or cycloalkyl may be partially halogenated or perhalogenated, whereby in the case of multiple halogenation, the halogen substituents may be identical or different.

Any cycloalkyl group, alone or as a structural element of other groups may contain from 3 to 8 carbon atoms, e.g. from 3 to 7 carbon atoms, preferably from 3 to 6 carbon atoms.

Acyl may be a residue R-CO wherein R is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenylC_{1-4}alkyl. Halogen may be F, Cl or Br, preferably F or Cl.

Aryl means a monocyclic or fused bicyclic aromatic ring assembly, e.g. containing six to ten ring carbon atoms. For example aryl may be naphthyl, phenyl, or phenyl optionally substituted, preferably a residue of formula (k):



(k)

wherein each of R_{13}, R_{14} and R_{15}, independently, is H; halogen; C_{1-8}alkyl optionally substituted by one or more halogen, hydroxy, or C_{1-4}alkoxy or optionally interrupted by one oxy or by one or more oxygen atoms; C_{1-8}alkoxy; C_{2-8}alkenyl; C_{2-8}alkynyl; C_{1-8}alkylthio; C_{1-8}alkylsulfonyl; C_{1-8}alkylsulfinyl; phenylC_{1-6}alkyl; phenylC_{1-6}alkoxy; phenyl optionally substituted by halogen, CF_3, C_{1-4}alkyl and/or C_{1-4}alkoxy. When ring A is monosubstituted, the substitutent is preferably in the *para* position.

Arylene means a divalent radical derived from an aryl group. For example arylene as used in this application may be phenylene or naphthylene, preferably phenylene, more preferably 1,4-phenylene.

Aryl-C_{2-4}alkenyl may be e.g. styryl.

Heteroaryl means aryl, as defined in this application, optionally substituted, provided that one or more of the ring carbon atoms indicated are replaced by a heteroatom, e.g. 1 to 3 heteroatoms, selected from N, O or S, and, e.g. each ring is comprised of 5 to 9 ring atoms. Examples include thienyl, pyridinyl, isoxazolyl, benzoxazolyl, benzo[1,3]dioxolyl, furyl, pyrrolyl, benzothienyl, benzofuryl, indolyl or benzoxadiazolyl, preferably thienyl or pyridinyl. Suitable substitutents are e.g. methyl, halogen or formyl. When substituted, it is preferably monosubstituted. Heteroarylene means heteroaryl, as defined in this application, provided that the ring assembly comprises a divalent radical.

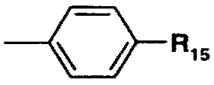
Compounds of the present invention are often active with free hydroxy and free amine groups. Forms of the compound that have the hydroxy or amine group present in a protected form often function as prodrugs. Prodrugs are compounds that are converted into an active drug form after administration, through one or more chemical or biochemical transformations. Forms of the compounds of the present invention that are readily converted into the claimed compound under physiological conditions are prodrugs of the claimed compounds and are within the scope of the present invention. Examples of prodrugs include forms where a hydroxy group is acylated to form a relatively labile ester such as an acetate ester, and forms where an amine group is acylated with the carboxylate group of glycine or an L-amino acid such as serine, forming an amide bond that is particularly susceptible to hydrolysis by common metabolic enzymes. Some molecules of the present invention may themselves be prodrugs, such as those comprising a phosphate residue of formula (h) which may be enzymatically dephosphorylated to a hydroxy group. Alternatively, a compound of the invention wherein R_2 and/or R_5 comprises a free hydroxy group may be enzymatically phosphorylated to a compound comprising a phosphate residue of formula (h). The present invention also includes both the enzymatically phosphorylated or dephosphorylated compounds of formula I, optionally in equilibrium.

Compounds of formula I may exist in free form or in salt form, e.g. addition salts with e.g. inorganic acids, such as hydrochloride, hydrobromide or sulfate, salts with organic acids, such as acetate, fumarate, maleate, benzoate, citrate, malate, methanesulfonate or benzenesulfonate salts; when group (h) is present and R_9 or R_{10} is –OH, group (h) may also be present in salt form, e.g. an ammonium salt or salts with metals such as sodium, potassium, calcium, zinc or magnesium, or a mixture thereof. Compounds of formula I and their salts in hydrate or solvate form are also part of the invention.

When the compounds of formula I have asymmetric centers in the molecule, various optical isomers are obtained. The present invention also encompasses enantiomers, racemates, diastereoisomers and mixtures thereof. For example the central carbon atom bearing R_2, CH_2-R_5 and NR_3R_4 may have the R or S configuration. Compounds having the R configuration at this central carbon atom are preferred. Moreover, when the compounds of formula I include geometric isomers, the present invention embraces cis-compounds, trans-compounds and mixtures thereof. Similar considerations apply in relation to starting materials exhibiting asymmetric carbon atoms or unsaturated bonds as mentioned above.

In the compounds of formula (I), the following significances are preferred individually or in any sub-combination:

1. Y is $-CH_2-CH_2-$ or $-CH(OH)-CH_2-$, preferably $-CH_2-CH_2-$;
2. X is 1,4-phenylene;
3. R_1 is mono- or di-substituted phenyl or thienyl, preferably para-monosubstituted phenyl, e.g. substituted by a group R_{15}, as defined below; e.g. R_1 is a group of formula (k)



(k)

where R_{15} is straight chain C_{5-8}alkyl; C_{2-8}alkenyl; or straight chain or branched C_{1-8}alkoxy optionally substituted by one C_{3-6}cycloalkyl or by a phenyl group optionally substituted by up to three halogens;

4. R_1 is mono- or di-substituted phenyl or thienyl, preferably para-monosubstituted phenyl, e.g. substituted by a group of formula (a), (b) or (c) as defined above;
5. R_1 is phenyl monosubstituted by a group of formula (a), preferably in the trans configuration;
6. In the group of formula (a), R_6 is C_{1-6}alkyl or C_{3-6}cycloalkyl, preferably straight chain C_{1-4}alkyl, cyclopropyl or cyclopropylmethyl;
7. In the group of formula (a), R_7 is H, C_{1-6}alkyl, C_{2-6}alkenyl, or C_{2-6}alkynyl, preferably straight chain C_{1-6}alkyl, vinyl, allyl or propyn-2-yl;
8. R_2 is C_{1-4}alkyl optionally substituted on the terminal C atom by OH or a residue of formula (h), preferably R_2 is methyl or hydroxymethyl, more preferably hydroxymethyl;

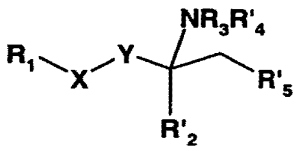
9. At least one of R_3 and R_4 is hydrogen, preferably both are hydrogen;

10. R_5 is hydrogen, –OH, –NHC(O)C_{1-4}alkyl or a residue of formula (h);

11. Each of R_9 and R_{10} is –OH;

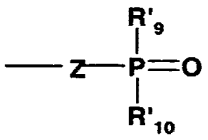
12. Z is O.

The present invention also includes a process for the preparation of a compound of formula I which process comprises removing the hydrolysable groups present in a compound of formula II



II

wherein X, Y, R_1 and R_3 are as defined above, R_4' is an amino protecting group, R_2' has one of the significances given for R_2 above except that the terminal OH when present in the OH-substituted C_{1-4}alkyl is in protected form or the residue of formula (h) is replaced by a residue of formula (h') and R_5' is R_5" in which R_5" is H, -OH in protected form or a residue of formula (h'), provided that at least one of R_2' and R_5' is OH in protected form or a residue of formula (h'), the residue of formula (h') being:



(h')

wherein Z is as described above, and each of R_9' and R_{10}' is a hydrolysable group and, where required, converting the compounds of formula I obtained in free form into the desired salt form, or vice versa.

The process may be carried out in accordance with methods known in the art. Hydrolysable groups may be hydroxy and amino protecting groups, e.g. when compounds of formula I are free of a residue of formula (h), and/or groups such as R'_9 and R'_{10}. Examples of protecting groups for hydroxy and amino groups are, e.g. as disclosed in "Protective Groups in Organic Synthesis" T.W. Greene, J. Wiley & Sons NY, 2nd ed., chapter 7, 1991, and references therein, e.g. benzyl, p-methoxybenzyl, methoxymethyl, tetrahydropyranyl, trialkylsilyl, acyl, *tert*-butoxy-carbonyl, benzyloxy-carbonyl, 9-fluorenylmethoxycarbonyl, trifluoroacetyl, trimethylsilyl-ethanesulfonyl and the like.

Preferably R_9' and R_{10}' are identical and have the significance of, e.g. phenoxy or benzoxy or form together a cyclic system such as in 1,5-dihydro-2,4,3-benzodioxaphosphepin.

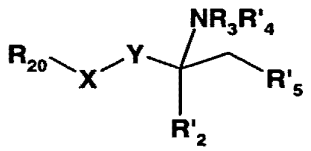
The removal of the hydroxy and amino protecting groups and/or of R'_4 or R'_5 groups in the compounds of formula II may conveniently be performed according to methods known in the art, e.g. by hydrolysis, e.g. in a basic medium, e.g. using a hydroxide such as barium hydroxide. It may also be performed by hydrogenolysis, e.g. in the presence of Pearlman's catalyst, e.g. as disclosed in J. Org. Chem., 1998, 63, 2375-2377. When the compounds of formula II are free of a residue of formula (h'), the removal of the hydroxy and amino protecting groups may also be performed in an acidic medium.

Compounds of formula II, used as starting materials, and salts thereof are also novel and form part of the invention.

The present invention also includes a process for the preparation of a compound of formula II which process comprises coupling a compound of formula III:

$$R_1—Q \qquad \text{III}$$

wherein R_1 is as defined above, Q is boron, silicon, magnesium, tin, lithium, copper or zinc, where each of these elements is bound to one or more suitable ligands, e.g. hydroxy, C_{1-8}alkoxy, C_{1-8}alkyl optionally substituted by a terminal carboxyl group, halogen or pseudohalogen, e.g. triflate (trifluoromethylsulfonate), mesylate, tosylate or cyanide; with a compound of formula IV:



IV

wherein X, Y, R'_2, R_3, R'_4 and R'_5 are as specified above, and R_{20} is halogen, preferably Cl, Br, I, triflate, tosylate or mesylate;

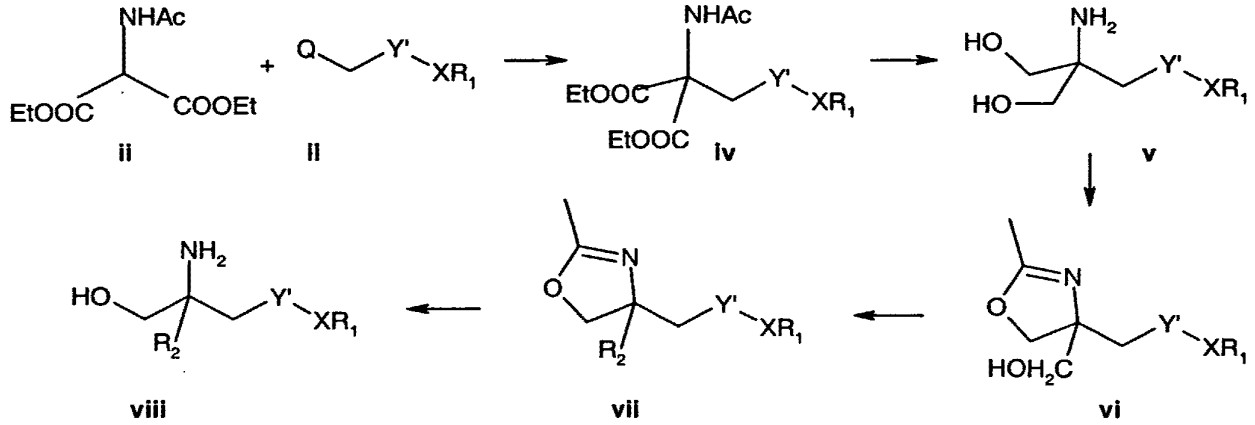
under the catalysis of a transition metal or salt thereof, e.g. palladium, rhodium or platinum, e.g. in the presence of a suitable ligand, e.g. a phosphine, carboxylate or heterocyclic carbene.

Compounds of formula II wherein R'_5 is a residue of formula (h') may also be prepared by reacting a compound of formula II wherein R'_5 is hydroxy in protected or unprotected form, with a corresponding phosphorylating agent, e.g. a phosphorochloridate, e.g. diphenylchlorophosphate or dibenzylchlorophosphate, cyanoethylphosphate, a phosphoramidate such as

N-phenyl phosphoramidate, 3-(diethylamino)-1,5-dihydro-2,4,3-benzodioxaphosphepin and the like.

Many compounds of the present invention, having general structure of Formula I, may be synthesized from protected aminomalonate esters such as ii (Scheme I). This compound may be readily alkylated by alkylating agents such as iii (wherein Y' is CH_2, CH(OH) or C=O) having leaving groups (-Q) such as bromide, iodide, or an alkyl or aryl sulfonate ester. These alkylating agents and methods for their preparation are generally well known in the art. The products of these alkylations are compounds such as iv, which may be reduced to produce compounds v of the present invention. This approach enables synthesis of compounds v, having various X-R_1 groups and linking groups between X and the aminopropanediol.

Scheme I.

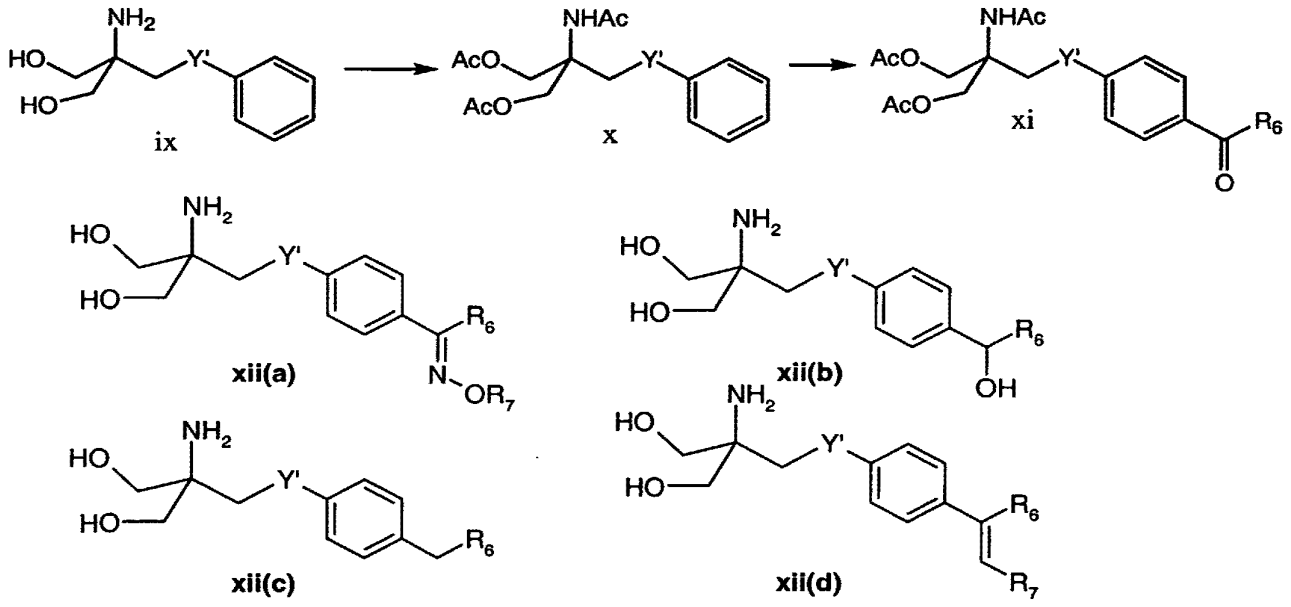


Compounds such as v may be used to prepare other compounds of the present invention, using well known protection strategies (Scheme I) to differentiate the two hydroxy groups. For example, v may be protected as an oxazoline (vi), leaving one hydroxy group free for further functionalization. Methods well known in the art (alkylation, acylation, oxidation, reduction, and combinations of these steps) may be used to convert the CH_2OH group of compound vi into various R_2 groups to provide other compounds that are within the scope of the present invention such as vii and viii.

Alternatively, a compound of formula ix may be protected by acylation, for example, allowing functionalization of the aryl group (Scheme II, wherein Y', R_6 and R_7 are as described above). When the aryl group is a phenyl, as shown for example with ix, it may be acylated to produce a compound x that may undergo Friedel-Crafts acylation under conventional conditions to produce a compound such as xi. This acylated compound may then be further transformed into compounds such as xii(a), xii(b), xii(c) and xii(d) by procedures well known

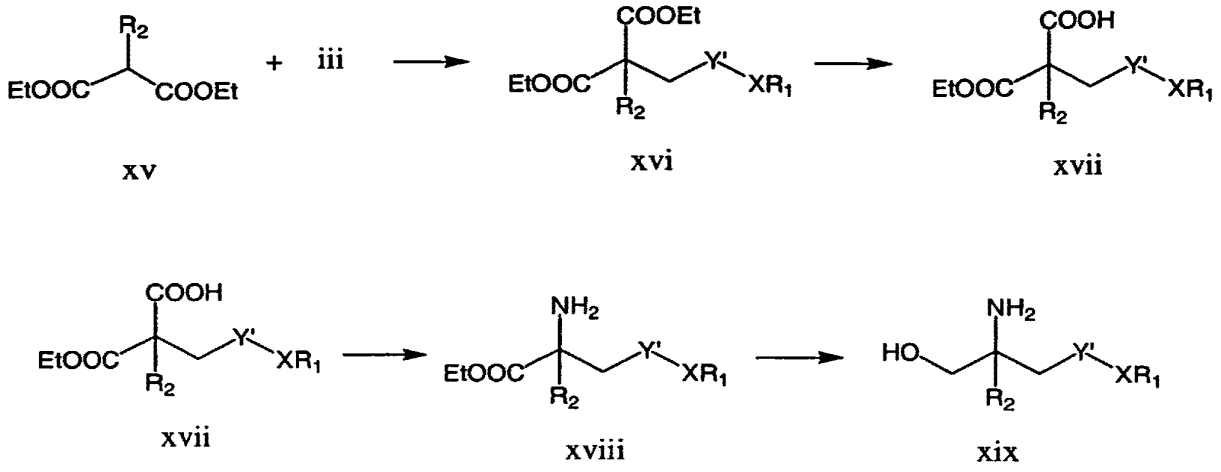
to those of skill in the art. For example, conversion to an oxime (xii(a)) is accomplished by treatment with an alkoxyamine as described below for Example 5. Reduction to the alcohol xii(b) may be accomplished with sodium borohydride, for example; further reduction to remove the hydroxy group (producing xii(c)) may be achieved with catalytic hydrogenation or with triethylsilane and trifluoroacetic acid. Olefination to form xii(d) may be achieved with Wittig or Horner-Emmons conditions, via Petersen olefination, or by a Grignard addition followed by elimination of the benzylic alcohol. Such transformations enable the incorporation of diverse substituents on the aryl groups of the compounds of the present invention.

Scheme II



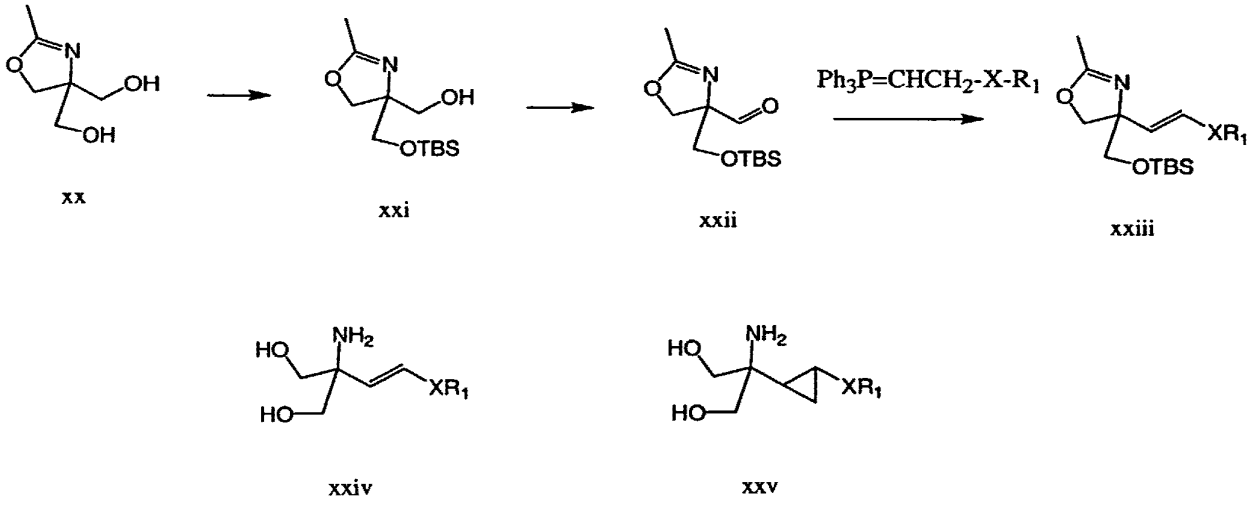
Another general method for preparing compounds where R_2 is other than CH_2OH or a residue of formula (h) (referred to herein as R''_2), begins with a malonate ester such as xv, which may be alkylated with an alkylating agent such as iii (Scheme III, wherein Y' is as described above). This provides intermediate xvi, which may be selectively hydrolyzed under conditions known in the art to give xvii. Compounds of this general structure may be converted to an amide or acyl azide, for example, which may be used to prepare compounds xix. Reduction of the ester group then provides compounds xix of the present invention. This enables access to compounds wherein R_2 is an aryl group such as e.g. 2-hydroxyphenyl.

Scheme III



Another versatile method for preparing compounds of the present invention utilizes xx, which is a known compound that may be selectively protected as xxi and oxidized to provide xxii (Scheme IV, wherein Y' is as described above). Aldehyde xxii may be used for a Wittig olefination reaction, e.g. to produce xxiv. This compound, after deprotection, may provide compounds xxv of the present invention. Alternatively, it may be used to synthesize other compounds such as e.g. compound xxvi, which is produced by cyclopropanation of the olefin of xxiv followed by deprotection.

Scheme IV

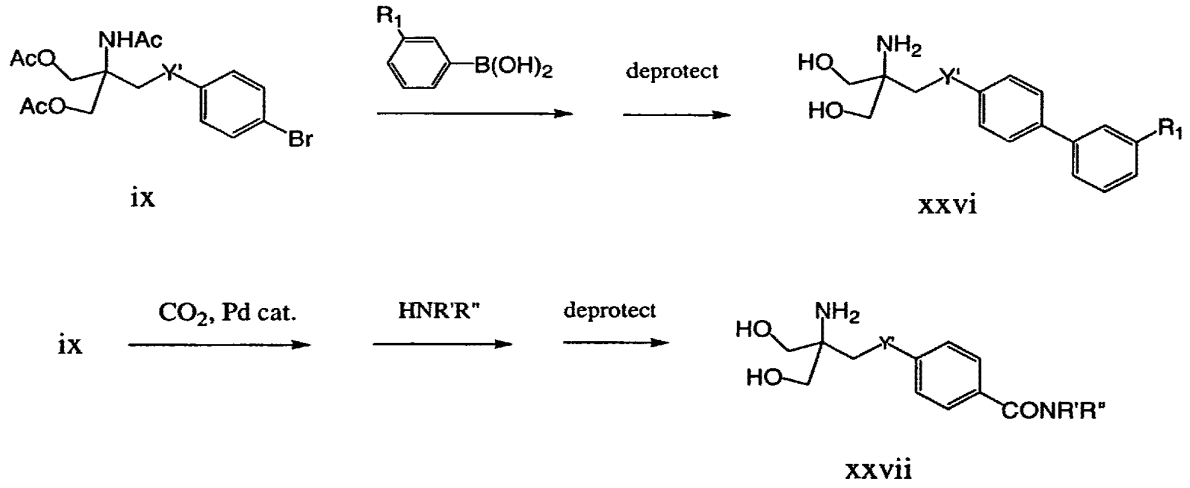


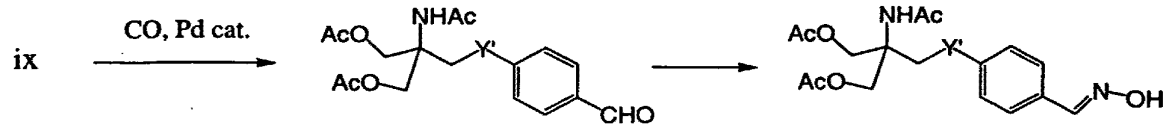
Certain compounds from the above schemes serve as versatile intermediates that allow further functionalization of the X group (Scheme V, wherein Y' is as described above). For

example, compounds such as iv, x, xvi, and xxiii have their hydroxy and amine groups protected; when X in such compounds is X' containing certain functional groups, they may be used to introduce new features on X. For example, if X' is a bromophenyl, bromopyridyl or similar group suitable for Suzuki reactions and similar palladium-catalyzed coupling reactions, X' may be arylated to provide biaryl compounds of the present invention, e.g. compounds of Formula I containing a biaryl group, like xxvi.

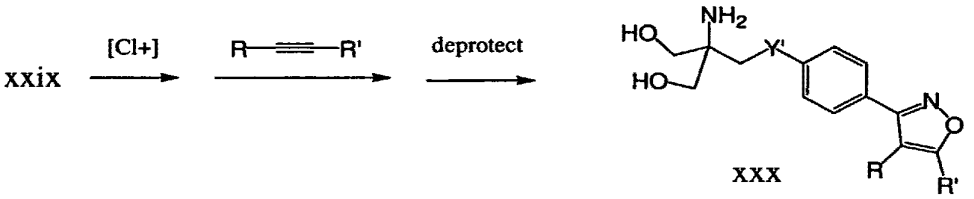
Alternatively, such bromophenyl and similar compounds may be carboxylated in the presence of a palladium catalyst and CO_2, and the carboxyl group may be used to introduce features such as an amide group. Furthermore, such bromophenyl and similar compounds may be carbonylated in the presence of palladium catalyst and CO, to introduce an aldehyde group. The aldehyde may then be used e.g. in Grignard or Wittig reactions to introduce new alkyl or aryl groups, or it may e.g. be converted into an oxime by reaction with hydroxylamine. Oximes such as xxix may be used to generate nitrile oxide intermediates by procedures well known in the art, and these readily undergo [3+2] cycloaddition reactions with olefins and acetylenes to produce isoxazolines and isoxazoles, respectively.

Scheme V





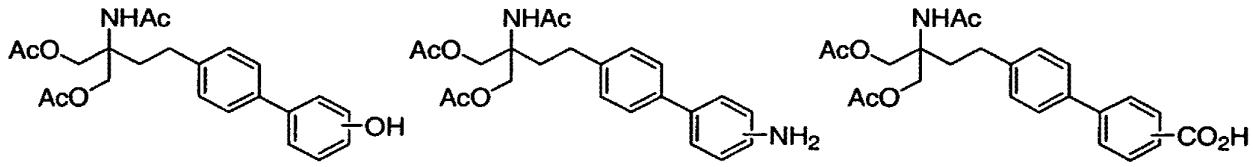
xxviii xxix



xxx

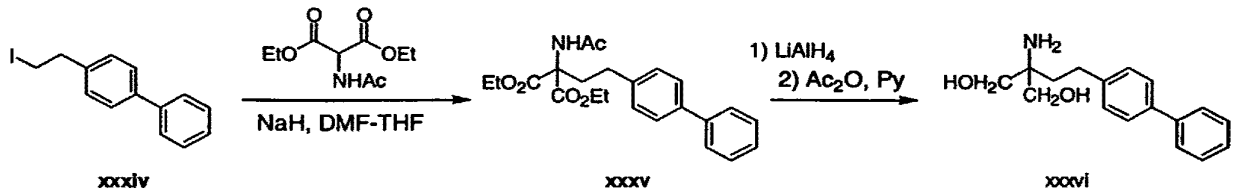
Furthermore, using protected intermediates such as xxiii above, an aryl ring X may be converted readily by methods known in the art to an arylboronic acid or an aryltrimethyltin species that may be used in Suzuki or Stille type coupling reactions to produce other biaryl compounds of the present invention.

Alternatively, a starting compound wherein X contains a nitro group as a substituent, that group may be reduced and alkylated, acylated or sulfonylated to produce other compounds of the present invention. A hydroxy group present in protected form may be deprotected and alkylated or otherwise modified, including being converted into a trifluoromethylsulfonate ("triflate") or similar functional group that is useful for palladium-catalyzed replacement reactions. Other substituents may likewise be incorporated on the aryl groups of intermediates such as those illustrated, as those of skill in the art will appreciate, and may also be transformed using well known methods into other groups to provide other compounds of the present invention. Examples of some very versatile intermediates of this type are shown below:



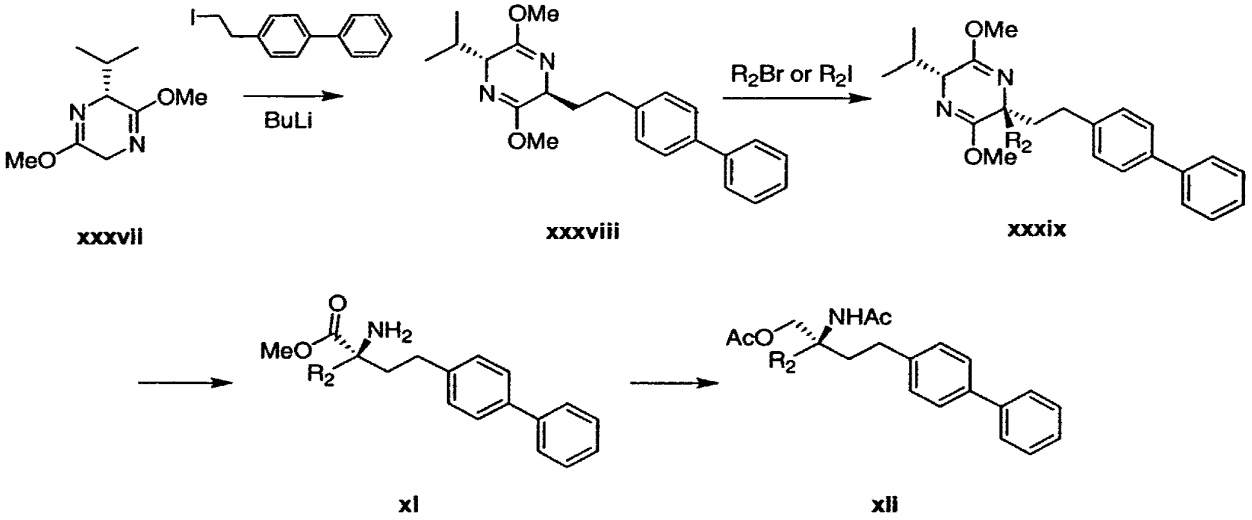
xxxi xxxii xxxiii

Certain key intermediates that are particularly useful for practicing the present invention are known in the art. For example, compound xxxvi, the preparation of which is described by Kiuche *et al.* in *J. Med. Chem., 43*:2946-2961 (2000).



xxxiv xxxv xxxvi

For some embodiments of the present invention, it is desirable to prepare compounds wherein R_2 is R''_2 as individual enantiomers. These may be obtained by methods described herein, and the individual enantiomers may be separated by methods such as crystallization or chiral chromatography as is known in the art. However, it is also possible to synthesize the individual enantiomers by chiral synthetic methods, using Schöllkopt methodology, for example. Both enantiomers may be prepared using this synthetic route and proper selection of the chiral auxiliary group. By sequential alkylation of the chiral template xxxvii, compound xxxviii is produced diastereoselectively. The chiral intermediate xli may be obtained therefrom by subsequent transformations including hydrolysis, reduction and protection.



xxxvii xxxviii xxxix

xl xli

Insofar as the production of the starting materials is not particularly described, the compounds are known or may be prepared analogously to methods known in the art or as disclosed in the Examples hereinafter.

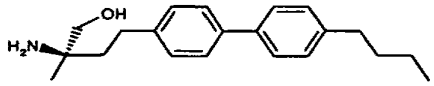
The following Examples are illustrative of the invention.

RT = room temperature

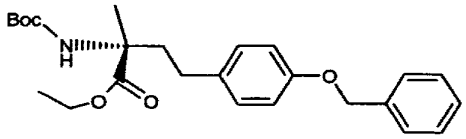
DCM = dichloromethane

Bn = benzyl

Example 1: (R)-2-Amino-4-(4'-butyl-biphenyl-4-yl)-2-methyl-butan-1-ol

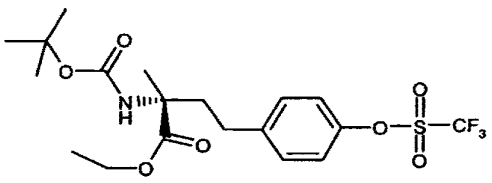


a) (R)-4-(4-Benzyloxy-phenyl)-2-tert-butoxycarbonylamino-2-methyl-butyric acid ethyl ester



To a solution of (2R,5R)-2-[2-(4-benzyloxy-phenyl)-ethyl]-3,6-diethoxy-5-isopropyl-2-methyl-2,5-dihydro-pyrazine (6.9 g, prepared as disclosed in WO 02/76695 the contents of which being herein incorporated by reference) in dry dioxane (170 ml) is added 105 ml of 0.5N HCl in water. After the homogenous solution is left standing overnight, ethyl acetate (300 ml) is added and the mixture is extracted with water (3 x 150 ml). The organic phase is dried ($MgSO_4$) and the solvent is evaporated. The crude product is dissolved in DCM and after addition of t-butyloxycarbonylanhydride (5.17 g) is left standing overnight. The solvent is removed in vacuo and the crude residue is purified by chromatography using diethyl ether/hexane (1/5) (R_f = 0.2, MS: (ES+): 428.5 $(M+H)^+$).

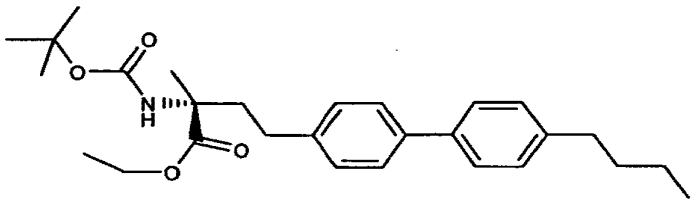
b) (R)-2-tert-Butoxycarbonylamino-2-methyl-4-(4-trifluoromethanesulfonyloxy-phenyl)-butyric acid ethyl ester



(R)-4-(4-Benzyloxy-phenyl)-2-tert-butoxycarbonylamino-2-methyl-butyric acid ethyl ester (2.78 g) is dissolved in ethyl acetate (100 ml) and hydrogenated at atmospheric pressure and RT using Pd/C (500 mg) for 16h. Filtration over talcum is followed by removal of the solvent in vacuo to yield a colorless oil (R_f (diethyl ether/hexane = 1/1) = 0.32, MS: (ES+): 338.4 $(M+H)^+$). The crude phenol (2.20g) and pyridine (2.6 ml) are dissolved in DCM and cooled to 0°C. Trifluoromethane sulfonic anhydride (1.3 ml) is added dropwise and the mixture is stirred at 0°C for 30 min. After addition of water (20 ml) and DCM (30 ml), the mixture is washed with 0.5N NaOH (15 ml), water (20 ml), 1M citric acid (2 x 25 ml) and

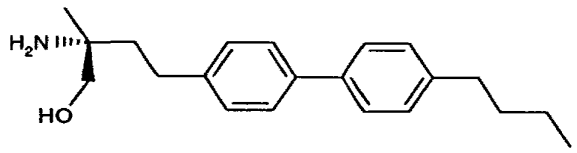
water (20 ml). The organic phase is dried over $MgSO_4$, the solvent removed and the crude material purified by chromatography using diethyl ether/hexane (1/2) giving the desired product as colorless oil (R_f = 0.44, MS: (ES+): 470.5 $(M+H)^+$).

c) (R)-2-tert-Butoxycarbonylamino-4-(4'-butyl-biphenyl-4-yl)-2-methyl-butyric acid ethyl ester



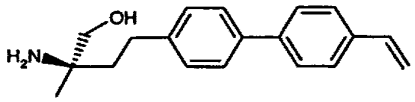
(R)-2-tert-Butoxycarbonylamino-2-methyl-4-(4-trifluoromethane sulfonyloxy-phenyl)-butyric acid ethyl ester (100 mg), 4-butylboronic acid (75 mg) and K_2CO_3 (44 mg) are suspended in dry toluene (3 ml). Argon is bubbled through the mixture for 10 min., tetrakispalladiumtriphenyl-phosphine (5mg) is added and the mixture is stirred at 95°C under Argon for 16h. After cooling to RT, ethyl acetate (5 ml) is added and the mixture is washed with 0.5N NaOH (2 ml), water (2 ml), 1M citric acid (2 x 2 ml) and water (2 ml). The organic phase is dried over $MgSO_4$, the solvent removed and the crude material purified by chromatography using diethylether/hexane = 1/5 (R_f = 0.14, MS: (ES+): 454.6 $(M+H)^+$).

d) (R)-2-Amino-4-(4'-butyl-biphenyl-4-yl)-2-methyl-butan-1-ol



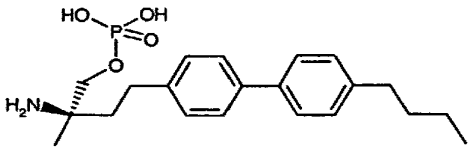
To a solution of (R)-2-tert-butoxycarbonylamino-4-(4'-butyl-biphenyl-4-yl)-2-methyl-butyric acid ethyl ester (22 mg) in diethylether is added lithium borohydride (20 mg). After stirring the suspension for 9h at RT, ethyl acetate (5 ml) is added and the mixture is washed with water (2 ml), 1M citric acid (2 x 2 ml) and water (2 ml). The organic phase is dried over $MgSO_4$, the solvent removed and the crude material purified by chromatography using diethyl ether/hexane (1/1) (R_f = 0.31, MS: (ES+): 412.6 $(M+H)^+$). The purified product is dissolved in dioxane containing 4M HCl and left at room temperature for 16h. After lyophilisation, the desired compound is obtained as a white solid in the hydrochloride salt form (R_f = 0.48 in DCM/methanol 100/15, MS: (ES+): 312.5 $(M+H)^+$).

Example 2: (R)-2-Amino-4-(4'-vinyl-biphenyl-4-yl)-2-methyl-butan-1-ol



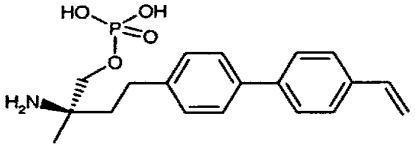
The title compound is obtained by following the procedure as disclosed in Example 1, but using the appropriate starting materials, e.g. vinylphenylboronic acid instead of 4-butylboronic acid in step c). The compound is obtained as an off-white solid, in the hydrochloride salt form. MS: (ES+): 282.4 $(M+H)^+$

Example 3: Phosphoric acid mono-{(R)-2-amino-4-(4'-butyl-biphenyl-4-yl)-2-methyl butyl} ester



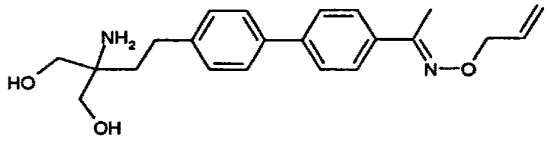
The compound of Example 1c) is converted into the corresponding phosphoric acid monoester by following a procedure as disclosed in WO 02/18395.

Example 4: Phosphoric acid mono-{(R)-2-amino-4-(4'-vinyl-biphenyl-4-yl)-2-methyl-butyl} ester



(R)-2-tert-Butoxycarbonylamino-4-(4'-vinyl-biphenyl-4-yl)-2-methyl-butyric acid ethyl ester is converted into the corresponding phosphoric acid monoester by following a procedure as disclosed in WO 02/18395.

Example 5: 1-[4'-(3-Amino-4-hydroxy-3-hydroxymethyl-butyl)-biphenyl-4yl]-ethanone-*O*-allyl-oxime



Step A: 2-Acetylamino-2-(2-biphenyl-4-yl-2-oxo-ethyl)-malonic acid diethyl ester

Sodium hydride (15 mmol) is added to anhydrous ethanol (50 mL). To this resulting sodium ethoxide solution is added 2-acetylaminomalonic acid diethyl ester (15 mmol) in one portion. The resulting mixture is stirred at room temperature for 30 min. A solution of 4'-phenyl-2-bromoacetophenone (10 mmol) in ethanol (10 mL) is then added and the resulting mixture is stirred at room temperature for 12 h. After concentrating under reduced pressure, the residue is dissolved in EtOAc and water. The organic phase is washed with brine and dried over Na_2SO_4. After removal of the solvent, the crude material is purified by column chromatography using EtOAc/hexane (1/3) giving the desired product as white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.6 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.45 (m, 3H), 7.13 (s, 1H), 4.28 (m, 6H), 1.98 (s, 2H), 1.26 (t, J = 7.1 Hz, 6H); MS: (ES^+): 412.2 $(M+1)^+$.

Step B: Acetic acid 4-acetoxy-2-acetoxymethyl-2-acetylamino-4-biphenyl-4-yl-butyl ester

To a solution of 2-acetylamino-2-(2-biphenyl-4-yl-2-oxo-ethyl)-malonic acid diethyl ester (5 mmol) in 95% EtOH (50 mL) is added $NaBH_4$ (25 mmol) in portions. After stirring at room temperature for 3 h, the reaction is quenched with saturated NH_4Cl. After removal of EtOH under reduced pressure, the aqueous solution is extracted with EtOAc. The organic phase is washed with brine and dried over Na_2SO_4. After concentrating, the residue is dissolved in anhydrous CH_2Cl_2 (25 mL). Ac_2O (30 mmol) and pyridine (60 mmol) are then added. After stirring at room temperature for 12 h, it is sequentially washed with 1 N HCl, saturated $NaHCO_3$, and brine and dried over Na_2SO_4. After removal of the solvent, the crude material is purified by column chromatography using EtOAc/hexane (1/1) to give desired product as a white solid. MS: (ES^+): 456.2 $(M+1)^+$.

Step C: Acetic acid 2-acetoxymethyl-2-acetylamino-4-biphenyl-4-yl-butyl ester

Acetic acid 4-acetoxy-2-acetoxymethyl-2-acetylamino-4-biphenyl-4-yl-butyl ester (5 mmol) is dissolved in EtOH (50 mL) and hydrogenated at atmospheric pressure using 10% Pd-C (10 %) at room temperature for 12 h. After filtration and concentration, the crude product is obtained as a white solid and used in the next step without further purification. MS: (ES^+): 398.2 $(M+1)^+$.

Step D: Acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-acetylbiphenyl-4-yl)-butyl ester

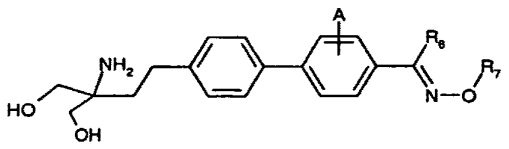
To a suspension of $AlCl_3$ (16 mmol) in DCE (20 mL) is added AcCl (8 mmol) in one portion. After stirring at room temperature for 30 min, to the solution is added acetic acid 2-acetoxymethyl-2-acetylamino-4-biphenyl-4-yl-butyl ester (2 mmol) in DCE (5 mL). After an additional 30 min, the mixture is poured into ice-cold 1 N NaOH and is extracted with DCM. The organic phase is washed with 1 N HCl, brine and dried over Na_2SO_4. After concentrating, the crude material is purified by column chromatography using EtOAc/hexane (2/1) to give the desired product as a white solid. MS: (ES^+): 439.2 $(M+1)^+$.

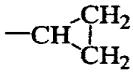
Step E: 1-[4'-(3-Amino-4-hydroxy-3-hydroxymethyl-butyl)-biphenyl-4-yl]ethanone-*O*-allyl-oxime

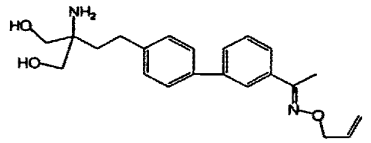
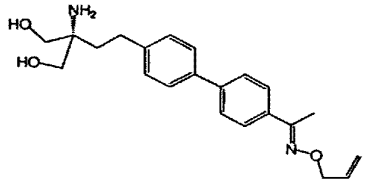
To a solution of 1-[4'-(3-amino-4-hydroxy-3-hydroxymethyl-butyl)-biphenyl-4-yl]ethanone-*O*-allyl-oxime (0.2 mmol) in MeOH (1 mL) is added *O*-allylalkoxylamine hydrochloride salt (0.24 mmol) and Et_3N (0.23 mmol). After stirring at room temperature for 12 h, it is concentrated and the residue is dissolved in DCM, which is washed with brine and dried over Na_2SO_4. After the concentration, the crude product is dissolved in THF (1 mL) and treated with 2 N LiOH aqueous solution (0.5 mL). The resulting mixture is stirred at reflux for 1 h and diluted with H_2O (10 mL). It is then extracted with EtOAc (3 x 5 mL) and the combined organic phase is washed with brine and dried over Na_2SO_4. After concentrating, the crude product is purified with LC-MS to give the desired product as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.6 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.45 (m, 3H), 7.13 (s, 1H), 4.28 (m, 6H), 1.98 (s, 2H), 1.26 (t, J = 7.1 Hz, 6H); MS: (ES^+): 369.2 $(M+1)^+$.

By repeating the procedure described in Example 5, using appropriate starting materials, the following compounds of Formula I are obtained as identified in Table I.

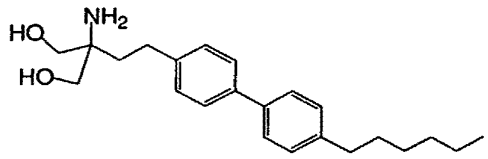
TABLE I

Example No.				Physical Data
	A	R_6	R_7	MS (M+1)

6	H	$-(CH_2)_2CH_3$	$-CH_3$	371.2
7	H	$-CH_3$	-H	329.2
8	H	$-CH_3$	$-CH_3$	343.2
9	H	$-CH_2CH_3$	$-CH_3$	357.2
10	H	$-(CH_2)_3CH_3$	$-CH_3$	385.2
11	H	$-(CH_2)_2CH_3$	$-CH_2CH_3$	385.2
12	H	$-(CH_2)_2CH_3$	$-CH_2CH=CH_2$	397.2
13	H	$-CH_2CH_3$	-H	343.2
14	H	$-CH_2CH_3$	$-CH_2CH_3$	371.2
15	H	$-CH_2CH_3$	$-CH_2CH=CH_2$	383.2
16	H		$-CH_2CH=CH_2$	395.3
17	H	$-(CH_2)_3CH_3$	$-CH_2CH=CH_2$	411.3
18	H	$-(CH_2)_3CH_3$	$-CH_2CH_3$	399.3
19	H	$-(CH_2)_4CH_3$	$-CH_2CH=CH_2$	425.3
20	H	$-(CH_2)_4CH_3$	$-CH_2CH_3$	413.3
21	H	$-(CH_2)_6CH_3$	$-CH_2CH=CH_2$	453.3
22	$-CH_3$ (*meta*)	$-CH_3$	$-CH_2CH=CH_2$	383.2
23	H	H	$-CH_2CH=CH_2$	355.2
24	H	$-CH_3$	$-(CH_2)_3CH_3$	385.2
25	H	$-CH_3$	$-(CH_2)_2CH_3$	371.2
26	H	$-CH_3$	$-(CH_2)_4CH_3$	399.2
27	H	$-CH_3$	$-(CH_2)_5CH_3$	413.2
28	I (*meta*)	$-CH_3$	$-(CH_2)_2CH_3$	
28.1	F (*meta*)	$-CH_3$	$-(CH_2)_2CH_3$	389.2

29	I (*meta*)	$-CH_3$	$-CH_2CH=CH_2$	
29.1	F (*meta*)	$-CH_3$	$-CH_2CH=CH_2$	387.2
30	I (*ortho*)	$-CH_3$	$-CH_2CH=CH_2$	
30.1	F (*ortho*)	$-CH_3$	$-CH_2CH=CH_2$	387.2
31	I (*ortho*)	$-CH_3$	$-(CH_2)_2CH_3$	
31.1	F (*ortho*)	$-CH_3$	$-(CH_2)_2CH_3$	389.2
32	H	$-CH_3$	$-CH_2C\equiv CH$	367.2
33				369.2
34				369.2

Example 35: 2-Amino-2-[2-(4'-hexylbiphenyl-4-yl)-ethyl]propane-1,3-diol



Step A: Acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-hexyl-biphenyl-4-yl)-butyl ester

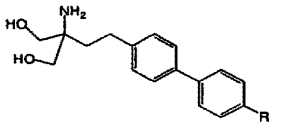
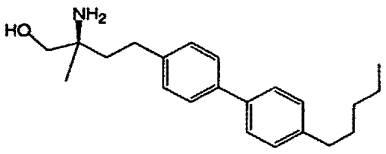
To a solution of acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-hexanoyl-biphenyl-4-yl)-butyl ester (prepared according to Scheme 1) (1 mmol) in trifluoroacetic acid (10 mL) is added triethylsilane (2.5 mmol). The resulting mixture is stirred at room temperature for 12 h. After concentrating under reduced pressure, the residue is dissolved in DCM and the organic solution is washed with saturated $NaHCO_3$ and brine and dried over Na_2SO_4. After concentration, the crude product is purified by column chromatography using EtOAc/hexane (1/1) to give the desired compound as a white solid. MS: (ES^+): 482.3 $(M+1)^+$.

Step B: 2-Amino-2-[2-(4'-hexylbiphenyl-4-yl)-ethyl]propane-1,3-diol

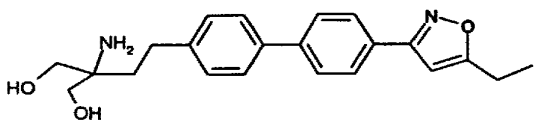
Acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-hexyl-biphenyl-4-yl)-butyl ester (0.2 mmol) is dissolved in THF (1 mL) and treated with 2 N LiOH aqueous solution (0.5 mL). The resulting mixture is stirred at reflux for 1 h and diluted with H_2O (10 mL). It is then extracted with EtOAc (3 x 5 mL) and the combined organic phase is washed with brine and dried over Na_2SO_4. After concentrating, the crude product is purified with LC-MS to give the desired product as a white solid. MS: (ES^+): 356.2 $(M+1)^+$.

By repeating the procedure described in Example 35, using appropriate starting materials, the following compounds of Formula I are obtained as identified in Table II.

TABLE II

Example No.	 R	Physical Data MS (M+1)
36	$-(CH_2)_4CH_3$	342.2
37	$-(CH_2)_7CH_3$	384.3
38	$-C(=CHCH_2CH_3)(CH_2)_2CH_3$	368.3
39		326.2

Example 40: 2-Amino-2-{2-[4'-(5-propyl-isoxazol-3-yl)-biphenyl-4-yl]-ethyl}-propane-1,3-diol



Step A: 4-[2-(4-Bromophenyl)vinyl]-4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazole

To a suspension of (4-bromobenzyl)triphenyl-phosphonium bromide (6 mmol) in dry THF (25 mL) is added NaH (6 mmol) in portions. After stirring at room temperature for 30 min, a solution of 4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazole-4-carbaldehyde (prepared according to Scheme 3 using well-known chemistry in the art) (5 mmol) in THF (10 mL) is added in one portion. The mixture is stirred at room temperature for 12 h. After concentration, the residue is treated with EtOAc/hexane (1/5) (100 mL) and the solid is filtrated. The filtrate is washed with brine and dried over Na_2SO_4. After concentration, the crude product is purified by column chromatography by EtOAc/hexane (1/5) to give the desired product as a colorless oil. MS: (ES^+): 410.1 $(M+1)^+$.

Step B: 4-[2-(4-Bromophenyl)ethyl]-4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazole

4-[2-(4-Bromophenyl)vinyl]-4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazole (3 mmol) is dissolved in ethanol (15 mL) and hydrogenated at atomospheric pressure in the presence of chlorotris(triphenylphosphine)rhodium(I) (10 %). The mixture is stirred at 40 ^{0}C for 12 h. After filtration and concentration, the crude product is obtained as colorless oil, which is used directly in the next step without further purification. MS: (ES^+): 412.1 $(M+1)^+$.

a) Step C: 4-{2-[4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazol-4-yl]ethyl}-biphenyl-4-carbaldehyde

The mixture of 4-[2-(4-bromophenyl)ethyl]-4-(*t*-butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazole (2 mmol), 4-fomylphenylboronic acid (2.4 mmol), $Pd(PPh_3)_4$ (0.2 mmol) and Na_2CO_3 (9.6 mmol) in toluene (5 mL), EtOH (1.5 mL) and H_2O (5 mL) is stirred at 90°C for 5 h. It is diluted with H_2O (15 mL) and EtOAc (15 mL) and the organic phase is washed with brine and dried over Na_2SO_4. After concentration, the crude product is purified by column chromatography using EtOAc/hexane (1/4) to give the desired product as a white solid. MS: (ES^+): 438.2 $(M+1)^+$.

b) Step D: Acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-formylbiphenyl-4-yl)butyl ester

To a solution of 4-{2-[4-(*t*-Butyldimethylsilanyloxymethyl)-2-methyl-4,5-dihydrooxazol-4-yl]ethyl}-biphenyl-4-carbaldehyde (2 mmol) in THF (10 mL) is added 1 N HCl aqueous solution (5 mL). The mixture is refluxed for 2 hours. After cooling to room temperature, it is

neutralized by saturated Na_2CO_3 and extracted with EtOAc (20 X 3). The combined organic phase is washed with brine and dried over Na_2SO_4. After concentrating, the residue is dissolved in dry DCM (10 mL) and is treated with Ac_2O (8 mmol) and pyridine (16 mmol). After stirring at room temperature for 12 h, the solution is washed with 1 N HCl and brine and dried over Na_2SO_4. After concentrating, the crude product is purified by column chromatography using EtOAc/hexane (1/1) to give the desired product as white solid. MS: (ES^+): 426.2 $(M+1)^+$.

c) Step E: Acetic acid 2-acetoxymethyl-2-acetylamino-4-[4'-(hydroxyiminomethyl)-biphenyl-4-yl]butyl ester

To a solution of acetic acid 2-acetoxymethyl-2-acetylamino-4-(4'-formyl-biphenyl-4-yl) butyl ester (1 mmol) in methanol (10 mL) is added $NH_2OH.HCl$ (1.2 mmol) and Et_3N (1.1 mmol). The mixture is stirred at room temperature 12 hours. After concentrating, the residue is dissolved in DCM (20 mL) and washed with H_2O and brine. The crude product, after concentration, is used in the next step without further purification. MS: (ES^+): 441.2 $(M+1)^+$.

d) Step F: Acetic acid 2-acetoxymethyl-2-acetylamino-4-[4'-(5-propyl-isoxazol-3-yl)biphenyl-4-yl]butyl ester

A mixture of acetic acid 2-acetoxymethyl-2-acetylamino-4-[4'-(hydroxyimino-methyl)biphenyl-4-yl]butyl ester (0.2 mmol), NaOCl (2 mmol), Et_3N (3 mmol) and pentyne (40 mmol) in DCM (4 mL) and H_2O (1 mL) is stirred at room temperature for 12 h. It is diluted with DCM (5 mL) and H_2O (10 mL) and the organic phase is washed with brine and dried over Na_2SO_4. After concentrating, the crude product is purified by column chromatography using EtOAc/hexane (1/1) to give the desired product as a white solid. MS: (ES^+): 507.2 $(M+1)^+$.

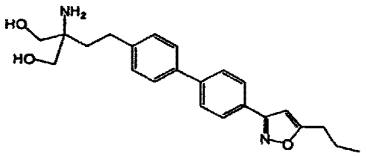
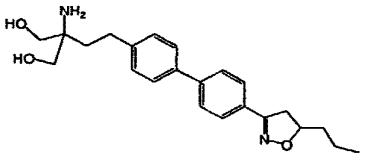
e) Step G: 2-Amino-2-{2-[4'-(5-propyl-isoxazol-3-yl)-biphenyl-4-yl]ethyl}propane-1,3-diol

Acetic acid 2-acetoxymethyl-2-acetylamino-4-[4'-(5-propyl-isoxazol-3-yl)-biphenyl-4-yl]butyl ester (0.1 mmol) is dissolved in THF (1 mL) and treated with 2 N LiOH aqueous solution (0.5 mL). The resulting mixture is stirred at reflux for 1 h and diluted with H_2O (10 mL). It is then extracted with EtOAc (3 x 5 mL) and the combined organic phase is washed with brine and dried over Na_2SO_4. After concentrated, the crude product is purified with LC-MS to give the desired product as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 7.86 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 3.53 (q, J = 11.0

Hz, 4H), 2.81 (t, J = 7.4 Hz, 2H), 2.71 (m, 2H), 1.79 (m, 4H), 1.04 (t, J = 7.4 Hz, 3H). MS: (ES$^+$): 381.2 $(M+1)^+$.

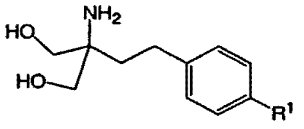
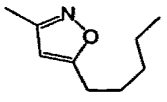
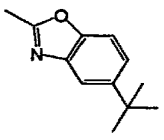
By repeating the procedure described in Example 40, using appropriate starting materials, the following compounds of formula I are obtained as identified in Table III.

TABLE III

Example No.	Structure	Physical Data MS (M+1)
41		381.2
42		383.2

By repeating the appropriate procedure described above, using appropriate starting materials, the following compounds of formula I are obtained as identified in Tables IV, V and VI.

TABLE IV

Example No.	 R^1	Physical Data MS (M+1)
43		333.2
44		369.2

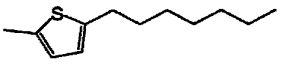
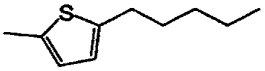
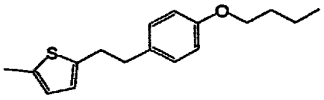
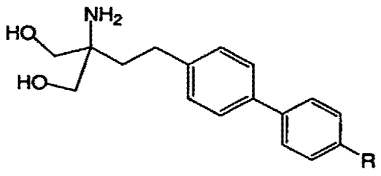
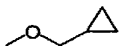
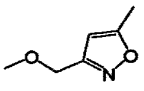
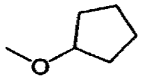
45		376.2
46		348.2
47		454.2

TABLE V

Example No.	 R	Physical Data MS (M+1)
48	$-O(CH_2)_2CH_3$	330.2
49		342.2
50	$-O(CH_2)_3CH_3$	344.2
51	$-OCH_2CH_3$	316.2
52	$-O(CH_2)_2CH(CH_3)_2$	358.2
53	$-O(C_6H_5)$	364.2
54	$-O(CH_2)_4CH_3$	358.2
55		383.2
56	$-O(CH_2)_2(C_6H_5)$	392.2
57		356.2
58	$-O(CH_2)_2OCH_2CH_3$	360.2
59	$-O(CH_2)_2OCH_3$	346.2

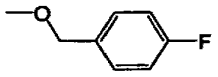
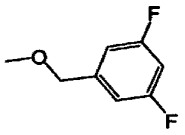
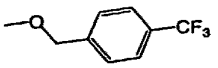
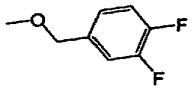
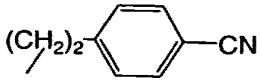
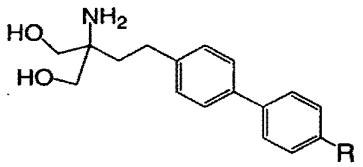
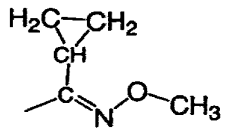
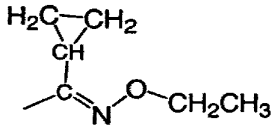
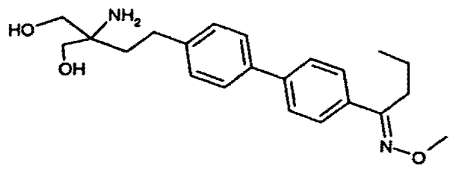
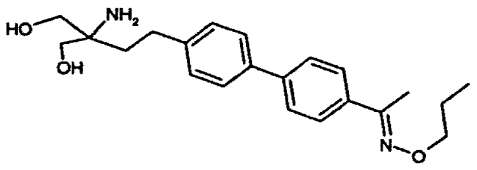
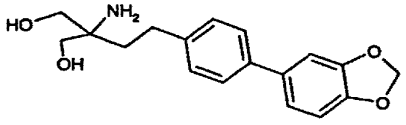
60		396.2
61		414.2
62		446.2
63		414.2
64		401.2

TABLE VI

Example No.	 R	Physical Data MS (M+1)
65	H	272.2
66	-C(O)$(CH_2)_2CH_3$	342.2
67	-CH(OH)$(CH_2)_2CH_3$	344.2
68	-C(O)CH_2CH_3	328.2
69	-C(O)$(CH_2)_3CH_3$	356.2
70	-$(CH_2)_3CH_3$	328.2
71	-CH(OH)CH_2CH_3	330.2
72	-CH(OH)$(CH_2)_3CH_3$	358.2
73	-$(CH_2)_2CH_3$	314.2
74	-C(=NOH)$(CH_2)_2CH_3$	357.2

75	-C(=NOH)$(CH_2)_3CH_3$	371.2
76	-C(=NOCH_2CH_3)CH_3	357.2
77		369.2
78		383.2
79	-C(=NOCH_2CH_3)$(CH_2)_6CH_3$	441.3
80	-CH(CH_2CH_3)$(CH_2)_2CH_3$	356.3
81	-CH($(CH_2)_2CH_3)_2$	370.3
82	-NHS$(O)_2CH_3$	365.2
83	-NH_2	287.2
84	-C(O)NH$(CH_2)_3CH_3$	371.2
85	-CH_2CN	311.2
86	-OCH_2CN	327.2
87	-OCH_2C≡CH	326.2
88	-OH	288.2
89	-O$(CH_2)_3$F	348.2
90	-O$(CH_2)_7CH_3$	400.3
91	-O$(CH_2)_6CH_3$	386.3
92		371.2

93		371.2
94		316.2

The compounds of formula I in free form or in pharmaceutically acceptable salt form, exhibit valuable pharmacological properties, e.g. lymphocyte recirculation modulating or anti-angiogenic properties, e.g. as indicated in in vitro and in vivo tests and are therefore indicated for therapy.

A. In vitro: Binding affinity of compounds of formula I to individual human S1P receptors may be determined in the following assays:

Transient transfection of human S1P receptors into HEK293 cells

EDG receptors and G_i proteins are cloned, and equal amounts of 4 cDNAs for the EDG receptor, G_i-α, G_i-β and G_i-γ are mixed and used to transfect monolayers of HEK293 cells using the calcium phosphate precipitate method (M. Wigler et al., Cell. 1977;11;223 and DS. Im et al., Mol. Pharmacol. 2000;57;753). Briefly, a DNA mixture containing 25 μg of DNA and 0.25 M CaCl is added to HEPES-buffered 2 mM Na_2HPO_4. Subconfluent monolayers of HEK293 cells are poisoned with 25 mM chloroquine, and the DNA precipitate is then applied to the cells. After 4 hours, the monolayers are washed with phosphate-buffered saline and re-fed media (90% 1:1 Dulbecco's modified essential media (DMEM):F-12 + 10% fetal bovine serum). The cells are harvested 48-72 hours after addition of the DNA by scraping in HME buffer (in mM: 20 HEPES, 5 $MgCl_2$, 1 EDTA, pH 7.4) containing 10% sucrose on ice, and disrupted using a Dounce homogenizer. After centrifugation at 800xg, the supernatant is diluted with HME without sucrose and centrifuged at 100,000xg for 1 hour. The resulting pellet is rehomogenized and centrifuged a second hour at 100,000xg. This crude membrane pellet is resuspended in HME with sucrose, aliquoted, and snap-frozen by immersion in liquid nitrogen. The membranes are stored at 70°C. Protein concentration is determined spectroscopically by the Bradford protein assay.

Example	**$S1P_1$** EC_{50} [nM]	**$S1P_2$** EC_{50} [nM]	**$S1P_3$** EC_{50} [nM]	**$S1P_4$** EC_{50} [nM]	**$S1P_5$** EC_{50} [nM]
36	0.33	>10000	>10000	1.2	1.1

41	0.16	>10000	53.8	>10000	2.1
63	0.07	>10000	1.9	>10000	0.1

GTPγS binding assay using S1P receptor/HEK293 membrane preparations

GTPγS binding experiments are performed as described by DS. Im et al., Mol. Pharmacol. 2000; 57:753. Ligand-mediated GTPγS binding to G-proteins is measured in GTP binding buffer (in mM: 50 HEPES, 100 NaCl, 10 $MgCl_2$, pH 7.5) using 25 μg of a membrane preparation from transiently transfected HEK293 cells. Ligand is added to membranes in the presence of 10 μM GDP and 0.1 nM [^{35}S]GTPγS (1200 Ci/mmol) and incubated at 30°C for 30 min. Bound GTPγS is separated from unbound using the Brandel harvester (Gaithersburg, MD) and counted with a liquid scintillation counter.

In these assays, the compounds of formula I wherein R_2 or R_5 is a residue of formula (h) have binding affinities to S1P receptors in the sub-microM range.

B. In Vitro: Antitumor Activity

A mouse breast cancer cell line originally isolated from mammary carcinomas is used, e.g. JygMC(A). The cell number is adjusted to $5x10^5$ for plating in fresh medium before the procedure. Cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12 hours and then washed twice with PBS, followed by addition of fresh medium with 10% FCS and additionally incubated for another 12 hours. Thereafter the cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12 hours. To release the cells from the block, the cells are washed twice with PBS and replated in fresh medium with 10% FCS. After synchronisation, the cells are incubated with or without various concentrations of a compound of formula I for 3, 6, 9, 12, 18 or 24 hours. The cells are harvested after treatment with 0.2% EDTA, fixed with ice-cold 70% ethanol solution, hydrolyzed with 250μg/ml of RNaseA (type 1-A: Sigma Chem. Co.) at 37°C for 30 minutes and stained with propidium iodide at 10mg/ml for 20 minutes. After the incubation period, the number of cells is determined both by counting cells in a Coulter counter and by the SRB colorimetric assay. Under these conditions compounds of formula I inhibit the proliferation of the tumor cells at concentrations ranging from 10^{-12} to 10^{-6} M.

C. In vitro: S1P-Mediated HUVEC Migration Assay

The migration assay is performed using Fluoro-Blok 24-Multiwell Insert Plates coated with fibronectin (8 μm pore size, Falcon #351147) instead of the individual inserts in a 24-well

plate. Cells and test compounds are prepared and pre-incubated as described above, then 100 µl is added to each approriate well in the Insert Plate. 300 µl of the EBM-2 + 2 % charcoal-stripped media without S1P is added to the bottoms of the wells marked for no stimulation (-), and 300 µl of the media containing S1P (500 nM) is added to the bottoms of the wells marked for stimulation (+). The plate is then incubated for 4 hours at 37 ºC, 5 % CO_2.

Calcein AM, 50 µg/vial, (Molecular Probes #C3100) is prepared by first adding 20 µl DMSO to the vial. Then 12.5 ml of HBSS (per plate) is warmed to 37ºC and 150 µl is added to the vial. The contents of the vial are then transferred back to the remaining HBSS to make the final concentration 4 µg/ml Calcein AM.

The Fluoro-Blok plate is removed from the incubator and the top insert plate is separated and "flicked" to remove excess media clinging to the inserts. The insert plate is then transferred to a fresh 24-well plate containing 500 µl/well of the 4 µg/ml Calcein AM. The plate is then incubated for 1.5 hours at 37 ºC, 5 % CO_2.

After incubation, the plate is read on a Cytofluor II at an excitation of 485 nm and emission of 530 nm. The Fluoro-Blok coating in the inserts allows only the cells that have migrated to the bottom to be counted. Data are transferred to Excel for calculations, graphs are created using SigmaPlot, and SigmaStat is used for significance tests (t-test).

D. In vivo: Blood Lymphocyte Depletion

A compound of formula I or the vehicle is administered orally by gavage to rats. Tail blood for hematological monitoring is obtained on day –1 to give the baseline individual values, and at 2, 6, 24, 48 and 72 hours after drug application. In this assay, the compounds of formula I deplete peripheral blood lymphocytes when administered at a dose of 0.03 to 3 mg/kg. For example compounds of Example 2 and 9 deplete peripheral blood lymphocytes by more than 50% 6 hours after administration of a dose of 0.8 mg/kg and 0.2 mg/kg, respectively.

E. In vivo: Screening Assays for measurement of circulating lymphocytes and assessment of heart effect

Measurement of Circulating Lymphocytes: Compounds are dissolved in DMSO and further diluted with deionized water. Mice (C57bl/6 male, 6-10 week-old) are administered 20 μg of compounds (diluted in 200 μl water, 4% DMSO) via intra-peritoneal (IP) injection under short isoflurane anesthesia. 200 μl water, 4% DMSO, and FTY720 (10 μg) are included as negative and positive controls.

Blood is collected from the retro-orbital sinus 18 hours after drug administration under short isoflurane anesthesia. Whole blood samples are subjected to hematology analysis. Peripheral lymphocyte counts are determined using an automated analyzer (Hemavet 3700). Subpopulations of peripheral blood lymphocytes are stained by fluorochrome-conjugated specific antibodies and analyzed using a fluorescent activating cell sorter (Facscalibur). Two mice are used to assess the lymphocyte depletion activity of each compound screened.

Assessment of Heart Effect: The effects of compounds on cardiac function are monitored using the AnonyMOUSE ECG recording system. ECGs are recorded in conscious mice (C57bl/6 male, 6-10 week-old) before and after compound administration. 90 μg of compound further diluted in 200 μl water and 15% DMSO are injected IP. Four mice are used to assess heart rate effect of each compound.

F. In vivo: Anti-angiogenic Activity

Porous chambers containing (i) sphingosine-1-phosphate (5 μM/chamber) or (ii) human VEGF (1 μg/chamber) in 0.5 ml of 0.8% w/v agar (containing heparin, 20 U/ml) are implanted subcutaneously in the flank of mice. S1P or VEGF induces the growth of vascularized tissue around the chamber. This response is dose-dependent and can be quantified by measuring the weight and blood content of the tissue. Mice are treated once a day orally or intravenously with a compound of formula I starting 4-6 hours before implantation of the chambers and continuing for 4 days. The animals are sacrificed for measurement of the vascularized tissues 24 hours after the last dose. The weight and blood content of the vascularized tissues around the chamber is determined. Animals treated with a compound of formula I show reduced weight and/or blood content of the vascularized tissues compared to animals treated with vehicle alone. Compounds of formula I are anti-angiogenic when administered at a dose of about 0.3 to about 3 mg/kg.

The compounds of formula I are, therefore, useful in the treatment and/or prevention of diseases or disorders mediated by lymphocytes interactions, e.g. in transplantation, such as acute or chronic rejection of cell, tissue or organ allo- or xenografts or delayed graft function,

graft versus host disease, autoimmune diseases, e.g. rheumatoid arthritis, systemic lupus erythematosus, hashimoto's thyroidis, multiple sclerosis, myasthenia gravis, diabetes type I or II and the disorders associated therewith, vasculitis, pernicious anemia, Sjoegren syndrome, uveitis, psoriasis, Graves ophthalmopathy, alopecia areata and others, allergic diseases, e.g. allergic asthma, atopic dermatitis, allergic rhinitis/conjunctivitis, allergic contact dermatitis, inflammatory diseases optionally with underlying aberrant reactions, e.g. inflammatory bowel disease, Crohn's disease or ulcerative colitis, intrinsic asthma, inflammatory lung injury, inflammatory liver injury, inflammatory glomerular injury, atherosclerosis, osteoarthritis, irritant contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, cutaneous manifestations of immunologically-mediated disorders, inflammatory eye disease, keratoconjunctivitis, myocarditis or hepatitis, ischemia/reperfusion injury, e.g. myocardial infarction, stroke, gut ischemia, renal failure or hemorrhage shock, traumatic shock, T cell lymphomas or T cell leukemias, infectious diseases, e.g. toxic shock (e.g. superantigen induced), septic shock, adult respiratory distress syndrome or viral infections, e.g. AIDS, viral hepatitis, chronic bacterial infection, or senile dementia. Examples of cell, tissue or solid organ transplants include e.g. pancreatic islets, stem cells, bone marrow, corneal tissue, neuronal tissue, heart, lung, combined heart-lung, kidney, liver, bowel, pancreas, trachea or oesophagus. For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired.

Furthermore, the compounds of formula I are useful in cancer chemotherapy, particularly for cancer chemotherapy of solid tumors, e.g. breast cancer, or as an anti-angiogenic agent.

In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.03 to 2.5 mg/kg per body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 100 mg, conveniently administered, e.g. in divided doses up to four times a day or in retard form. Suitable unit dosage forms for oral administration comprise from ca. 1 to 50 mg active ingredient.

The compounds of formula I may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or parenterally, e.g. in the form of injectable solutions or suspensions, topically, e.g. in the form of lotions, gels, ointments or creams, or in a nasal or a suppository form. Pharmaceutical compositions comprising a compound of formula I in free form or in pharmaceutically acceptable salt form in association

with at least one pharmaceutical acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent.

The compounds of formula I may be administered in free form or in pharmaceutically acceptable salt form e.g. as indicated above. Such salts may be prepared in a conventional manner and exhibit the same order of activity as the free compounds.

In accordance with the foregoing the present invention further provides:

1.1 A method for preventing or treating disorders or diseases mediated by lymphocytes, e.g. such as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

1.2 A method for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, e.g. as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof;

1.3 A method for inhibiting or controlling deregulated angiogenesis, e.g. sphingosine-1-phosphate (S1P) mediated angiogenesis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

1.4 A method for preventing or treating diseases mediated by a neo-angiogenesis process or associated with deregulated angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

2. A compound of formula I, in free form or in a pharmaceutically acceptable salt form for use as a pharmaceutical, e.g. in any of the methods as indicated under 1.1 to 1.4 above.

3. A pharmaceutical composition, e.g. for use in any of the methods as in 1.1 to 1.4 above comprising a compound of formula I in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier therefor.

4. A compound of formula I or a pharmaceutically acceptable salt thereof for use in the preparation of a pharmaceutical composition for use in any of the method as in 1.1 to 1.4 above.

The compounds of formula I may be administered as the sole active ingredient or in conjunction with, e.g. as an adjuvant to, other drugs e.g. immunosuppressive or immunomodulating agents or other anti-inflammatory agents, e.g. for the treatment or prevention of allo- or xenograft acute or chronic rejection or inflammatory or autoimmune disorders, or a chemotherapeutic agent, e.g. a malignant cell anti-proliferative agent. For example the compounds of formula I may be used in combination with a calcineurin inhibitor, e.g. cyclosporin A or FK 506; a mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, CCI779, ABT578 or AP23573; an ascomycin having immunosuppressive properties, e.g. ABT-281, ASM981, etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; leflunomide; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or an immunosuppressive homologue, analogue or derivative thereof; immunosuppressive monoclonal antibodies, e.g. monoclonal antibodies to leukocyte receptors, e.g. MHC, CD2, CD3, CD4, CD7, CD8, CD25, CD28, CD40. CD45, CD58, CD80, CD86 or their ligands; other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y ; adhesion molecule inhibitors, e.g. LFA-1 antagonists, ICAM-1 or -3 antagonists, VCAM-4 antagonists or VLA-4 antagonists; or a chemotherapeutic agent.

By the term "chemotherapeutic agent" is meant any chemotherapeutic agent and it includes but is not limited to,

i. an aromatase inhibitor,
ii. an anti-estrogen, an anti-androgen (especially in the case of prostate cancer) or a gonadorelin agonist,
iii. a topoisomerase I inhibitor or a topoisomerase II inhibitor,
iv. a microtubule active agent, an alkylating agent, an antineoplastic antimetabolite or a platin compound,
v. a compound targeting/decreasing a protein or lipid kinase activity or a protein or lipid phosphatase activity, a further anti-angiogenic compound or a compound which induces cell differentiation processes,

vi. a bradykinin 1 receptor or an angiotensin II antagonist,

vii. a cyclooxygenase inhibitor, a bisphosphonate, a histone deacetylase inhibitor, a heparanase inhibitor (prevents heparan sulphate degradation), e.g. PI-88, a biological response modifier, preferably a lymphokine or interferons, e.g. interferon γ, an ubiquitination inhibitor, or an inhibitor which blocks anti-apoptotic pathways,

viii. an inhibitor of Ras oncogenic isoforms, e.g. H-Ras, K-Ras or N-Ras, or a farnesyl transferase inhibitor, e.g. L-744,832 or DK8G557,

ix. a telomerase inhibitor, e.g. telomestatin,

x. a protease inhibitor, a matrix metalloproteinase inhibitor, a methionine aminopeptidase inhibitor, e.g. bengamide or a derivative thereof, or a proteosome inhibitor, e.g. PS-341, and/or

xi. a mTOR inhibitor.

The term "aromatase inhibitor" as used herein relates to a compound which inhibits the estrogen production, i.e. the conversion of the substrates androstenedione and testosterone to estrone and estradiol, respectively. The term includes, but is not limited to steroids, especially atamestane, exemestane and formestane and, in particular, non-steroids, especially aminoglutethimide, roglethimide, pyridoglutethimide, trilostane, testolactone, ketokonazole, vorozole, fadrozole, anastrozole and letrozole. A combination of the invention comprising a chemotherapeutic agent which is an aromatase inhibitor is particularly useful for the treatment of hormone receptor positive tumors, e.g. breast tumors.

The term "anti-estrogen" as used herein relates to a compound which antagonizes the effect of estrogens at the estrogen receptor level. The term includes, but is not limited to tamoxifen, fulvestrant, raloxifene and raloxifene hydrochloride. A combination of the invention comprising a chemotherapeutic agent which is an anti-estrogen is particularly useful for the treatment of estrogen receptor positive tumors, e.g. breast tumors.

The term "anti-androgen" as used herein relates to any substance which is capable of inhibiting the biological effects of androgenic hormones and includes, but is not limited to, bicalutamide.

The term "gonadorelin agonist" as used herein includes, but is not limited to abarelix, goserelin and goserelin acetate.

The term "topoisomerase I inhibitor" as used herein includes, but is not limited to topotecan, irinotecan, 9-nitrocamptothecin and the macromolecular camptothecin conjugate PNU-166148 (compound A1 in WO99/17804).

The term "topoisomerase II inhibitor" as used herein includes, but is not limited to the anthracyclines such as doxorubicin, daunorubicin, epirubicin, idarubicin and nemorubicin, the anthraquinones mitoxantrone and losoxantrone, and the podophillotoxines etoposide and teniposide.

The term "microtubule active agent" relates to microtubule stabilizing and microtubule destabilizing agents including, but not limited to taxanes, e.g. paclitaxel and docetaxel, vinca alkaloids, e.g., vinblastine, especially vinblastine sulfate, vincristine especially vincristine sulfate, and vinorelbine, discodermolides and epothilones and derivatives thereof, e.g. epothilone B or a derivative thereof.

The term "alkylating agent" as used herein includes, but is not limited to busulfan, chlorambucil, cyclophosphamide, ifosfamide, melphalan or nitrosourea (BCNU or Gliadel™).

The term "antineoplastic antimetabolite" includes, but is not limited to 5-fluorouracil, capecitabine, gemcitabine, cytarabine, fludarabine, thioguanine, methotrexate and edatrexate.

The term "platin compound" as used herein includes, but is not limited to carboplatin, cis-platin and oxaliplatin.

The term "compounds targeting/decreasing a protein or lipid kinase activity or further anti-angiogenic compounds" as used herein includes, but is not limited to protein tyrosine kinase and/or serine and/or threonine kinase inhibitors or lipid kinase inhibitors, e.g. compounds targeting, decreasing or inhibiting the activity of the epidermal growth factor family of receptor tyrosine kinases (EGFR, ErbB2, ErbB3, ErbB4 as homo- or heterodimers), the vascular endothelial growth factor family of receptor tyrosine kinases (VEGFR), the platelet-derived growth factor-receptors (PDGFR), the fibroblast growth factor-receptors (FGFR), the insulin-like growth factor receptor 1 (IGF-1R), the Trk receptor tyrosine kinase family, the Axl receptor tyrosine kinase family, the Ret receptor tyrosine kinase, the Kit/SCFR receptor tyrosine kinase, members of the c-Abl family and their gene-fusion products (e.g. BCR-Abl), members of the protein kinase C (PKC) and Raf family of serine/threonine kinases, members of the MEK, SRC, JAK, FAK, PDK or PI(3) kinase family, or of the PI(3)-kinase-related kinase family, and/or members of the cyclin-dependent kinase family (CDK) and anti-angiogenic compounds having another mechanism for their activity, e.g. unrelated to protein or lipid kinase inhibition.

Compounds which target, decrease or inhibit the activity of VEGFR are especially compounds, proteins or antibodies which inhibit the VEGF receptor tyrosine kinase, inhibit a VEGF receptor or bind to VEGF, and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 98/35958, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or a pharmaceutically acceptable salt thereof, e.g. the succinate, in WO 00/27820, e.g. a N-aryl(thio) anthranilic acid amide derivative e.g. 2-[(4-pyridyl)methyl]amino-N-[3-methoxy-5-(trifluoromethyl)phenyl]benzamide or 2-[(1-oxido-4-pyridyl)methyl]amino-N-[3-trifluoromethylphenyl]benzamide, or in WO 00/09495, WO 00/59509, WO 98/11223, WO 00/27819 and EP 0 769 947; those as described by M. Prewett et al in Cancer Research 59 (1999) 5209-5218, by F. Yuan et al in Proc. Natl. Acad. Sci. USA, vol. 93, pp. 14765-14770, Dec. 1996, by Z. Zhu et al in Cancer Res. 58, 1998, 3209-3214, and by J. Mordenti et al in Toxicologic Pathology, Vol. 27, no. 1, pp 14-21, 1999; in WO 00/37502 and WO 94/10202; Angiostatin™, described by M. S. O'Reilly et al, Cell 79, 1994, 315-328; Endostatin™, described by M. S. O'Reilly et al, Cell 88, 1997, 277-285; anthranilic acid amides; ZD4190; ZD6474; SU5416; SU6668; or anti-VEGF antibodies or anti-VEGF receptor antibodies,e.g. RhuMab.

By antibody is meant intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies formed from at least 2 intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

Compounds which target, decrease or inhibit the activity of the epidermal growth factor receptor family are especially compounds, proteins or antibodies which inhibit members of the EGF receptor tyrosine kinase family, e.g. EGF receptor, ErbB2, ErbB3 and ErbB4 or bind to EGF or EGF related ligands, or which have a dual inhibiting effect on the ErbB and VEGF receptor kinase and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 97/02266, e.g. the compound of ex. 39, or in EP 0 564 409, WO 99/03854, EP 0520722, EP 0 566 226, EP 0 787 722, EP 0 837 063, US 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and, especially, WO 96/30347 (e.g. compound known as CP 358774), WO 96/33980 (e.g. compound ZD 1839) and WO 95/03283 (e.g. compound ZM105180) or PCT/EP02/08780; e.g. trastuzumab (Herpetin®), cetuximab, Iressa, OSI-774, CI-1033, EKB-569, GW-2016, E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6.3 or E7.6.3.

Compounds which target, decrease or inhibit the activity of PDGFR are especially compounds which inhibit the PDGF receptor, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib.

Compounds which target, decrease or inhibit the activity of c-Abl family members and their gene fusion products are, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib; PD180970; AG957; or NSC 680410.

Compounds which target, decrease or inhibit the activity of protein kinase C, Raf, MEK, SRC, JAK, FAK and PDK family members, or PI(3) kinase or PI(3) kinase-related family members, and/or members of the cyclin-dependent kinase family (CDK) are especially those staurosporine derivatives disclosed in EP 0 296 110, e.g. midostaurin; examples of further compounds include e.g. UCN-01, safingol, BAY 43-9006, Bryostatin 1, Perifosine; Ilmofosine; RO 318220 and RO 320432; GO 6976; Isis 3521; or LY333531/LY379196.

Further anti-angiogenic compounds are e.g. thalidomide (THALOMID) and TNP-470.

Compounds which target, decrease or inhibit the activity of a protein or lipid phosphatase are, e.g. inhibitors of phosphatase 1, phosphatase 2A, PTEN or CDC25, e.g. okadaic acid or a derivative thereof.

Compounds which induce cell differentiation processes are, e.g. retinoic acid, α-, γ- or δ-tocopherol or α-, γ- or δ-tocotrienol.

The term cyclooxygenase inhibitor as used herein includes, but is not limited to, e.g. celecoxib (Celebrex^R), rofecoxib (Vioxx^R), etoricoxib, valdecoxib or a 5-alkyl-2-arylaminophenylacetic acid, e.g. 5-methyl-2-(2'-chloro-6'-fluoroanilino)phenyl acetic acid.

The term "histone deacetylase inhibitor" as used herein includes, but is not limited to MS-27-275, SAHA, pyroxamide, FR-901228 or valproic acid.

The term "bisphosphonates" as used herein includes, but is not limited to, etridonic, clodronic, tiludronic, pamidronic, alendronic, ibandronic, risedronic and zoledronic acid.

The term "matrix metalloproteinase inhibitor" as used herein includes, but is not limited to collagen peptidomimetic and non-petidomimetic inhibitors, tetracycline derivatives, e.g. hydroxamate peptidomimetic inhibitor batimastat and its orally bioavailable analogue marimastat, prinomastat, BMS-279251, BAY 12-9566, TAA211 or AAJ996.

The term "mTOR inhibitor" as used herein includes, but is not limited to rapamycin (sirolimus) or a derivative thereof, e.g. 32-deoxorapamycin, 16-pent-2-ynyloxy-32-deoxo-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin and, more preferably, 40-0-(2-hydroxyethyl)-rapamycin. Further examples of rapamycin derivatives include e.g. CCI779 or 40- [3-hydroxy-2-(hydroxy-

methyl)-2-methylpropanoate]-rapamycin or a pharmaceutically acceptable salt thereof, as disclosed in USP 5,362,718, ABT578 or 40-(tetrazolyl)-rapamycin, particularly 40-epi-(tetrazolyl)-rapamycin, e.g. as disclosed in WO 99/15530, or rapalogs as disclosed e.g. in WO 98/02441 and WO01/14387, e.g. AP23573.

Where the compounds of formula I are administered in conjunction with other immunosuppressive / immunomodulatory, anti-inflammatory or chemotherapeutic therapy, dosages of the co-administered immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic compound will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a calcineurin inhibitor, on the specific drug employed, on the condition being treated and so forth.

In accordance with the foregoing the present invention provides in a yet further aspect:

5. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective non-toxic amount of a compound of formula I and at least a second drug substance, e.g. an immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic drug, e.g. as indicated above.

6. A pharmaceutical combination, e.g. a kit, comprising a) a first agent which is a compound of formula I as disclosed herein, in free form or in pharmaceutically acceptable salt form, and b) at least one co-agent, e.g. an immunosuppressant, immunomodulatory, anti-inflammatory or chemotherapeutic drug, e.g. as disclosed above. The kit may comprise instructions for its administration.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

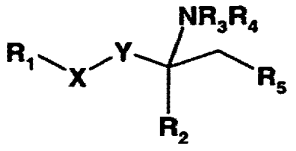
The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a compound of formula I and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a compound of formula I and a co-agent, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides

therapeutically effective levels of the 2 compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of 3 or more active ingredients.

In each case where citations of patent applications or scientific publications are given, the subject-matter relating to the compounds is hereby incorporated into the present application by reference. Comprised are likewise the pharmaceutically acceptable salts thereof, the corresponding racemates, diastereoisomers, enantiomers, tautomers as well as the corresponding crystal modifications of above disclosed compounds where present, e.g. solvates, hydrates and polymorphs, which are disclosed therein. The compounds used as active ingredients in the combinations of the invention can be prepared and administered as described in the cited documents, respectively. Also within the scope of this invention is the combination of more than two separate active ingredients as set forth above, i.e. a pharmaceutical combination within the scope of this invention could include three active ingredients or more. Further both the first agent and the co-agent are not the identical ingredient.

CLAIMS

1. A compound of formula I



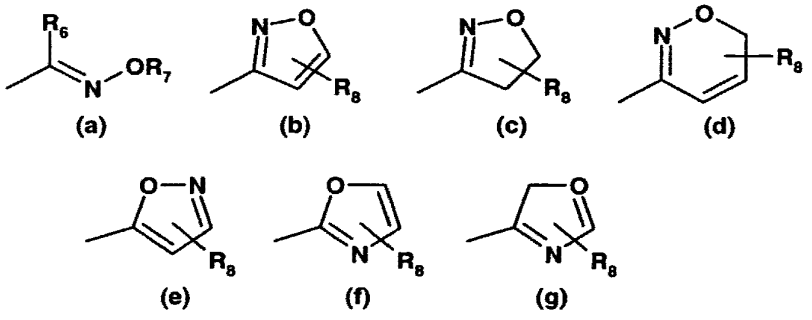
I

wherein

Y is $-CH_2CH_2-$, $-CH_2CH(OH)-$, $-CH(OH)CH_2-$, $-C(O)CH_2-$, $-CH_2C(O)-$, $-CH=CH-$; or 1,2-cyclopropylene;

X is arylene or C_{5-6}heteroarylene optionally substituted by one to three substituents selected from halogen, nitro, C_{1-10}alkyl and halogen-substituted C_{1-6}alkyl;

R_1 is aryl, aryl-C_{2-4}alkenyl, heteroaryl, or heteroaryl-C_{2-4}alkenyl each being substituted by (i) one to three substituents selected from hydrogen, halogen, amino, phenyl, heteroaryl, heteroaryl-C_{1-4}alkyl, C_{1-10}alkyl, cycloalkyl-C_{1-4}alkyl, cycloalkyl-C_{1-4}alkoxy, C_{1-10}alkoxy, C_{2-10}alkenyl, C_{2-10}alkynyl, C_{1-10}alkylthio, C_{1-10}alkylsulfonyl, C_{1-10}alkyl-sulfinyl, C_{1-4}alkyl-$S(O)_2NH$-, phenylC_{1-6}alkyl, or phenylC_{1-6}alkoxy, in each of which any aliphatic part of the group may be straight or branched chain and optionally substituted by up to three substituents selected from halogen, amino, hydroxy, cyano, or cycloalkyl groups and optionally interrupted by a double or triple bond or one or more C(O), NR_{12}, S, S(O), $S(O)_2$ or O groups, wherein R_{12} is hydrogen or C_{1-6}alkyl; and any aromatic group may be optionally substituted by one to three substituents selected from halogen, cyano, amino, C_{1-4}alkyl halogen-substituted-C_{1-4}alkyl and C_{1-8}alkoxy; and/or (ii) a group of formula (a), (b), (c), (d), (e), (f) or (g):

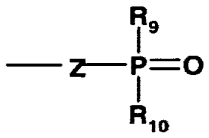


in which each of

R_6, R_7 and R_8 independently, is hydrogen; phenyl, C_{1-10}alkyl, cycloalkyl, heteroaryl, heteroaryl-C_{1-4}alkyl, C_{1-10}alkoxy, C_{2-10}alkenyl, C_{2-10}alkynyl, C_{1-10}alkylthio, C_{1-10}alkyl-sulfonyl, C_{1-10}alkylsulfinyl, phenylC_{1-8}alkyl, or phenylC_{1-6}alkoxy, in each of which any

aliphatic part of the group may be straight chain or branched and may be optionally substituted by up to three halogen, hydroxy, cycloalkyl, or C_{1-4}alkoxy groups and optionally interrupted by a double or triple bond or one or more C(O), NR_{12}, S, S(O), $S(O)_2$ or O groups, and any aromatic group may be optionally substituted by one to three substituents selected from halogen, CF_3, C_{1-8}alkyl and C_{1-8}alkoxy;

R_2 is hydrogen; halogen; C_{1-4}alkyl optionally substituted with one or more halogens; C_{2-6} alkenyl; C_{2-6}alkynyl; or cycloalkyl optionally substituted by halogen; aryl optionally substituted with hydroxy; or C_{1-4}alkyl optionally substituted on the terminal C atom by OH or a residue of formula (h):



(h)

in which Z is a direct bond, O, S, $(CH_2)_{1-2}$, CF_2, or NR_{11} where R_{11} is H, (C_{1-4})alkyl or halogen-substituted (C_{1-4})alkyl; and each of R_9 and R_{10}, independently, is H, OH, (C_{1-4})alkyl optionally substituted by one to three halogens, or (C_{1-4})alkoxy optionally substituted by halogen; with the proviso that R_9 and R_{10} are not both hydrogen;

each of R_3 and R_4, independently, is H or C_{1-4}alkyl optionally substituted by halogen or acyl; and R_5 is H, –OH, -Oacyl, –NHacyl, or a residue of formula (h) as defined above;

provided that at least either R_2 comprises a terminal OH or a residue of formula (h) or R_5 is OH or a residue of formula (h),

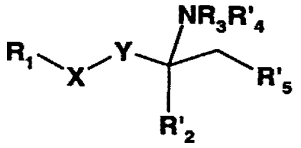
or a salt thereof.

2. A compound according to claim 1, wherein X is 1,4-phenylene, Y is $–CH_2-CH_2-$, R_1 is phenyl monosubstituted in position para by a group R_{15} wherein R_{15} is straight chain C_{5-8}alkyl; C_{2-8}alkenyl; or straight chain or branched C_{1-8}alkoxy optionally substituted by one C_{3-6}cycloalkyl or by a phenyl group optionally substituted by up to three halogens; or R_1 is para-monosubstituted phenyl substituted by a group of formula (a), (b) or (c), as defined in claim 1, R_2 is C_{1-4}alkyl optionally substituted on the terminal C atom by OH or a residue of formula (h) as defined above, R_3 and R_4 are hydrogen, and R_5 is OH, or a salt thereof.

3. A compound according to claim 1 or 2, wherein R_1 is phenyl monosubstituted in position para by a group of formula (a) as defined above, or a salt thereof.

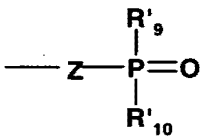
4. A compound according to any one of claims 1 to 3, wherein Z is O, or a salt thereof.

5. A process for preparing a compound according to any one of claims 1 to 5 which process comprises removing the hydrolysable groups present in a compound of formula II



II

wherein X, Y, R_1 and R_3 are as defined in claim 1, R_4' is an amino protecting group, R_2' has one of the significances given for R_2 above except that the terminal OH when present in the OH-substituted C_{1-4}alkyl is in protected form or the residue of formula (h) is replaced by a residue of formula (h') and R_5' is R_5" in which R_5" is H, -OH in protected form or a residue of formula (h'), provided that at least one of R_2' and R_5' is OH in protected form or a residue of formula (h'), the residue of formula (h') being:



(h')

wherein Z is as described above, and each of R_9' and R_{10}' is a hydrolysable group and, where required, converting the compounds of formula I obtained in free form into the desired salt form, or vice versa.

6. A compound according to any one of claims 1 to 4 or a pharmaceutically acceptable salt thereof for use as a pharmaceutical.

7. A compound according to any one of claims 1 to 4 or a pharmaceutically acceptable salt thereof for use in the preparation of a medicament.

8. A pharmaceutical composition comprising a compound according to any one of claims 1 to 4, or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable diluent or carrier therefor.

9. A pharmaceutical combination comprising a) a first agent which is a compound according to any one of claims 1 to 4, in free form or in pharmaceutically acceptable salt form, and b) at least one co-agent.

10. A method for preventing or treating disorders or diseases mediated by lymphocytes, for preventing or treating acute or chronic transplant rejection or T-cell mediated inflammatory or autoimmune diseases, for inhibiting or controlling deregulated

angiogenesis, or for preventing or treating diseases mediated by a neo-angiogenesis process or associated with deregulated angiogenesis in a subject comprising administering to the subject in need thereof an effective amount of a compound according to any one of claims 1 to 4, or a pharmaceutically acceptable salt thereof.

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(54) Title: USE OF EDG RECEPTOR BINDING AGENTS IN CANCER

(57) Abstract: Provided is a method for treating solid tumors, e.g. tumor invasiveness, and particularly inhibiting or controlling deregulated angiogenesis, using a sphingosine-1-phosphate receptor agonist, optionally in combination with a chemotherapeutic agent. The invention also comprises a combination of a sphingosine-1-phosphate receptor agonist with a chemotherapeutic agent.

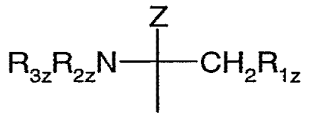
WO 03/097028 A1

Use of EDG receptor binding agents in cancer

The present invention relates to a new use for a sphingosine-1-phosphate (S1P) receptor agonist, particularly in the treatment of cancer.

S1P receptor agonists are accelerating lymphocyte homing (LH) agents which elicit a lymphopenia resulting from a re-distribution, preferably reversible, of lymphocytes from circulation to secondary lymphatic tissue, without evoking a generalized immunosuppression. Naïve cells are sequestered; CD4 and CD8 T-cells and B-cells from the blood are stimulated to migrate into lymph nodes (LN) and Peyer's patches (PP), and thus for example infiltration of cells into transplanted organs is inhibited.

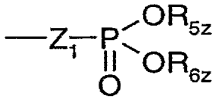
S1P receptor agonists are typically sphingosine analogues, such as 2-substituted 2-amino-propane-1,3-diol or 2-amino-propanol derivatives, e.g. a compound comprising a group of formula X



X

wherein

Z is H; C_{1-6}alkyl; C_{2-6}alkenyl; C_{2-6}alkynyl; phenyl; phenyl substituted by OH; C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH; or CH_2-R_{4z} wherein R_{4z} is OH, acyloxy or a residue of formula (a)



(a)

wherein Z_1 is a direct bond or O, preferably O; each of R_{5z} and R_{6z}, independently, is H, or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms;

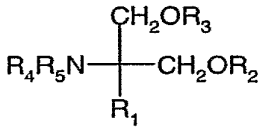
R_{1z} is OH, acyloxy or a residue of formula (a); and each of R_{2z} and R_{3z}, independently, is H, C_{1-4}alkyl or acyl.

Group of formula X is a functional group attached as a terminal group to a moiety which may be hydrophilic or lipophilic and comprise one or more aliphatic, alicyclic, aromatic and/or heterocyclic residues, to the extent that the resulting molecule wherein at least one of Z and R_{1z} is or comprises a residue of formula (a), signals as an agonist at one of more sphingosine-1-phosphate receptor.

S1P receptor agonists are compounds which signal as agonists at one or more sphingosine-1 phosphate receptors, e.g. S1P1 to S1P8. Agonist binding to a S1P receptor may e.g. result in dissociation of intracellular heterotrimeric G-proteins into Gα-GTP and Gβγ-GTP, and/or increased phosphorylation of the agonist-occupied receptor and activation of downstream signaling pathways/kinases. The binding affinity of S1P receptor agonists may be measured as described at paragraph **I.** below.

Examples of appropriate S1P receptor agonists are, for example:

- Compounds as disclosed in EP627406A1, e.g.a compound of formula I



I

wherein R_1 is a straight- or branched (C_{12-22})carbon chain

- which may have in the chain a bond or a hetero atom selected from a double bond, a triple bond, O, S, NR_6, wherein R_6 is H, alkyl, aralkyl, acyl or alkoxycarbonyl, and carbonyl, and/or
- which may have as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxyimino, hydroxy or carboxy; or

R_1 is

- a phenylalkyl wherein alkyl is a straight- or branched (C_{6-20})carbon chain; or
- a phenylalkyl wherein alkyl is a straight- or branched (C_{1-30})carbon chain wherein said phenylalkyl is substituted by
- a straight- or branched (C_{6-20})carbon chain optionally substituted by halogen,
- a straight- or branched (C_{6-20})alkoxy chain optionally substitued by halogen,
- a straight- or branched (C_{6-20})alkenyloxy,
- phenylalkoxy, halophenylalkoxy, phenylalkoxyalkyl, phenoxyalkoxy or phenoxyalkyl,
- cycloalkylalkyl substituted by C_{6-20}alkyl,
- heteroarylalkyl substituted by C_{6-20}alkyl,
- heterocyclic C_{6-20}alkyl or
- heterocyclic alkyl substituted by C_{2-20}alkyl,

and wherein

the alkyl moiety may have

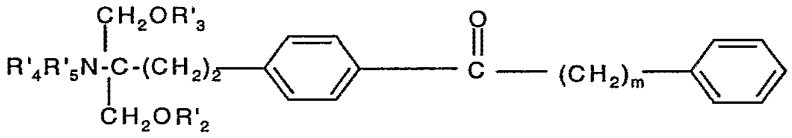
- in the carbon chain, a bond or a heteroatom selected from a double bond, a triple bond, O, S, sulfinyl, sulfonyl, or NR_6, wherein R_6 is as defined above, and

- as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxy or carboxy, and

each of R_2, R_3, R_4 and R_5, independently, is H, C_{1-4}alkyl or acyl

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in EP 1002792A1, e.g. a compound of formula II

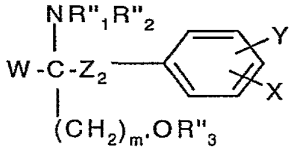


II

wherein m is 1 to 9 and each of R'_2, R'_3, R'_4 and R'_5, independently, is H, alkyl or acyl,

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in EP0778263 A1, e.g. a compound of formula III



III

wherein W is H; C_{1-6}alkyl, C_{2-6}alkenyl or C_{2-6}alkynyl; unsubstituted or by OH substituted phenyl; $R''_4O(CH_2)_n$; or C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH;

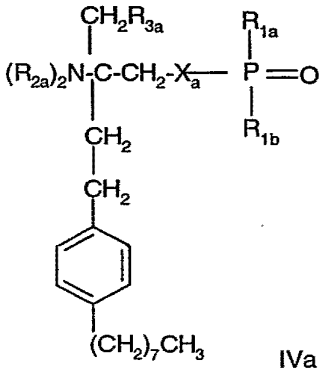
X is H or unsubstituted or substituted straight chain alkyl having a number p of carbon atoms or unsubstituted or substituted straight chain alkoxy having a number (p-1) of carbon atoms, e.g. substituted by 1 to 3 substitutents selected from the group consisting of C_{1-6} alkyl, OH, C_{1-6}alkoxy, acyloxy, amino, C_{1-6}alkylamino, acylamino, oxo, haloC_{1-6}alkyl, halogen, unsubstituted phenyl and phenyl substituted by 1 to 3 substituents selected from the group consisting of C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl and halogen; Y is H, C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl or halogen, Z_2 is a single bond or a straight chain alkylene having a number or carbon atoms of q,

each of p and q, independently, is an integer of 1 to 20, with the proviso of $6 \leq p+q \leq 23$, m' is 1, 2 or 3, n is 2 or 3,

each of R''_1, R''_2, R''_3 and R''_4, independently, is H, C_{1-4}alkyl or acyl,

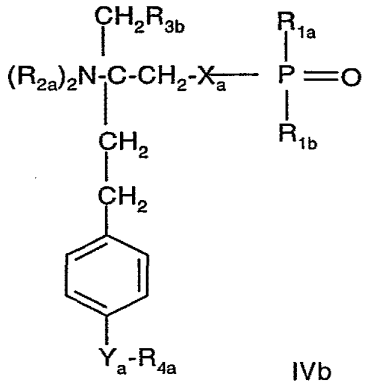
or a pharmaceutically acceptable salt thereof,

- Compounds as disclosed in WO02/18395, e.g. a compound of formula IVa or IVb



IVa

or

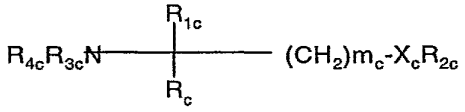


IVb

wherein X_a is O, S, NR_{1s} or a group $-(CH_2)_{na}-$, which group is unsubstituted or substituted by 1 to 4 halogen; n_a is 1 or 2, R_{1s} is H or (C_{1-4})alkyl, which alkyl is unsubstituted or substituted by halogen; R_{1a} is H, OH, (C_{1-4})alkyl or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by 1 to 3 halogen; R_{1b} is H, OH or (C_{1-4})alkyl, wherein alkyl is unsubstituted or substituted by halogen; each R_{2a} is independently selected from H or (C_{1-4})alkyl, which alkyl is unsubstituted or substitued by halogen; R_{3a} is H, OH, halogen or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; and R_{3b} is H, OH, halogen, (C_{1-4})alkyl wherein alkyl is unsubstituted or substituted by hydroxy, or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; Y_a is $-CH_2-$, -C(O)-, -CH(OH)-, -C(=NOH)-, O or S, and R_{4a} is (C_{4-14})alkyl or (C_{4-14})alkenyl;

or a pharmaceutically acceptable salt or hydrate thereof;

- Compounds as disclosed in WO 02/076995, e.g. a compound of formula V



V

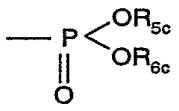
wherein

m_c is 1, 2 or 3;

X_c is O or a direct bond;

R_{1c} is H; C_{1-6} alkyl optionally substituted by OH, acyl, halogen, C_{3-10}cycloalkyl, phenyl or hydroxy-phenylene; C_{2-6}alkenyl; C_{2-6}alkynyl; or phenyl optionally substituted by OH;

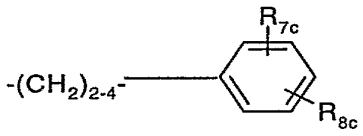
R_{2c} is



wherein R_{5c} is H or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms, and R_{6c} is H or C_{1-4}alkyl optionally substituted by halogen;

each of R_{3c} and R_{4c}, independently, is H, C_{1-4}alkyl optionally substituted by halogen, or acyl, and

R_c is C_{13-20}alkyl which may optionally have in the chain an oxygen atom and which may optionally be substituted by nitro, halogen, amino, hydroxy or carboxy; or a residue of formula (a)

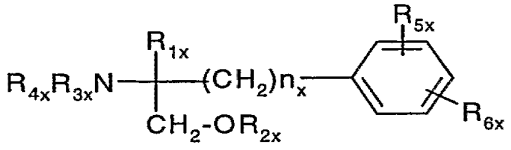


(a)

wherein R_{7c} is H, C_{1-4}alkyl or C_{1-4}alkoxy, and R_{8c} is substituted C_{1-20}alkanoyl, phenylC_{1-14}alkyl wherein the C_{1-14}alkyl is optionally substituted by halogen or OH, cycloalkylC_{1-14}alkoxy or phenylC_{1-14}alkoxy wherein the cycloalkyl or phenyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy, phenylC_{1-14}alkoxy-C_{1-14}alkyl, phenoxyC_{1-14}alkoxy or phenoxyC_{1-14}alkyl,

R_c being also a residue of formula (a) wherein R_{8c} is C_{1-14}alkoxy when R_{1c} is C_{1-4}alkyl, C_{2-6}alkenyl or C_{2-6}alkynyl,

or a compound of formula VI



VI

wherein

n_x is 2, 3 or 4

R_{1x} is H; C_{1-6}alkyl optionally substituted by OH, acyl, halogen, cycloalkyl, phenyl or hydroxy-phenylene; C_{2-6}alkenyl; C_{2-6}alkynyl; or phenyl optionally substituted by OH;

R_{2x} is H, C_{1-4} alkyl or acyl

each of R_{3x} and R_{4x}, independently is H, C_{1-4}alkyl optionally substituted by halogen or acyl,

R_{5x} is H, C_{1-4}alkyl or C_{1-4}alkoxy, and

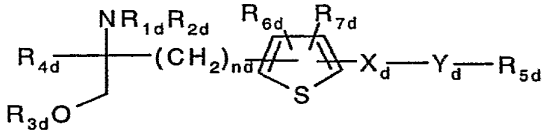
R_{6x} is C_{1-20}alkanoyl substituted by cycloalkyl; cyloalkylC_{1-14}alkoxy wherein the cycloalkyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy; phenylC_{1-14}alkoxy wherein the phenyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy,

R_{6x} being also C_{4-14}alkoxy when R_{1x} is C_{2-4}alkyl substituted by OH, or pentyloxy or hexyloxy when R_{1x} is C_{1-4}akyl,

provided that R_{6x} is other than phenyl-butylenoxy when either R_{5x} is H or R_{1x} is methyl,

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in WO02/06268Al, e.g. a compound of formula VII



VII

wherein each of R_{1d} and R_{2d}, independently, is H or an amino-protecting group;

R_{3d} is hydrogen or a hydroxy-protecting group;

R_{4d} is lower alkyl;

n_d is an integer of 1 to 6;

X_d is ethylene, vinylene, ethynylene, a group having a formula – D-CH_2- (wherein D is carbonyl, – CH(OH)-, O, S or N), aryl or aryl substituted by up to three substitutents selected from group a as defined hereinafter;

Y_d is single bond, C_{1-10}alkylene, C_{1-10}alkylene which is substituted by up to three substitutents selected from groups a and b, C_{1-10}alkylene having O or S in the middle or end of the carbon chain, or C_{1-10}alkylene having O or S in the middle or end of the carbon chain which is substituted by up to three substituents selected from groups a and b;

R_{5d} is hydrogen, cycloalkyl, aryl, heterocycle, cycloalkyl substituted by up to three substituents selected from groups a and b, aryl substituted by up to three substituents selected from groups a and b, or heterocycle substituted by up to three substituents selected from groups a and b; and

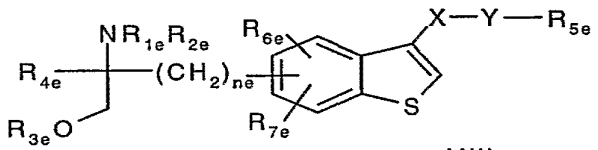
each of R_{6d} and R_{7d}, independently, is H or a substituent selected from group a;

<group a > is halogen, lower alkyl, halogeno lower alkyl, lower alkoxy, lower alkylthio, carboxyl, lower alkoxycarbonyl, hydroxy, lower aliphatic acyl, amino, mono-lower alkylamino, di-lower alkylamino, lower aliphatic acylamino, cyano or nitro;

<group b > is cycloalkyl, aryl, heterocycle, each being optionally substituted by up to three substituents selected from group a;

with the proviso that when R_{5d} is hydrogen, Y_d is a either a single bond or linear C_{1-10} alkylene, or a pharmacologically acceptable salt or ester thereof.

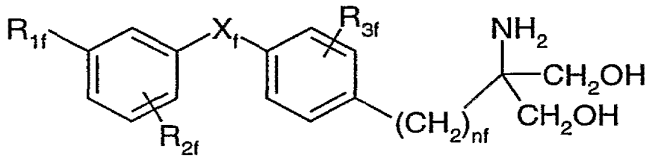
-Compounds as disclosed in JP-14316985 (JP2002316985), e.g. a compound of formula VIII:



VIII

wherein R_{1e},R_{2e},R_{3e},R_{4e},R_{5e},R_{6e},R_{7e}, n_e, X_e and Y_e are as disclosed in JP-14316985; or a pharmacologically acceptable salt or ester thereof.

-Compounds as disclosed in WO 03/29184 and WO 03/29205, e.g. compounds of formula IX



IX

wherein X_f is O or S, and R_{1f}, R_{2f}, R_{3f} and n_f are as disclosed in WO 03/29184 and O3/29205, e.g. 2-amino-2-[4-(3-benzyloxyphenoxy)-2-chlorophenyl]propyl-1,3-propane-diol or 2-amino-2-[4-(benzyloxyphenylthio)-2- chlorophenyl]propyl-1,3-propane-diol.

In each case where citations of patent applications are given, the subject matter relating to the compounds is hereby incorporated into the present application by reference.

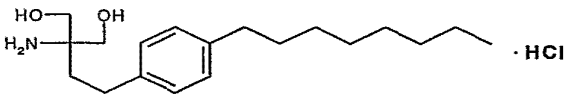
Acyl may be a residue R_y-CO- wherein R_y is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenyl-C_{1-4}alkyl. Unless otherwise stated, alkyl, alkoxy, alkenyl or alkynyl may be straight or branched.

When in the compounds of formula I the carbon chain as R_1 is substituted, it is preferably substituted by halogen, nitro, amino, hydroxy or carboxy. When the carbon chain is interrupted by an optionally substituted phenylene, the carbon chain is preferably unsubstituted. When the phenylene moiety is substituted, it is preferably substituted by halogen, nitro, amino, methoxy, hydroxy or carboxy.

Preferred compounds of formula I are those wherein R_1 is C_{13-20}alkyl, optionally substituted by nitro, halogen, amino, hydroxy or carboxy, and, more preferably those wherein R_1 is phenylalkyl substituted by C_{6-14}-alkyl chain optionally substituted by halogen and the alkyl moiety is a C_{1-6}alkyl optionally substituted by hydroxy. More preferably, R_1 is phenyl-C_{1-6}alkyl substituted on the phenyl by a straight or branched, preferably straight, C_{6-14}alkyl chain. The C_{6-14}alkyl chain may be in ortho, meta or para, preferably in para.

Preferably each of R_2 to R_5 is H.

A preferred compound of formula I is 2-amino-2-tetradecyl-1,3-propanediol. A particularly preferred S1P receptor agonist of formula I is FTY720, i.e. 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol in free form or in a pharmaceutically acceptable salt form (referred to hereinafter as Compound A), e.g. the hydrochloride, as shown:



A preferred compound of formula II is the one wherein each of R'_2 to R'_5 is H and m is 4, i.e. 2-amino-2-{2-[4-(1-oxo-5-phenylpentyl)phenyl]ethyl}propane-1,3-diol, in free form or in pharmaceutically acceptable salt form (referred to hereinafter as Compound B), e.g the hydrochloride.

A preferred compound of formula III is the one wherein W is CH_3, each of R''_1 to R''_3 is H, Z_2 is ethylene, X is heptyloxy and Y is H, i.e. 2-amino-4-(4-heptyloxyphenyl)-2-methyl-butanol, in free form or in pharmaceutically acceptable salt form (referred to hereinafter as Compound C), e.g. the hydrochloride. The R-enantiomer is particularly preferred.

A preferred compound of formula IVa is the FTY720-phosphate (R_{2a} is H, R_{3a} is OH, X_a is O, R_{1a} and R_{1b} are OH). A preferred compound of formula IVb is the Compound C-phosphate (R_{2a} is H, R_{3b} is OH, X_a is O, R_{1a} and R_{1b} are OH, Y_a is O and R_{4a} is heptyl). A preferred compound of formula V is Compound B-phosphate.

A preferred compound of formula V is phosphoric acid mono-[(R)-2-amino-2-methyl-4-(4-pentyloxy-phenyl)-butyl]ester.

A preferred compound of formula VIII is (2R)-2-amino-4-[3-(4-cyclohexyloxybutyl)benzo[b]thien-6-yl]-2-methylbutan-1-ol.

When the compounds of formulae I to IX have one or more asymmetric centers in the molecule, the present invention is to be understood as embracing the various optical isomers, as well as racemates, diastereoisomers and mixtures thereof are embraced. Compounds of formula III or IVb, when the carbon atom bearing the amino group is asymmetric, have preferably the R-configuration at this carbon atom.

Examples of pharmaceutically acceptable salts of the compounds of the formulae I to IX include salts with inorganic acids, such as hydrochloride, hydrobromide and sulfate, salts with organic acids, such as acetate, fumarate, maleate, benzoate, citrate, malate,

methanesulfonate and benzenesulfonate salts, or, when appropriate, salts with metals such as sodium, potassium, calcium and aluminium, salts with amines, such as triethylamine and salts with dibasic amino acids, such as lysine. The compounds and salts of the methods of the present invention encompass hydrate and solvate forms.

The S1P receptor agonists have, on the basis of observed activity, e.g. homing of lymphocytes, e.g. as described in EP627406A1 or USP 6,004,565, been found to be useful e.g. as immunosuppressant, e.g. in the treatment of acute allograft rejection. It has now been found that S1P receptor agonists have interesting properties which make them useful for cancer chemotherapy, particularly of solid tumors, especially of advanced solid tumors. There is still the need to expand the armamentarium of cancer treatment of solid tumors, especially in cases where treatment with anticancer compounds is not associated with disease regression or stabilization.

In accordance with the particular findings of the present invention, there is provided:

1.1 A method for treating solid tumors in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.2 A method for inhibiting growth of solid tumors in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.3 A method for inducing tumor regression, e.g. tumor mass reduction, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.4 A method for treating solid tumor invasiveness or symptoms associated with such tumor growth in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.5 A method for preventing metastatic spread of tumours or for preventing or inhibiting growth of micrometastasis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.6 A method for inhibiting or controlling deregulated angiogenesis, e.g. sphingosine-1-phosphate (S1P) mediated angiogenesis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

1.7 A method for preventing or treating diseases mediated by a neo-angiogenesis process or associated with deregulated angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof.

By "solid tumors" are meant tumors and/or metastasis (whereever located) other than lymphatic cancer, e.g. brain and other central nervous system tumors (eg. tumors of the meninges, brain, spinal cord, cranial nerves and other parts of central nervous system, e.g. glioblastomas or medulla blastomas); head and/or neck cancer; breast tumors; circulatory system tumors (e.g. heart, mediastinum and pleura, and other intrathoracic organs, vascular tumors and tumor-associated vascular tissue); excretory system tumors (e.g. kidney, renal pelvis, ureter, bladder, other and unspecified urinary organs); gastrointestinal tract tumors (e.g. oesophagus, stomach, small intestine, colon, colorectal, rectosigmoid junction, rectum, anus and anal canal), tumors involving the liver and intrahepatic bile ducts, gall bladder, other and unspecified parts of biliary tract, pancreas, other and digestive organs); oral cavity (lip, tongue, gum, floor of mouth, palate, and other parts of mouth, parotid gland, and other parts of the salivary glands, tonsil, oropharynx, nasopharynx, pyriform sinus, hypopharynx, and other sites in the lip, oral cavity and pharynx); reproductive system tumors (e.g. vulva, vagina, Cervix uteri, Corpus uteri, uterus, ovary, and other sites associated with female genital organs, placenta, penis, prostate, testis, and other sites associated with male genital organs); respiratory tract tumors (e.g. nasal cavity and middle ear, accessory sinuses, larynx, trachea, bronchus and lung, e.g. small cell lung cancer or non-small cell lung cancer); skeletal system tumors (e.g. bone and articular cartilage of limbs, bone articular cartilage and other sites); skin tumors (e.g. malignant melanoma of the skin, non-melanoma skin cancer, basal cell carcinoma of skin, squamous cell carcinoma of skin, mesothelioma, Kaposi's sarcoma); and tumors involving other tissues incluing peripheral nerves and autonomic nervous system, connective and soft tissue, retroperitoneum and peritoneum, eye and adnexa, thyroid, adrenal gland and other endocrine glands and related structures, secondary and unspecified malignant neoplasm of lymph nodes, secondary malignant neoplasm of respiratory and digestive systems and secondary malignant neoplasm of other sites.

Where hereinbefore and subsequently a tumor, a tumor disease, a carcinoma or a cancer is mentioned, also metastasis in the original organ or tissue and/or in any other location are implied alternatively or in addition, whatever the location of the tumor and/or metastasis is.

When the S1P receptor agonist is a compound of formula I, e.g. Compound A, or a compound of formula IVa or IVb, in one embodiment it is used in the treatment methods 1.1, 1.2, 1.3 or 1.4 for a solid tumor other than breast, prostate, bladder, kidney or lung tumor.

In a series of further specific or alternative embodiments, the present invention also provides

1.8 A method for enhancing the activity of a chemotherapeutic agent or for overcoming resistance to a chemotherapeutic agent in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist , e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, either concomitantly or sequentially with said chemotherapeutic agent.

1.9 A method according to 1.8 wherein the chemotherapeutic agent is an inhibitor of signal transduction pathways directed either against host cells or processes involved in tumor formation and/or metastases formation or utilised by tumour cells for proliferation, survival, differentiation or development of drug resistance.

1.10 A method as indicated above, wherein the S1P receptor agonist is administered intermittently.

In a series of further specific or alternative embodiments, the present invention also provides:

2.1 A S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, for use in any method as defined under 1.1 to 1.4 above, preferably for a solid tumor other than breast, prostate, bladder, kidney or lung when the S1P receptor agonist is a compound of formula I, e.g. Compound A, or a compound of formula IVa or IVb.

2.2 A S1P receptor agonist, e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, for use in any method as defined under 1.5 to 1.10 above or 7 below.

3.1 A S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, for use in the preparation of a pharmaceutical composition for use in any method as defined under 1.1 to 1.4 above, preferably for a solid tumor other than breast, prostate, bladder, kidney or lung when the S1P receptor agonist is a compound of formula I, e.g. Compound A, or a compound of formula IVa or IVb.

3.2 A S1P receptor agonist, e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, for use in the preparation of a pharmaceutical composition for use in any method as defined under 1.5 to 1.10 above or 7 below.

4.1 A pharmaceutical composition for use in any method as defined under 1.1 to 1.4 above comprising a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically acceptable diluents or carriers therefor, preferably for a solid tumor other than breast, prostate, bladder, kidney or lung when the S1P receptor agonist is a compound of formula I, e.g. Compound A, or a compound of formula IVa or IVb.

4.2 A pharmaceutical composition for use in any method as defined under 1.5 to 1.10 above or 7 below comprising a S1P receptor agonist, e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically acceptable diluents or carriers therefor.

5.1 A pharmaceutical combination comprising a) a first agent which is a S1P receptor agonist , e.g. a S1P receptor agonist comprising a group of formula X or a pharmaceutically acceptable salt thereof and b) a co-agent which is a chemotherapeutic agent, e.g. as defined hereinafter.

5.2 A pharmaceutical combination comprising an amount of a) a first agent which is a S1P receptor agonist , e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, and b) a co-agent which is a chemotherapeutic agent selected from the compounds defined under section xi) below, to produce a synergistic therapeutic effect.

6. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a S1P receptor agonist, e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, and a second drug substance, said second drug substance being a chemotherapeutic agent, e.g. as indicated hereinafter.

7. A method for treating lymphoproliferative or myeloproliferative disorders, e.g. for treating tumor invasiveness or symptoms associated with such tumor growth in a subject in need thereof, comprising co-administering to said subject, e.g. concomitantly or in sequence, of a S1P receptor agonist, e.g. a S1P receptor agonist comprising a group of formula X, or a pharmaceutically acceptable salt thereof, and a second drug substance, said second drug substance being a chemotherapeutic agent, e.g. as indicated hereinafter.

By "lymphatic cancer" are meant e.g. tumors of blood and lymphatic system (e.g. Hodgkin's disease, Non-Hodgkin's lymphoma, Burkitt's lymphoma, AIDS-related lymphomas, malignant immunoproliferative diseases, multiple myeloma and malignant plasma cell neoplasms, lymphoid leukemia, acute or chronic myeloid leukemia, acute or chronic lymphocytic leukemia, monocytic leukemia, other leukemias of specified cell type, leukemia of

unspecified cell type, other and unspecified malignant neoplasms of lymphoid, haematopoietic and related tissues, for example diffuse large cell lymphoma, T-cell lymphoma or cutaneous T-cell lymphoma). Myeloid cancer includes e.g. acute or chronic myeloid leukaemia.

By the term "chemotherapeutic agent" is meant especially any chemotherapeutic agent other than the S1P receptor agonist. It includes but is not limited to,

i. an aromatase inhibitor,
ii. an antiestrogen, an anti-androgen (especially in the case of prostate cancer) or a gonadorelin agonist,
iii. a topoisomerase I inhibitor or a topoisomerase II inhibitor,
iv. a microtubule active agent, an alkylating agent, an antineoplastic antimetabolite or a platin compound,
v. a compound targeting/decreasing a protein or lipid kinase activity or a protein or lipid phosphatase activity, a further anti-angiogenic compound or a compound which induces cell differentiation processes,
vi. a bradykinin 1 receptor or an angiotensin II antagonist,
vii. a cyclooxygenase inhibitor, a bisphosphonate, a histone deacetylase inhibitor, a heparanase inhibitor (prevents heparan sulphate degradation), e.g. PI-88, a biological response modifier, preferably a lymphokine or interferons, e.g. interferon γ, an ubiquitination inhibitor, or an inhibitor which blocks anti-apoptotic pathways,
viii. an inhibitor of Ras oncogenic isoforms, e.g. H-Ras, K-Ras or N-Ras, or a farnesyl transferase inhibitor, e.g. L-744,832 or DK8G557,
ix. a telomerase inhibitor, e.g. telomestatin,
x. a protease inhibitor, a matrix metalloproteinase inhibitor, a methionine aminopeptidase inhibitor, e.g. bengamide or a derivative thereof, or a proteosome inhibitor, e.g. PS-341, and/or
xi) a mTOR inhibitor.

The term "aromatase inhibitor" as used herein relates to a compound which inhibits the estrogen production, i.e. the conversion of the substrates androstenedione and testosterone to estrone and estradiol, respectively. The term includes, but is not limited to steroids, especially atamestane, exemestane and formestane and, in particular, non-steroids, especially aminoglutethimide, roglethimide, pyridoglutethimide, trilostane, testolactone, ketokonazole, vorozole, fadrozole, anastrozole and letrozole. Exemestane can be administered, e.g., in the form as it is marketed, e.g. under the trademark AROMASIN™. Formestane can be administered, e.g., in the form as it is marketed, e.g. under the

trademark LENTARON™. Fadrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark AFEMA™. Anastrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark ARIMIDEX™. Letrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark FEMARA™ or FEMAR™ Aminoglutethimide can be administered, e.g., in the form as it is marketed, e.g. under the trademark ORIMETEN™. A combination of the invention comprising a chemotherapeutic agent which is an aromatase inhibitor is particularly useful for the treatment of hormone receptor positive tumors, e.g. breast tumors.

The term "antiestrogen" as used herein relates to a compound which antagonizes the effect of estrogens at the estrogen receptor level. The term includes, but is not limited to tamoxifen, fulvestrant, raloxifene and raloxifene hydrochloride. Tamoxifen can be administered, e.g., in the form as it is marketed, e.g. under the trademark NOLVADEX™. Raloxifene hydrochloride can be administered, e.g., in the form as it is marketed, e.g. under the trademark EVISTA™. Fulvestrant can be formulated as disclosed in US 4,659,516 or it can be administered, e.g., in the form as it is marketed, e.g. under the trademark FASLODEX™. A combination of the invention comprising a chemotherapeutic agent which is an antiestrogen is particularly useful for the treatment of estrogen receptor positive tumors, e.g. breast tumors.

The term "anti-androgen" as used herein relates to any substance which is capable of inhibiting the biological effects of androgenic hormones and includes, but is not limited to, bicalutamide (CASODEX™), which can be formulated, e.g. as disclosed in US 4,636,505.

The term "gonadorelin agonist" as used herein includes, but is not limited to abarelix, goserelin and goserelin acetate. Goserelin is disclosed in US 4,100,274 and can be administered, e.g., in the form as it is marketed, e.g. under the trademark ZOLADEX™. Abarelix can be formulated, eg. as disclosed in US 5,843,901.

The term "topoisomerase I inhibitor" as used herein includes, but is not limited to topotecan, irinotecan, 9-nitrocamptothecin and the macromolecular camptothecin conjugate PNU-166148 (compound A1 in WO99/17804). Irinotecan can be administered, e.g. in the form as it is marketed, e.g. under the trademark CAMPTOSAR™. Topotecan can be administered, e.g., in the form as it is marketed, e.g. under the trademark HYCAMTIN™.

The term "topoisomerase II inhibitor" as used herein includes, but is not limited to the anthracyclines such as doxorubicin (including liposomal formulation, e.g. CAELYX™), daunorubicin, epirubicin, idarubicin and nemorubicin, the anthraquinones mitoxantrone and losoxantrone, and the podophillotoxines etoposide and teniposide. Etoposide can be

administered, e.g. in the form as it is marketed, e.g. under the trademark ETOPOPHOS™. Teniposide can be administered, e.g. in the form as it is marketed, e.g. under the trademark VM 26-BRISTOL™ Doxorubicin can be administered, e.g. in the form as it is marketed, e.g. under the trademark ADRIBLASTIN™. Epirubicin can be administered, e.g. in the form as it is marketed, e.g. under the trademark FARMORUBICIN™. Idarubicin can be administered, e.g. in the form as it is marketed, e.g. under the trademark ZAVEDOS™. Mitoxantrone can be administered, e.g. in the form as it is marketed, e.g. under the trademark NOVANTRON™.

The term "microtubule active agent" relates to microtubule stabilizing and microtubule destabilizing agents including, but not limited to taxanes, e.g. paclitaxel and docetaxel, vinca alkaloids, e.g., vinblastine, especially vinblastine sulfate, vincristine especially vincristine sulfate, and vinorelbine, discodermolides and epothilones and derivatives thereof, e.g. epothilone B or a derivative thereof. Paclitaxel may be administered e.g. in the form as it is marketed, e.g. TAXOL™. Docetaxel can be administered, e.g., in the form as it is marketed, e.g. under the trademark TAXOTERE™. Vinblastine sulfate can be administered, e.g., in the form as it is marketed, e.g. under the trademark VINBLASTIN R.P.™. Vincristine sulfate can be administered, e.g., in the form as it is marketed, e.g. under the trademark FARMISTIN™. Discodermolide can be obtained, e.g., as disclosed in US 5,010,099.

The term "alkylating agent" as used herein includes, but is not limited to busulfan, chlorambucil, cyclophosphamide, ifosfamide, melphalan or nitrosourea (BCNU or Gliadel™). Cyclophosphamide can be administered, e.g., in the form as it is marketed, e.g. under the trademark CYCLOSTIN™. Ifosfamide can be administered, e.g., in the form as it is marketed, e.g. under the trademark HOLOXAN™.

The term "antineoplastic antimetabolite" includes, but is not limited to 5-fluorouracil, capecitabine, gemcitabine, cytarabine, fludarabine, thioguanine, methotrexate and edatrexate. Capecitabine can be administered, e.g., in the form as it is marketed, e.g. under the trademark XELODA™. Gemcitabine can be administered, e.g., in the form as it is marketed, e.g. under the trademark GEMZAR™.

The term "platin compound" as used herein includes, but is not limited to carboplatin, cis-platin and oxaliplatin. Carboplatin can be administered, e.g., in the form as it is marketed, e.g. under the trademark CARBOPLAT™. Oxaliplatin can be administered, e.g., in the form as it is marketed, e.g. under the trademark ELOXATIN™.

The term "compounds targeting/decreasing a protein or lipid kinase activity or further anti-angiogenic compounds" as used herein includes, but is not limited to protein tyrosine kinase

and/or serine and/or threonine kinase inhibitors or lipid kinase inhibitors, e.g. compounds targeting, decreasing or inhibiting the activity of the epidermal growth factor family of receptor tyrosine kinases (EGFR, ErbB2, ErbB3, ErbB4 as homo- or heterodimers), the vascular endothelial growth factor family of receptor tyrosine kinases (VEGFR), the platelet-derived growth factor-receptors (PDGFR), the fibroblast growth factor-receptors (FGFR), the insulin-like growth factor receptor 1 (IGF-1R), the Trk receptor tyrosine kinase family, the Axl receptor tyrosine kinase family, the Ret receptor tyrosine kinase, the Kit/SCFR receptor tyrosine kinase, members of the c-Abl family and their gene-fusion products (e.g. BCR-Abl), members of the protein kinase C (PKC) and Raf family of serine/threonine kinases, members of the MEK, SRC, JAK, FAK, PDK or PI(3) kinase family, or of the PI(3)-kinase-related kinase family, and/or members of the cyclin-dependent kinase family (CDK) and anti-angiogenic compounds having another mechanism for their activity, e.g. unrelated to protein or lipid kinase inhibition.

Compounds which target, decrease or inhibit the activity of VEGFR are especially compounds, proteins or antibodies which inhibit the VEGF receptor tyrosine kinase, inhibit a VEGF receptor or bind to VEGF, and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 98/35958, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or a pharmaceutically acceptable salt thereof, e.g. the succinate, in WO 00/27820, e.g. a N-aryl(thio) anthranilic acid amide derivative e.g. 2-[(4-pyridyl)methyl]amino-N-[3-methoxy-5-(trifluoromethyl)phenyl]benzamide or 2-[(1-oxido-4-pyridyl)methyl]amino-N-[3-trifluoromethylphenyl]benzamide, or in WO 00/09495, WO 00/59509, WO 98/11223, WO 00/27819 and EP 0 769 947; those as described by M. Prewett et al in Cancer Research 59 (1999) 5209-5218, by F. Yuan et al in Proc. Natl. Acad. Sci. USA, vol. 93, pp. 14765-14770, Dec. 1996, by Z. Zhu et al in Cancer Res. 58, 1998, 3209-3214, and by J. Mordenti et al in Toxicologic Pathology, Vol. 27, no. 1, pp 14-21, 1999; in WO 00/37502 and WO 94/10202; Angiostatin™, described by M. S. O'Reilly et al, Cell 79, 1994, 315-328; Endostatin™, described by M. S. O'Reilly et al, Cell 88, 1997, 277-285; anthranilic acid amides; ZD4190; ZD6474; SU5416; SU6668; or anti-VEGF antibodies or anti-VEGF receptor antibodies,e.g. RhuMab.

By antibody is meant intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies formed from at least 2 intact antibodies, and antibodies fragments so long as they exhibit the desired biological activity.

Compounds which target, decrease or inhibit the activity of the epidermal growth factor receptor family are especially compounds, proteins or antibodies which inhibit members of the EGF receptor tyrosine kinase family, e.g. EGF receptor, ErbB2, ErbB3 and ErbB4 or bind

to EGF or EGF related ligands, or which have a dual inhibiting effect on the ErbB and VEGF receptor kinase and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 97/02266, e.g. the compound of ex. 39, or in EP 0 564 409, WO 99/03854, EP 0520722, EP 0 566 226, EP 0 787 722, EP 0 837 063, US 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and, especially, WO 96/30347 (e.g. compound known as CP 358774), WO 96/33980 (e.g. compound ZD 1839) and WO 95/03283 (e.g. compound ZM105180) or PCT/EP02/08780; e.g. trastuzumab (Herpetin^{R}), cetuximab, Iressa, OSI-774, CI-1033, EKB-569, GW-2016, E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6.3 or E7.6.3.

Compounds which target, decrease or inhibit the activity of PDGFR are especially compounds which inhibit the PDGF receptor, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib.

Compounds which target, decrease or inhibit the activity of c-Abl family members and their gene fusion products, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib; PD180970; AG957; or NSC 680410.

Compounds which target, decrease or inhibit the activity of protein kinase C, Raf, MEK, SRC, JAK, FAK and PDK family members, or PI(3) kinase or PI(3) kinase-related family members, and/or members of the cyclin-dependent kinase family (CDK) are especially those staurosporine derivatives disclosed in EP 0 296 110, e.g. midostaurin; examples of further compounds include e.g. UCN-01, safingol, BAY 43-9006, Bryostatin 1, Perifosine; UO126; Ilmofosine; RO 318220 and RO 320432; GO 6976; Isis 3521; or LY333531/LY379196.

Further anti-angiogenic compounds are e.g. thalidomide (THALOMID) and TNP-470.

Compounds which target, decrease or inhibit the activity of a protein or lipid phosphatase are e.g. inhibitors of phosphatase 1, phosphatase 2A, PTEN or CDC25, e.g. okadaic acid or a derivative thereof.

Compounds which induce cell differentiation processes are e.g. retinoic acid, α–, γ– or δ-tocopherol or α–, γ– or δ-tocotrienol.

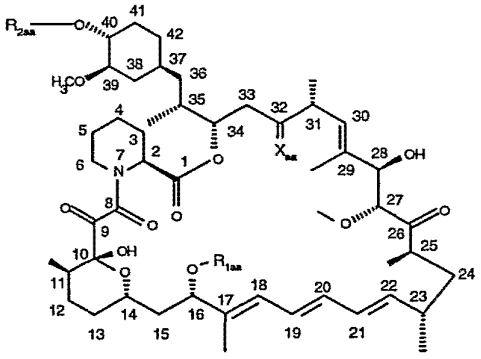
The term cyclooxygenase inhibitor as used herein includes, but is not limited to, e.g. celecoxib (Celebrex^{R}), rofecoxib (Vioxx^{R}), etoricoxib, valdecoxib or a 5-alkyl-2-arylaminophenylacetic acid, e.g. 5-methyl-2-(2'-chloro-6'-fluoroanilino)phenyl acetic acid.

The term "histone deacetylase inhibitor" as used herein includes, but is not limited to MS-27-275, SAHA, pyroxamide, FR-901228 or valproic acid.

The term "bisphosphonates" as used herein includes, but is not limited to, etridonic, clodronic, tiludronic, pamidronic, alendronic, ibandronic, risedronic and zoledronic acid. "Etridonic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark DIDRONEL™. "Clodronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark BONEFOS™. "Tiludronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark SKELID™. "Pamidronic acid" can be administered, e.g. in the form as it is marketed, e.g. under the trademark AREDIA™. "Alendronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark FOSAMAX™. "Ibandronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark BONDRANAT™. "Risedronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark ACTONEL™. "Zoledronic acid" can be administered, e.g. in the form as it is marketed, e.g. under the trademark ZOMETA™

The term "matrix metalloproteinase inhibitor" as used herein includes, but is not limited to collagen peptidomimetic and nonpetidomimetic inhibitors, tetracycline derivatives, e.g. hydroxamate peptidomimetic inhibitor batimastat and its orally bioavailable analogue marimastat, prinomastat, BMS-279251, BAY 12-9566, TAA211 or AAJ996.

The term "mTOR inhibitor" as used herein includes, but is not limited to rapamycin (sirolimus) or a derivative thereof. Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus. Suitable derivatives of rapamycin include e.g. compounds of formula A



A

wherein

R_{1aa} is CH_3 or C_{3-6}alkynyl,

R_{2aa} is H or $-CH_2-CH_2-OH$, 3-hydroxy-2-(hydroxymethyl)-2-methyl-propanoyl or tetrazolyl, and

X_{aa} is =O, (H,H) or (H,OH)

provided that R_{2aa} is other than H when X_{aa} is =O and R_{1aa} is CH_3.

or a prodrug thereof when R_{2aa} is $-CH_2-CH_2-OH$, e.g. a physiologically hydrolysable ether thereof.

Compounds of formula A are disclosed e.g. in WO 94/09010, WO 95/16691, WO 96/41807, USP 5,362,718 or WO 99/15530 which are incorporated herein by reference. They may be prepared as diclosed or by analogy to the procedures described in these references

Preferred rapamycin derivatives are 32-deoxorapamycin, 16-pent-2-ynyloxy-32-deoxorapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin and, more preferably, 40-0-(2-hydroxyethyl)-rapamycin. Further examples of rapamycin derivatives include e.g. CCI779 or 40- [3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]-rapamycin or a pharmaceutically acceptable salt thereof, as disclosed in USP 5,362,718, ABT578 or 40-(tetrazolyl)-rapamycin, particularly 40-epi-(tetrazolyl)-rapamycin, e.g. as disclosed in WO 99/15530, or rapalogs as disclosed e.g. in WO 98/02441 and WO01/14387, e.g. AP23573.

In each case where citations of patent applications or scientific publications are given, the subject-matter relating to the compounds is hereby incorporated into the present application by reference. Comprised are likewise the pharmaceutically acceptable salts thereof, the corresponding racemates, diastereoisomers, enantiomers, tautomers as well as the corresponding crystal modifications of above disclosed compounds where present, e.g. solvates, hydrates and polymorphs, which are disclosed therein. The compounds used as active ingredients in the combinations of the invention can be prepared and administered as described in the cited documents, respectively. Also within the scope of this invention is the combination of more than two separate active ingredients as set forth above, i.e. a pharmaceutical combination within the scope of this invention could include three active ingredients or more. Further both the first agent and the co-agent are not the identical ingredient.

Utility of the S1P agonists, e.g. the S1P agonists comprising a group of formula X, in treating solid tumors as hereinabove specified, may be demonstrated in animal test methods as well as in clinic, for example in accordance with the methods hereinafter described.

A. In Vitro

A.1 Antitumor Activity

A mouse breast cancer cell line originally isolated from mammary carcinomas is used, e.g. JygMC(A). The cell number is adjusted to $5x10^5$ for plating in fresh medium before the procedure. Cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12 h and then washed twice with PBS, followed by addition of

fresh medium with 10% FCS and additionally incubated for another 12h. Thereafter the cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12h. To release the cells from the block, the cells are washed twice with PBS and replated in fresh medium with 10% FCS. After synchronisation, the cells are incubated with or without various concentrations of a compound of formula I for 3, 6, 9, 12, 18 or 24h. The cells are harvested after treatment with 0.2% EDTA, fixed with ice-cold 70% ethanol solution, hydrolyzed with 250µg/ml of RNaseA (type 1-A: Sigma Chem. Co.) at 37°C for 30 mn and stained with propidium iodide at 10mg/ml for 20 mn. After the incubation period, the number of cells is determined both by counting cells in a Coulter counter and by the SRB colorimetric assay. Under these conditions an S1P agonist, e.g. Compound B in hydrochloride salt form, inhibits the proliferation of the tumor cells at concentrations ranging from 10^{-12} to 10^{-6} M.

A.2 S1P-Mediated HUVEC Tube Formation Assay

For the tube formation assay, HUVEC from passage 2-8 are used and are never greater than 70% confluent before harvesting. Cells are prepared for the assay by washing with Herpes Balanced Saline Solution (HBSS from Clonetics) and then trypsinizing with Trypsin/EDTA (0.25 mg/ml, from Clonetics). After approximately 90 % of the cells have lifted off the plate, an equal volume of Trypsin Neutralizing Solution (TNS from Clonetics) is added and the cells are collected into a conical tube containing at least 10 ml of EBM-2 (Clonetics) + 0.1 % BSA (Sigma) media. Cells are centrifuged at 1000 rpm for 5 minutes and the supernatant is removed and replaced with 5 ml of fresh EBM-2 + 0.1 % BSA. Cells are counted using a hemacytometer and the volume of the cell suspension is adjusted to achieve a concentration of 500,000 cells/ml. Conical tubes are prepared with test compounds at 100 nM, and pertussin toxin (PTx) at 10 ng/ml in each, then 1 ml of the cell suspension is added to each tube. Tubes are then incubated for ½ hour at 37 ºC, 5 % CO_2. The migration assay is performed using Fluoro-Blok 24-Multiwell Insert Plates coated with fibronectin (8 µm pore size, Falcon #351147) instead of the individual inserts in a 24-well plate. Cells and test compounds are prepared and pre-incubated as described above, then 100 µl is added to each approriate well in the Insert Plate. 300 µl of the EBM-2 + 2 % charcoal-stripped media without S1P is added to the bottoms of the wells marked for no stimulation (-), and 300 µl of the media containing S1P (500 nM) is added to the bottoms of the wells marked for stimulation (+). The plate is then incubated for 4 hours at 37 ºC, 5 % CO_2.

Calcein AM, 50 µg/vial, (Molecular Probes #C3100) is prepared by first adding 20 µl DMSO to the vial. Then 12.5 ml of HBSS (per plate) is warmed to 37 ºC and 150 µl is added to the

vial. The contents of the vial are then transferred back to the remaining HBSS to make the final concentration 4 µg/ml Calcein AM.

The Fluoro-Blok plate is removed from the incubator and the top insert plate is separated and "flicked" to remove excess media clinging to the inserts. The insert plate is then transferred to a fresh 24-well plate containing 500 µl/well of the 4 µg/ml Calcein AM. The plate is then incubated for 1½ hours at 37 ºC, 5 % CO_2.

After incubation, the plate is read on a Cytofluor II at an excitation of 485 nm and emission of 530 nm. The Fluoro-Blok coating in the inserts allows only the cells that have migrated to the bottom to be counted. Data are transferred to Excel for calculations, graphs are created using SigmaPlot, and SigmaStat is used for significance tests (t-test). (Figure 7).

Tube formation is quantitated by counting the number of branching points (two independent cords connecting) in 3 independent fields at 4x magnification. The results are reported as follows:

Treatment	Branching Points
PBS	8 ± 5
S1P	42 ± 13
FTY720-Phosphate	48 ± 15
FTY720-Phosphate + S1P	14 ± 7
Compound C-Phosphate	44 ± 16
Compound C-Phosphate + S1P	18 ± 6

These results demonstrate the unique ability of FTY720-Phosphate or Compound C-Phosphate to act as an agonist of angiogenesis on its own, but then surprisingly, as an antagonist of S1P-mediated angiogenesis. Compound C-Phosphate is preferably the racemate or the R-enantiomer. PTx is used as a control to inhibit Giα (EDG-1) mediated activity.

B. In Vivo

B.1 Antitumor Activity

Antitumor activity is expressed as T/C% (mean increase in tumor volumes of treated animals divided by the mean increase of tumor volumes of control animals multiplied by 100).

Aliquots of cancer cells ($1x10^7$), e.g. human A375 melanoma cells, are transplanted into BALB/c-*nu/nu* mice. When the tumors have reached ca. 10x10 mm in size, the animals are assigned randomly to four subgroups and the treatment with a compound of formula I is initiated. Animals are sacrificed after 2 week treatment, at which times tumors and tissues are harvested and prepared for morphological and molecular analysis. The size of the tumors is determined with a caliper. In this assay, an S1P agonist, e.g. Compound B or C (in the hydrochloride salt form), slows tumor growth when administered at a dose of from 0.5 to 5 mg/kg vs saline control: for example, Compound C-HCl when administered at a dose of 2.5mg/kg 5x/week results in a final T/C value of 30%.

B.2 Combination with a VEGF-R protein tyrosin kinase inhibitor

Nude mice transplanted with human MDA-MB-435 breast tumors are treated for 2 weeks with a VEGF-R protein tyrosin kinase inhibitor, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine succinate, at a dose of 100 mg/kg p.o. 5x/week, a S1P receptor agonist, e.g. Compound C (hydrochloride salt), at a dose of 2.5 mg/kg i.v. 5x/week, or a combination of both. Antitumor is expressed as T/C% as indicated above. A combination of Compound C-HCl with 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine succinate produces a greater antitumor effect (T/C% 27) as compared to either agent alone (Compound C-HCl, T/C 66%; 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine succinate, T/C% 91).
Good antitumor responses are also obtained when nude mice are transplanted with human A375 melanoma cells and treated in a similar way with the same combination: the combined treatment results in a T/C% 15 whereas treatment with each agent alone results in a T/C% 35 and 44, respectively.

B.3 Antiangiogenic Activity

Porous chambers containing (i) sphingosine-1-phosphate (5 µM/chamber) or (ii) human VEGF (1 µg/chamber) in 0.5 ml of 0.8% w/v agar (containing heparin, 20 U/ml) are implanted subcutaneously in the flank of mice. S1P or VEGF induces the growth of vascularized tissue around the chamber. This response is dose-dependent and can be quantified by measuring the weight and blood content of the tissue. Mice are treated once a day (i) orally with Compound A (0.3, 3, 30 or 50 mg/kg) or (ii) intravenously with the R enantiomer of Compound C (2.5 mg/kg) or (iii) intravenously with the S enantiomer of Compound C (2.5 mg/kg) or (iv) orally or intravenously with vehicle (5% glucose, 10 ml/kg), starting 4-6 hours before implantation of the chambers and continuing for 4 days. The animals are sacrificed for measurement of the vascularized tissues 24 h after the last dose. The weight and blood content of the vascularized tissues around the chamber is determined.

Animals treated with Compound A or with the R or S enantiomer of Compound C show reduced weight and/or blood content of the vascularized tissues compared to animals treated with vehicle alone.

C. Clinical Trial

C.1 Investigation of clinical benefit of a S1P receptor agonist, e.g. a compound of formula I, II or III, e.g. Compound A, B or C

20 patients with progressing, advanced-stage solid tumors, resistant or refractory to standard therapies, to receive said compound at a dosage as determined by a dose escalating study. The general clinical state of the patient is investigated weekly by physical and laboratory examination. Changes in tumor and metastases burden are assessed every 2 months by radiological examination. Initially patients receive treatment for 2 months. Thereafter, they remain on treatment for as long as their disease does not progress and the drug is satisfactorily tolerated.

Main variables for evaluation: Safety (adverse events), standard serum biochemistry and haematology, tumor dimensions by computerised tomographic (CT) scan or magnetic resonance imaging (MRI).

C.2 Combined Treatment

Suitable clinical studies are, for example, open label non-randomized, dose escalation studies in patients with advanced solid tumors. Such studies prove in particular the synergism of the active ingredients of the combination of the invention. The beneficial effects on proliferative diseases can be determined directly through the results of these studies or by changes in the study design which are known as such to a person skilled in the art. Such studies are, in particular, suitable to compare the effects of a monotherapy using the active ingredients and a combination of the invention. Preferably, the dose of agent (a) is escalated until the Maximum Tolerated Dosage is reached, and the co-agent (b) is administered with a fixed dose. Alternatively, the agent (a) is administered in a fixed dose and the dose of co-agent (b) is escalated. Each patient receives doses of the agent (a) either daily or intermittent. The efficacy of the treatment can be determined in such studies, e.g., after 12, 18 or 24 weeks by radiologic evaluation of the tumors every 6 weeks.

Alternatively, a placebo-controlled, double blind study can be used in order to prove the benefits of the combination of the invention mentioned herein.

Daily dosages required in practicing the method of the present invention when a S1P receptor agonist alone is used will vary depending upon, for example, the compound used, the host, the mode of administration and the severity of the condition to be treated. A preferred daily dosage range is about from 0.1 to 100 mg as a single dose or in divided

doses. Suitable daily dosages for patients are on the order of from e.g. 0.1 to 50 mg p.o. The S1P receptor agonist may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, nasally, pulmonary (by inhalation) or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise from ca. 0.1 to 30 mg, usually 0.25 to 30 mg S1P receptor agonist, together with one or more pharmaceutically acceptable diluents or carriers therefore. In order to inhibit angiogenesis it is important to select a sufficiently high dose of the S1P receptor agonist, as low concentrations of S1P receptor agonists promote angiogenesis. A suitable dose for providing an anti-angiogenic effect when a S1P agonist is administered to a patient may be selected by concentration- and dose-escalating studies as described at A, B, and C above.

The combination of the invention can also be applied in combination with surgical intervention, mild prolonged whole body hyperthermia and/or irradiation therapy.

The administration of a pharmaceutical combination of the invention results in a beneficial effect, e.g. a synergistic therapeutic effect, e.g. with regard to slowing down, arresting or reversing the neoplasm formation, metastases spread or growth or a longer duration of tumor response or inhibition of angiogenesis; it may also result in other beneficial effects, e.g. less side-effects, an improved quality of life or a decreased mortality and morbidity, compared to a monotherapy applying only one of the pharmaceutically active ingredients used in the combination of the invention, in particular in the treatment of a tumor that is refractory to other chemotherapeutics known as anti-cancer agents.

A further benefit is that lower doses of the active ingredients of the combination of the invention can be used, for example, that the dosages need not only often be smaller but are also applied less frequently, or can be used in order to diminish the incidence of side-effects, while controlling the growth of neoplasm formation. This is in accordance with the desires and requirements of the patients to be treated.

According to one embodiment of the invention, a preferred pharmaceutical combination comprises

a) a compound of formula I, II, III, IVa, IVb, V or VI, e.g. Compound A, B or C, and

b) as a co-agent, one or more compounds as indicated in paragraphs (ii), (iii), (iv), (v), (vii) or (xi) above, e.g. carboplatin, cisplatinum, paclitaxel, docetaxel, gemcitabine, doxorubicin, a compound targeting, decreasing or inhibiting the activity of the vascular endothelial growth factor family of receptor tyrosine kinases (VEGFR) or the platelet-derived growth factor-receptors (PDGFR), a bisphosphonate or a mTOR inhibitor.

A further embodiment of the invention relates to the use of S1P receptor agonist (a) in combination with a chemotherapeutic agent (b) in the treatment of a lymphatic or myeloid cancer, e.g. as disclosed above. The combination may comprise as a further co-agent b) e.g. busulfan, cytarabine, 6-thioguanine, fludarabine, hydroxyurea, procarbazine, bleomycin or methotrexate. Topoisomerase II inhibitors e,g. daunorubicin or, particularly, compounds which target, decrease or inhibit the activity of PDGFR or of c-Abl family members and their gene fusion products, e.g. imatinib, are preferred as co-agent (b), e.g. for use in the treatment of a lymphatic cancer.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

It is one objective of this invention to provide a pharmaceutical composition comprising a quantity, which is jointly therapeutically effective against a proliferative malignant disease comprising a combination of the invention. In this composition, the first agent a) and co-agent (b) can be administered together, one after the other or separately in one combined unit dosage form or in two separate unit dosage forms. The unit dosage form may also be a fixed combination.

The pharmaceutical compositions according to the invention can be prepared in a manner known per se and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including humans, comprising a therapeutically effective amount of at least one pharmacologically active combination partner alone, e.g. as indicated above, or in combination with one or more pharmaceutically acceptable carriers or diluents, especially suitable for enteral or parenteral application.

Suitable pharmaceutical compositions contain, for example, from about 0.1 % to about 99.9%, preferably from about 1 % to about 60 %, of the active ingredient(s). Pharmaceutical preparations for the combination therapy for enteral or parenteral administration are, for example, those in unit dosage forms, such as sugar-coated tablets, tablets, capsules or suppositories, or ampoules. If not indicated otherwise, these are prepared in a manner known per se, for example by means of conventional mixing, granulating, sugar-coating, dissolving or lyophilizing processes. It will be appreciated that the unit content of a combination partner contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached by administration of a plurality of dosage units.

In particular, a therapeutically effective amount of each of the combination partner of the combination of the invention may be administered simultaneously or sequentially and in any order, and the components may be administered separately or as a fixed combination. For example, the method of delay of progression or treatment of a proliferative malignant disease according to the invention may comprise (i) administration of the first agent a) in free or pharmaceutically acceptable salt form and (ii) administration of a co-agent b) in free or pharmaceutically acceptable salt form, simultaneously or sequentially in any order, in jointly therapeutically effective amounts, preferably in synergistically effective amounts, e.g. in daily or intermittently dosages corresponding to the amounts described herein. The individual combination partners of the combination of the invention may be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. Furthermore, the term administering also encompasses the use of a pro-drug of a combination partner that convert *in vivo* to the combination partner as such. The instant invention is therefore to be understood as embracing all such regimens of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

The effective dosage of each of the combination partners employed in the combination of the invention may vary depending on the particular compound or pharmaceutical composition employed, the mode of administration, the condition being treated, the severity of the condition being treated. Thus, the dosage regimen of the combination of the invention is selected in accordance with a variety of factors including the route of administration and the renal and hepatic function of the patient. A physician, clinician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the single active ingredients required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentration of the active ingredients within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the active ingredients' availability to target sites.

Daily dosages for the first agent or component (a) will, of course, vary depending on a variety of factors, for example the compound chosen, the particular condition to be treated and the desired effect. In general, however, satisfactory results are achieved on administration of a S1P receptor agonist, e.g. Compound A, B or C, at daily dosage rates of the order of ca. 0.1 to 100 mg as a single dose or in divided doses. The S1P receptor agonist may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration

comprise from ca. 0.1 to 30 mg component (a), e.g. 0.1 to 25 mg, together with one or more pharmaceutically acceptable diluents or carriers therefor.

Fadrozole may be administered orally to a human in a dosage range varying from about 0.5 to about 10 mg/day, preferably from about 1 to about 2.5 mg/day. Exemestane may be administered orally to a human in a dosage range varying from about 5 to about 200 mg/day, preferably from about 10 to about 25 mg/day, or parenterally from about 50 to 500 mg/day, preferably from about 100 to about 250 mg/day. If the drug shall be administered in a separate pharmaceutical composition, it can be administered in the form disclosed in GB 2,177,700. Formestane may be administered parenterally to a human in a dosage range varying from about 100 to 500 mg/day, preferably from about 250 to about 300 mg/day. Anastrozole may be administered orallly to a human in a dosage range varying from about 0.25 to 20 mg/day, preferably from about 0.5 to about 2.5 mg/day. Aminogluthemide may be administered to a human in a dosage range varying from about 200 to 500 mg/day.

Tamoxifen citrate may be administered to a human in a dosage range varying from about 10 to 40 mg/day.

Vinblastine may be administered to a human in a dosage range varying from about 1.5 to 10 mg/m^{2}day. Vincristine sulfate may be administered parenterally to a human in a dosage range varying from about 0.025 to 0.05 mg/kg body weight * week. Vinorelbine may be administered to a human in a dosage range varying from about 10 to 50 mg/m^{2}day.

Etoposide phosphate may be administered to a human in a dosage range varying from about 25 to 115 mg/m^{2}day, e.g. 56.8 or 113.6 mg/m^{2}day.

Teniposide may be administered to a human in a dosage range varying from about 75 to 150 mg about every two weeks. Doxorubicin may be administered to a human in a dosage range varying from about 10 to 100 mg/m^{2}day, e.g. 25 or 50 mg/m^{2}day. Epirubicin may be administered to a human in a dosage range varying from about 10 to 200 mg/m^{2}day. Idarubicin may be administered to a human in a dosage range varying from about 0.5 to 50 mg/m^{2}day. Mitoxantrone may be administered to a human in a dosage range varying from about 2.5 to 25 mg/m^{2}day.

Paclitaxel may be administered to a human in a dosage range varying from about 50 to 300 mg/m^{2}day. Docetaxel may be administered to a human in a dosage range varying from about 25 to 100 mg/m^{2}day.

Cyclophosphamide may be administered to a human in a dosage range varying from about 50 to 1500 mg/m^{2}day. Melphalan may be administered to a human in a dosage range varying from about 0.5 to 10 mg/m^{2}day.

5-Fluorouracil may be administered to a human in a dosage range varying from about 50 to 1000 mg/m^{2}day, e.g. 500 mg/m^{2}day. Capecitabine may be administered to a human in a dosage range varying from about 10 to 1000 mg/m^{2}day. Gemcitabine hydrochloride may be administered to a human in a dosage range varying from about 1000 mg/m^2/week. Methotrexate may be administered to a human in a dosage range varying from about 5 to 500 mg/m^{2}day.

Topotecan may be administered to a human in a dosage range varying from about 1 to 5 mg/m^{2}day. Irinotecan may be administered to a human in a dosage range varying from about 50 to 350 mg/m^{2}day.

Carboplatin may be administered to a human in a dosage range varying from about 200 to 400 mg/m^2 about every four weeks. Cisplatin may be administered to a human in a dosage range varying from about 25 to 75 mg/m^2 about every three weeks. Oxaliplatin may be administered to a human in a dosage range varying from about 50 to 85 mg/m^2 every two weeks.

Imatinib may be administered to a human in a dosage in the range of about 2.5 to 850 mg/day, more preferably 5 to 600 mg/day and most preferably 20 to 300 mg/day.

Alendronic acid may be administered to a human in a dosage range varying from about 5 to 10 mg/day. Clodronic acid may be administered to a human e.g. in a dosage range varying from about 750 to 1500 mg/day. Etridonic acid may be administered to a human in a dosage range varying from about 200 to 400 mg/day. Ibandronic acid may be administered to a human in a dosage range varying from about 1 to 4 mg every three to four weeks. Risedronic acid may be administered to a human in a dosage range varying from about 20 to 30 mg/day. Pamidronic acid may be administered to a human in a dosage range varying from about 15 to 90 mg every three to four weeks. Tiludronic acid may be administered to a human in a dosage range varying from about 200 to 400 mg/day.

Trastuzumab may be administered to a human in a dosage range varying from about 1 to 4 mg/m^2/week.

Bicalutamide may be administered to a human in a dosage range varying from about 25 to 50 mg/m^{2}day.

1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or salt thereof, e.g. succinate, may be administered to a human in a dosage range of about 50 to 1500, more preferably about 100 to 750, and most preferably 250 to 500, mg/day.

Rapamycin or a derivative thereof, e.g. 40-O-(2-hydroxyethyl)-rapamycin, may be administered in a dosage range varying from about 0.1 to 25 mg.

Formulation Example: soft capsules

Compound of formula I,	
e.g. Compound A, HCl	30 mg
Polyethylene glycol 300	300 mg
Polysorbate 80	20 mg
Total	350 mg

The S1P receptor agonists, e.g. a S1P receptor agonist comprising a group of formula X, are well tolerated at dosages required for use in accordance with the present invention. For example, the acute LD_{50} for Compound A is > 10 mg/kg p.o. in rats and monkeys.

In a further aspect, the present invention relates to the use of S1P agonists as pro-angiogenic drugs. Induction of neo-angiogenesis has lately been recognized as an excellent target in a number of conditions (e.g.myocardial angiogenesis, wound healing or diabetic vascular dysfunction/vasculopathy).

As described above, high concentrations of S1P receptor agonists (2 μM or greater, e.g. 2-5 μM or around 5 μM) exhibit anti-angiogenic effects, and S1P receptor agonists can inhibit VEGF-induced angiogenesis. In contrast, low concentrations (0.1 -1 μM, e.g. 0.1 – 0.5 μM or 0.5 – 1 μM) of S1P agonists have an enhancing effect on angiogenesis and are able to potentiate VEGF-mediated angiogenesis. Thus, S1P agonists may have biphasic effects in angiogenesis.

Accordingly, the present invention further provides:

8. Use of a S1P agonist, e.g. a S1P agonist comprising a group of formula X, e.g. Compound A or Compound A-phosphate, in the induction of the neo-angiogenesis process,

e.g. as a pro-angionenic agent, e.g. in indications where a promotion of angiogenesis is indicated;

9. A process for the preparation of a medicament for the treatment or prevention of diseases mediated by the inhibition of the neo-angiogenesis process, e.g. mediated by anti-angionenic factors, e.g. in indications where a promotion of angiogenesis is indicated, e.g. in wound healing or in the treatment of myocardial infarction or diabetic vascular dysfunction/vasculopathy, comprising using a S1P receptor agonist, e.g. a S1P agonist comprising a group of formula X, e.g. Compound A or Compound A-phosphate, as an active ingredient;

10. A method of treating or preventing diseases mediated by the inhibition of the neo-angiogenesis process, e.g. mediated by anti-angionenic factors, e.g. in indications where a promotion of angiogenesis is indicated, such as e.g. in wound healing or in the treatment of myocardial infarction or diabetic vascular dysfunction/vasculopathy, comprising administering an effective amount of a S1P receptor agonist, e.g. a S1P agonist comprising a group of formula X, e.g. Compound A or Compound A-phosphate, to a subject in need of such treatment.

S1P agonists suitable for promoting angiogenesis include those defined above in relation to the treatment of cancer, e.g. S1P agonists comprising a group of formula X or compounds according to formulae I to IX, or pharmaceutically acceptable salts or esters thereof. Preferably the S1P agonist is Compound A-phosphate. The S1P agonist may be used alone, or in combination with one or more further agents which promote angiogenesis, e.g. VEGF.

In order to promote angiogenesis it is important to select a sufficiently low dose of the S1P receptor agonist, as high concentrations of S1P receptor agonists inhibit angiogenesis. A suitable dose for providing a pro-angiogenic effect when a S1P agonist is administered to a patient may be selected by concentration- and dose-escalating studies as described at A, B, and C above.

Description of the Figures

Figure 1

shows that Compound A-phosphate strongly promotes capillary-like network formation in a bell-shape dose-dependent manner showing maximal activity around 0.5 µM.

Figure 2

shows that both Compound A-phosphate and Compound A at 0.5 - 1 µM do not attenuate VEGF-mediated remodelling but rather cooperate with polypeptide growth factor.

Figure 3

Shows that Compound A-phosphate as well as S1P-stimulated tube formation is practically completely inhibited by pertussis toxin (PTX, 50 ng/ml), an inhibitor of heterotrimeric G proteins of $\alpha_{i/o}$-type. This may be interpreted as a possible involvement of EDG-1 ($S1P_1$) receptor-mediated signaling events in Compound A-phosphate-stimulated bioresponses.

Figure 4

Shows, that sphingosine at 1 µM, which itself seems to be less potent than S1P, attenuates the ability of both S1P and Compound A-phosphate to induce capillary-like structures, without having an inhibitory effect on the VEGF-induced tube formation. In this respect, sphingosine behaves different from Compound A. The data indicate that the balance between sphingosine and S1P seems to be critically important for endothelial cell activation/angiogenesis most likely via the EDG receptor family. Importantly, high concentrations of sphingosine and Compound A (2 – 5 µM) inhibits VEGF-triggered tube formation.

Figure 5

Shows that the treatment of HUVEC with Compound A-phosphate at 0.5 µM may result in transient activation of ERK1/2 with a peak of phosphorylation/activation at 10 minutes and returning to baseline by 20 minutes

Figure 6

It was tested whether Compound A, Compound A-phosphate, sphingosine or S1P also do induce tissue factor on HUVEC. The data found demonstrate that none of these compounds alone or in combinations may elevate tissue factor activity as shown in Figure 6. Compound A and Compound A-phosphate may slightly enhance the VEGF- but not TNF-α-induced tissue factor.

Figure 7

Shows the effect of Compound C in a S1P-mediated HUVEC tube formation assay.

The following abbreviations are used:

BSA: bovine serum albumine

ECGS: endothelial cell growth factor set

ECL: enhanced chemiluminescence

S: sphingosine

PBS: phosphate-buffered saline

JNK1/2: c-jun-N-terminal kinase1/2

RT: room temperature

TF equivalents: tissue factor equivalents

EGR-1/NFAT: early growth response protein 1/nuclear factor of activated T-cells

F1P: Compound A-phosphate (FTY720-phosphate)

Utility of the S1P receptor agonists, e.g. the S1P agonists comprising a group of formula X, in the promotion of angiogenesis may be demonstrated for example in accordance with the methods described hereinafter.

D. Cell culture and Materials

Human umbilical vein endothelial cells (HUVEC) are cultured at 37°C and 5% CO_2 in medium M199 supplemented with 20% SCS (HyClone, Logan, UT), 1U/ml heparin, 50 µg/ml ECGS, 2 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin. Cells are used for experiments up to passage number 5. Short-starved HUVEC are obtained by starving with 1% SCS-containing M199 for 5 h. Recombinant human $VEGF_{165}$ is obtained from PromoCell (Heidelberg, Germany). Phospho-specific ERK1/2, p38 kinase polyclonal antibodies, nonphospho ERK1/2 antibodies and LumiGLO chemiluminescent reagent are from New England BioLabs (Beverly, MA), polyclonal IκB antibodies from Santa Cruz Biotechnology (Santa Cruz, Calif.). Peroxidase-conjugated donkey anti-rabbit immunoglobulin G (IgG) and sheep anti-mouse IgG are purchased from Amersham LIFE SCIENCE (Amersham Place, England). Immobilon-P transfer membranes are products of Millipore (Bedford, MA). S is obtained from Sigma Chemical Co.; S1P is from Biomol. Compound A-phosphate stock solution is prepared by the following protocol. Compound A-phosphate is dissolved in methanol tracing with concentrated HCl (0.5 mg Compound A-phosphate in 500 µl of methanol plus 2 µl of HCl). Solvent from the resulting solution is evaporated under vacuum and the residue obtained is redissolved (variant 1) in 0.1 % of defatted BSA solution in sterile deionized water (500 µl) or (variant 2) in 0.5 % Triton X-100 in deionized water. The resulting stock solutions (2.5 mM) are sonicated and stored at 4°C.

Clotting Assay

Cells are seeded in 6-well plates at 80-90% confluency and grown overnight. Cells are scraped from the plates and analyzed for tissue factor activity according to the method as described in Clauss, M., J. Biol. Chem. 271,17629-17634 (1996), Mechtcheriakova, D., Blood 93,3811-3823 (1999). Briefly, after induction for 4 hours with VEGF (1.5 nM), TNF-α (100 U/ml), S (0.5-2 µM), S1P (0.5-2 µM), Compound A (0.5-2 µM), and Compound A-phosphate (0.5-2 µM), cells are washed twice and then scraped in 1ml clotting buffer (12 mM sodium acetate, 7 mM diethylbarbitate and 130 mM sodium chloride; pH 7.4). 50 µl of resuspended cells are mixed with 50 µl of citrated plasma, and clotting times are determined after recalcification with 50 µl of 20 mM $CaCl_2$ solution at 37°C. TF-equivalents are determined by using a standard curve obtained from rabbit brain thromboplastin.

E. Western Blot Analysis

After various treatments, the cells are washed twice with cold PBS, lysed in 100 µl of Laemmli buffer, scraped and heated for 5 min at 95°C. Total cell lysates are separated by SDS-PAGE and transferred to Immobilon-P membrane. The membrane is blocked for 30 minutes with PBS containing 0.1% Tween-20 and 3% skim milk and incubated for 1 hour at RT with a primary antibody diluted in blocking buffer. The membrane obtained is washed three times for 5 minutes with PBS containing 0.1% Tween-20 and incubated with peroxidase-conjugated secondary antibody for 1 hour at RT. After a washing step, the membrane is incubated for 1 minute with ECL reagent and exposed to film as required. For reprobing with another antibody, the membrane is washed twice in PBS, stripped for 30 min at 55°C with stripping buffer (62.5 mM Tris-HCL, pH 6.8, 2% SDS, 100 mM 2-mercaptoethanol) and washed three times for 5 minutes with PBS at RT. The membrane is stored wet wrapped in SaranWrap at 4°C after each immunodetection.

In vitro angiogenesis assay on Matrigel

Morphogenesis of endothelial cells into capillary-like structures on Growth Factor Reduced Matrigel Matrix (BD Bioscience) is performed according to the manufacture procedure. Briefly, HUVEC are trypsinized, resuspended in serum-free M199 medium containing soybean trypsin inhibitor (1mg/ml, Sigma). After centrifugation cells are resuspended in serum-free medium at a density 0.5 x 10^5 cells/ml, and cell suspension is seeded in 96-well cell culture plates (Costar, Corning Incorporated) precoated with 50 µl of Matrigel in the absence or presence of various stimuli: VEGF at 1.5 nM, S1P at 0.1-2 µM, S at 0.5-2 µM, Compound A at 0.5-2 µM, and Compound A-phosphate at 0.1-2 µM. Eight hours later, cells

on Matrigel are fixed with 3 % formaldehyde in PBS and kept at 4°C. Results are quantitated from images made with a Nikon Diaphot microscope equipped with a cooled CCD camera (Kappa GmbH, Gleichen, Germany) by direct counting of branching points on two microscopic fields from each well done in duplicates.

F. Compound A-phosphate-induces morphogenesis of endothelial cells in in vitro tube formation assay on Matrigel and a possible involvment of G_i-mediated signaling pathway(s)

The effect of Compound A and Compound A-phosphate on morphogenic differentiation of endothelial cells is determined using an in vitro angiogenesis assay on Matrigel. Endothelial cell morphogenesis is a complex process that requires cell-extracellular matrix interactions, followed by matrix remodelling, stimulated migration, cell-cell interactions, and perivascular proteolysis. As shown in Figure 1, Compound A-phosphate strongly may promote capillary-like network formation in a bell-shape dose-dependent manner showing maximal activity around 0.5 µM. The number of branching points per microscopic field, which reflects the induction potency of the stimulus, is comparable for Compound A-phosphate and S1P, and may exceed significantly the VEGF-triggered effects. Compound A itself at 0.5 - 1 µM has a weak, in comparison to Compound A-phosphate, but consistent enhancing effect. Both Compound A-phosphate and Compound A at 0.5 - 1 µM does not attenuate VEGF-mediated remodelling but rather cooperates with polypeptide growth factor (see e.g. Figure 2). Furthermore, Compound A-phosphate- as well as S1P-stimulated tube formation is completely inhibited by pertussis toxin (PTX, 50 ng/ml), an inhibitor of heterotrimeric G proteins of $\alpha_{i/o}$-type. This may be interpreted as a possible involvement of EDG-1 ($S1P_1$) receptor-mediated signaling events in Compound A-phosphate-stimulated bioresponses (see e.g. Figure 3). S at 1 µM, which itself seems to be less potent than S1P, attenuates the ability of both S1P and Compound A-phosphate to induce capillary-like structures, without having an inhibitory effect on the VEGF-induced tube formation (see e.g. Figure 4). In this respect, S behaves differently from Compound A. The data indicate that the balance between S and S1P seems to be critically important for endothelial cell activation/angiogenesis most likely via the EDG receptor family. Importantly, high concentrations of S and Compound A (2 – 5 µM) inhibited VEGF-triggered tube formation. That data suggest biphasic dose-dependent effects of Compound A and Compound A-phosphate on angiogenesis in vitro.

G. Activation of ERK1/2 MAP kinases by Compound A-phosphate

Signal transduction via MAP kinases plays a key role in a variety of endothelial cell functions. Treatment of HUVEC with Compound A-phosphate at 0.5 µM may result in transient activation of ERK1/2 with a peak of phosphorylation/activation at 10 minutes and returning to baseline by 20 minutes (see e.g. Figure 5). No activation of p38 kinase and JNK1/2 by Compound A-phosphate is detectable in HUVEC. Furthermore, Compound A-phosphate may trigger ERK1/2 activation in a dose-dependent manner, showing stronger activity at 2 µM. This is in contrast to the results from the tube formation assay, where Compound A-phosphate at 2 µM may be less potent than at 0.5 µM. Neither Compound A nor S are able to induce MAP kinase activation in endothelial cells in a kinetics ranging from 5 minute to 60 minute treatment. To estimate the possible role of inflammatory/NFκB-dependent program in Compound A-phosphate-stimulated bioresponses of endothelial cells, the membranes are reprobed with anti-IκB antibodies. IκB levels are not affected by Compound A-phosphate treatment. Moreover the treatment of endothelial cells with Compound A-phosphate may fail to induce E-Selectin expression as a NFκB-dependent secondary responsive gene. Thus, the data strongly indicate that Compound A-phosphate signaling does not involve NFκB activation – the main cascade in the acute inflammatory response in endothelial cells.

H. Compound A and Compound A-phosphate do not induce tissue factor expression on endothelial cells

An important characteristic feature of both classical inflammatory stimulus TNF-α and the main angiogenic growth factor VEGF on endothelial cells is their potency to upregulate tissue factor. Compound A, Compound A-phosphate, S or S1P are tested whether they also do induce tissue factor on HUVEC. The data found demonstrate that none of these compounds alone or in combinations may elevate tissue factor activity (see e.g. Figure 6). Compound A and Compound A-phosphate may slightly enhance the VEGF- but not TNF-α-induced tissue factor. The data obtained together indicate that Compound A, Compound A-phosphate, S and S1P mechanistically work distinctly to angiogenic VEGF and inflammatory TNF-α.

I. Binding affinity of S1P receptor agonists to individual human S1P receptors may be determined in following assays:

Transient transfection of human S1P receptors into HEK293 cells

EDG receptors and G_i proteins are cloned, and equal amounts of 4 cDNAs for the EDG receptor, G_i-α, G_i-β and G_i-γ are mixed and used to transfect monolayers of HEK293 cells using the calcium phosphate precipitate method (M. Wigler et al., Cell. 1977;11;223 and DS.

Im et al., Mol. Pharmacol. 2000;57;753). Briefly, a DNA mixture containing 25 µg of DNA and 0.25 M CaCl is added to HEPES-buffered 2 mM Na_2HPO_4. Subconfluent monolayers of HEK293 cells are poisoned with 25 mM chloroquine, and the DNA precipitate is then applied to the cells. After 4 h, the monolayers are washed with phosphate-buffered saline and refed media (90% 1:1 Dulbecco's modified essential media (DMEM):F-12 + 10% fetal bovine serum). The cells are harvested 48-72 h after addition of the DNA by scraping in HME buffer (in mM: 20 HEPES, 5 $MgCl_2$, 1 EDTA, pH 7.4) containing 10% sucrose on ice, and disrupted using a Dounce homogenizer. After centrifugation at 800xg, the supernatant is diluted with HME without sucrose and centrifuged at 100,000xg for 1h. The resulting pellet is rehomogenized and centrifuged a second hour at 100,000xg. This crude membrane pellet is resuspended in HME with sucrose, aliquoted, and snap-frozen by immersion in liquid nitrogen. The membranes are stored at 70°C. Protein concentration is determined spectroscopically by Bradford protein assay.

GTPγS binding assay using S1P receptor/HEK293 membrane preparations

GTPγS binding experiments are performed as described by DS. Im et al., Mol. Pharmacol. 2000; 57:753. Ligand-mediated GTPγS binding to G-proteins is measured in GTP binding buffer (in mM: 50 HEPES, 100 NaCl, 10 $MgCl_2$, pH 7.5) using 25 µg of a membrane preparation from transiently transfected HEK293 cells. Ligand is added to membranes in the presence of 10 µM GDP and 0.1 nM [^{35}S]GTPγS (1200 Ci/mmol) and incubated at 30°C for 30 min. Bound GTPγS is separated from unbound using the Brandel harvester (Gaithersburg, MD) and counted with a liquid scintillation counter.

Claims

1. A method for treating solid tumors or inhibiting growth of solid tumors in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist, with the proviso that when the S1P receptor agonist is FTY720 or FTY720-phosphate, the tumor is other than breast, prostate, bladder, kidney or lung tumor.

2. A method for treating solid tumor invasiveness or symptoms associated with such tumor growth, preventing metastatic spread of tumours or for preventing or inhibiting growth of micrometastasis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist.

3. A method for inhibiting or controlling deregulated angiogenesis, e.g. sphingosine-1-phosphate (S1P) mediated angiogenesis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist.

4. A method for preventing or treating diseases mediated by a neo-angiogenesis process or associated with deregulated angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist.

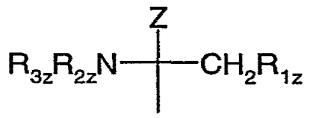
5. A method for enhancing the activity of a chemotherapeutic agent or for overcoming resistance to a chemotherapeutic agent in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist, either concomitantly or sequentially with said chemotherapeutic agent.

6. A method according to any preceding claim wherein the S1P receptor agonist is administered intermittently.

7. A method according to any preceding claim comprising co-administration, concomitantly or in sequence, of a therapeutically effective amount of a S1P receptor agonist and a second drug substance, said second drug substance being a chemotherapeutic agent.

8. A method for treating lymphoproliferative or myeloproliferative disorders comprising co-administering to said subject, concomitantly or in sequence, of a S1P receptor agonist, and a second drug substance, said second drug substance being a chemotherapeutic agent.

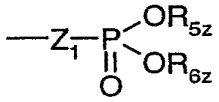
9. A method according to any preceding claim, wherein the S1P receptor agonist comprises a group of formula X:



X

wherein

Z is H; C_{1-6}alkyl; C_{2-6}alkenyl; C_{2-6}alkynyl; phenyl; phenyl substituted by OH; C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH; or CH_2-R_{4z} wherein R_{4z} is OH, acyloxy or a residue of formula (a)



(a)

wherein Z_1 is a direct bond or O, preferably O; each of R_{5z} and R_{6z}, independently, is H, or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms;

R_{1z} is OH, acyloxy or a residue of formula (a); and each of R_{2z} and R_{3z}, independently, is H, C_{1-4}alkyl or acyl.

10. A pharmaceutical combination comprising a) a first agent which is a S1P receptor agonist and b) a co-agent which is a chemotherapeutic agent.

11. A combination according to claim 10, wherein the co-agent is selected from

i. an aromatase inhibitor,
ii. an antiestrogen, an anti-androgen or a gonadorelin agonist,
iii. a topoisomerase I inhibitor or a topoisomerase II inhibitor,
iv. a microtubule active agent, an alkylating agent, an antineoplastic antimetabolite or a platin compound,
v. a compound targeting/decreasing a protein or lipid kinase activity or a protein or lipid phosphatase activity, a further anti-angiogenic compound or a compound which induces cell differentiation processes,
vi. a bradykinin 1 receptor or an angiotensin II antagonist,
vii. a cyclooxygenase inhibitor, a bisphosphonate, a histone deacetylase inhibitor, a heparanase inhibitor, a biological response modifier, an ubiquitination inhibitor, or an inhibitor which blocks anti-apoptotic pathways,
viii. an inhibitor of Ras oncogenic isoforms,
ix. a telomerase inhibitor,

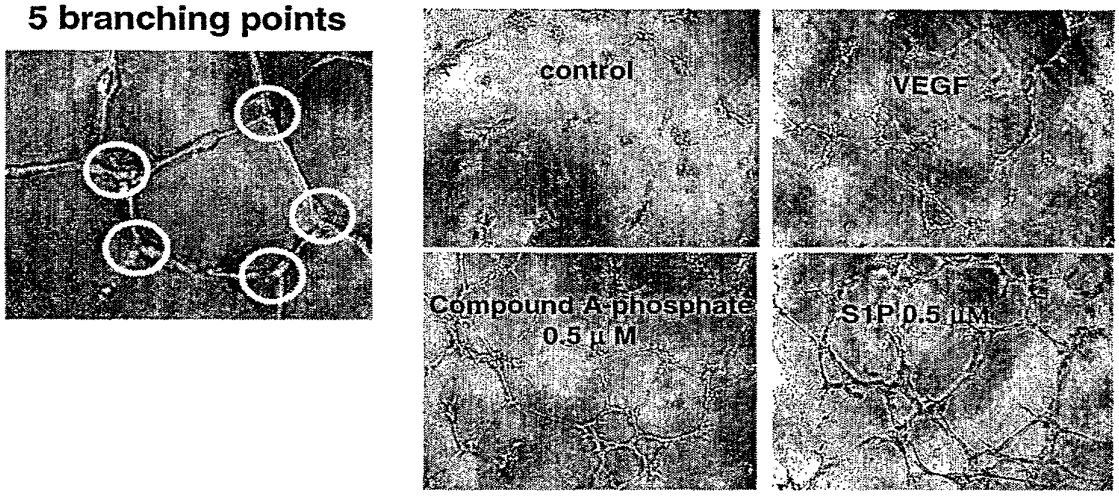
x. a protease inhibitor, a matrix metalloproteinase inhibitor, a methionine aminopeptidase inhibitor, or a proteosome inhibitor, and/or

xi) a mTOR inhibitor.

12. A method of treating or preventing diseases mediated by inhibition of the neo-angiogenesis process comprising administering an effective amount of an S1P receptor agonist to a subject in need of such treatment.

13. A method according to claim 12, wherein the S1P receptor agonist comprises a group of formula X as defined in claim 9.

Fig. 1


5 branching points
control
VEGF
Compound A-phosphate 0.5 µM
S1P 0.5 µM

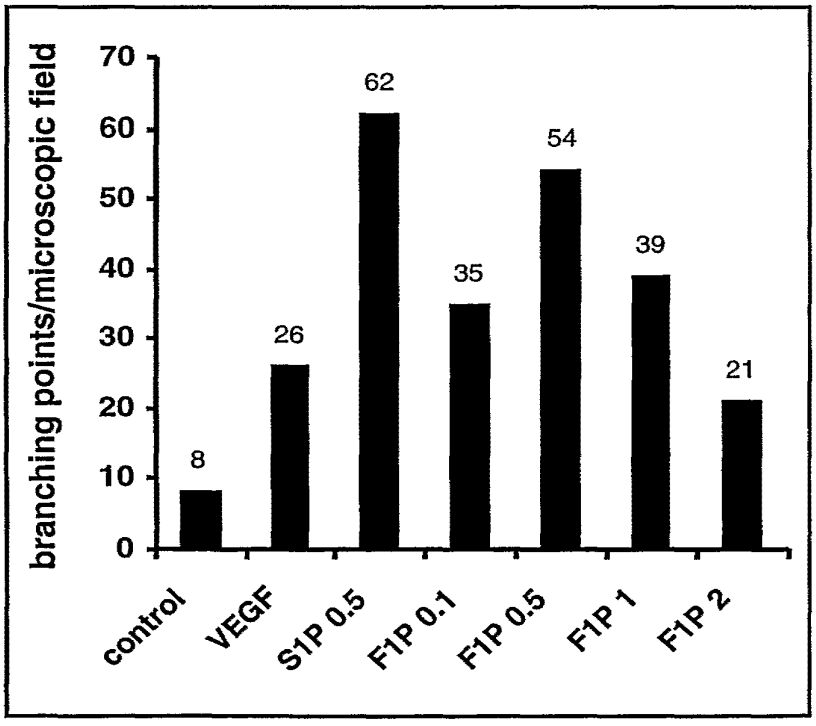

branching points/microscopic field
0
10
20
30
40
50
60
70
8
26
62
35
54
39
21
control
VEGF
S1P 0.5
F1P 0.1
F1P 0.5
F1P 1
F1P 2

Fig. 2

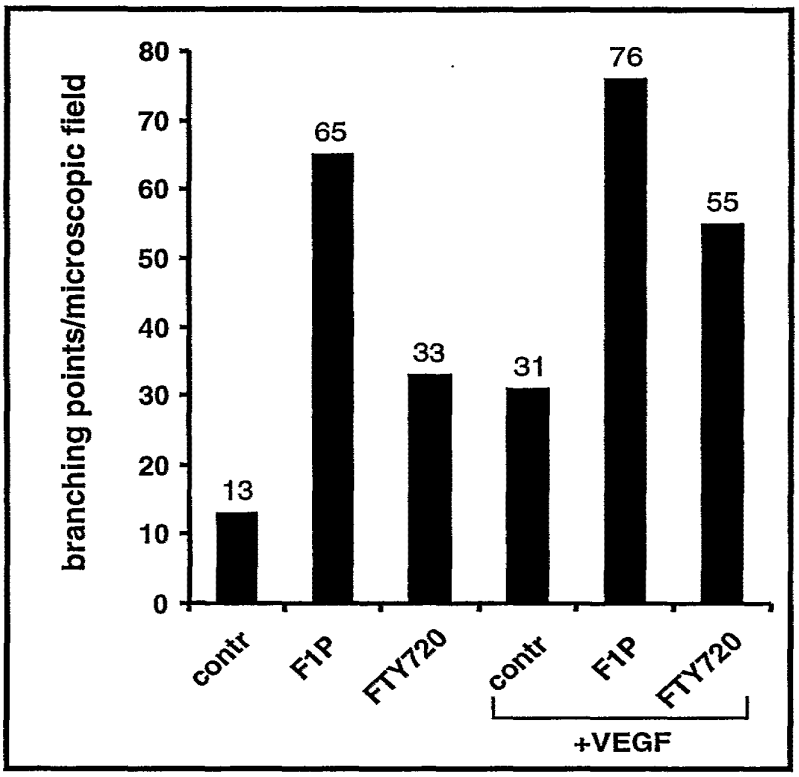

branching points/microscopic field
80
70
60
50
40
30
20
10
0
13
65
33
31
76
55
contr
F1P
FTY720
contr
F1P
FTY720
+VEGF

Fig 3

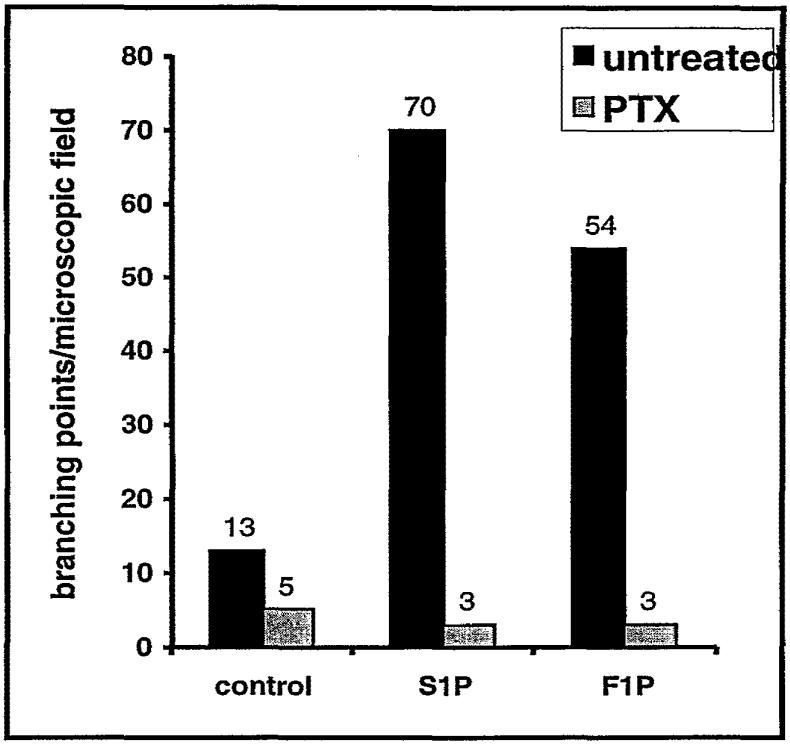

branching points/microscopic field
80
70
60
50
40
30
20
10
0
untreated
PTX
13
5
70
3
54
3
control
S1P
F1P

Fig 4

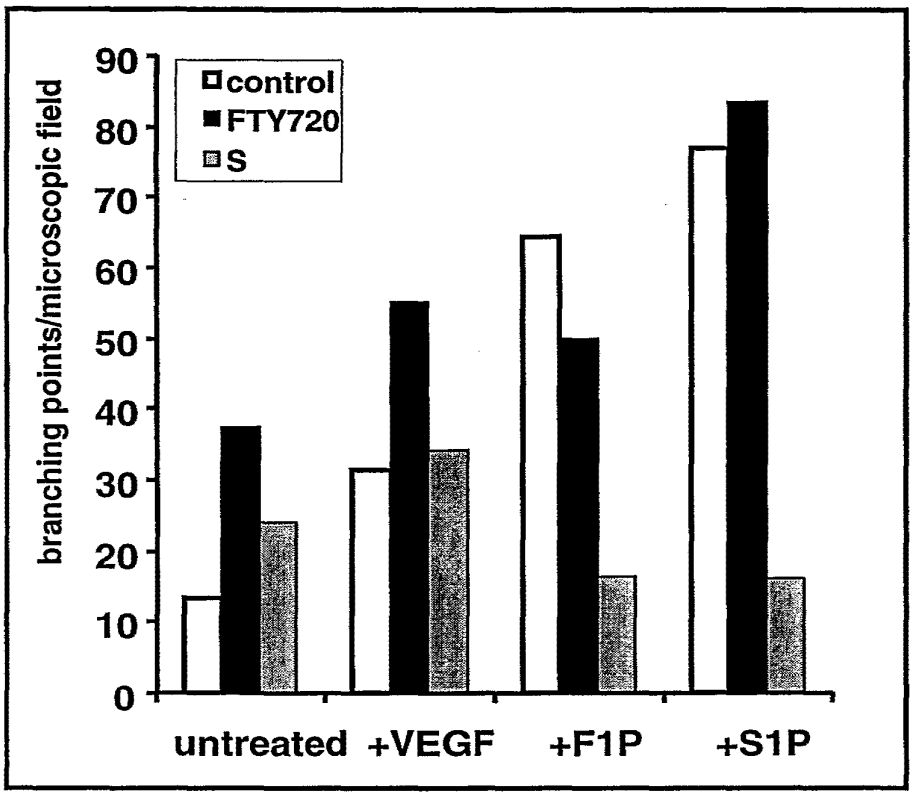

branching points/microscopic field
90
80
70
60
50
40
30
20
10
0
control
FTY720
S
untreated
+VEGF
+F1P
+S1P

Fig 5

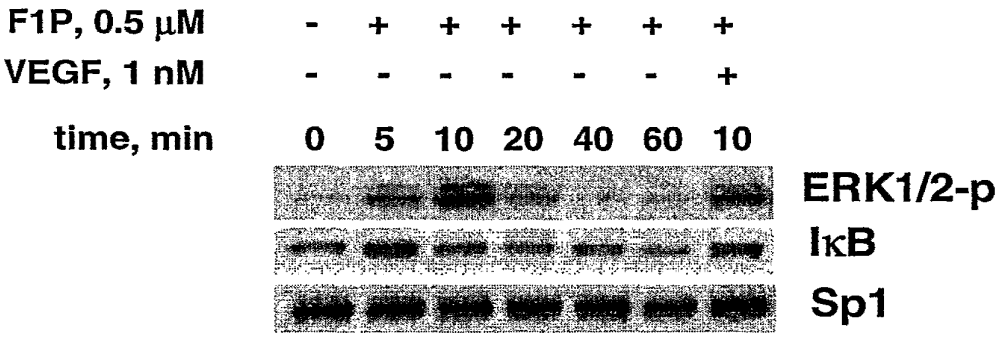

F1P, 0.5 µM - + + + + + +
VEGF, 1 nM - - - - - - +
time, min 0 5 10 20 40 60 10
ERK1/2-p
IκB
Sp1

Fig 6

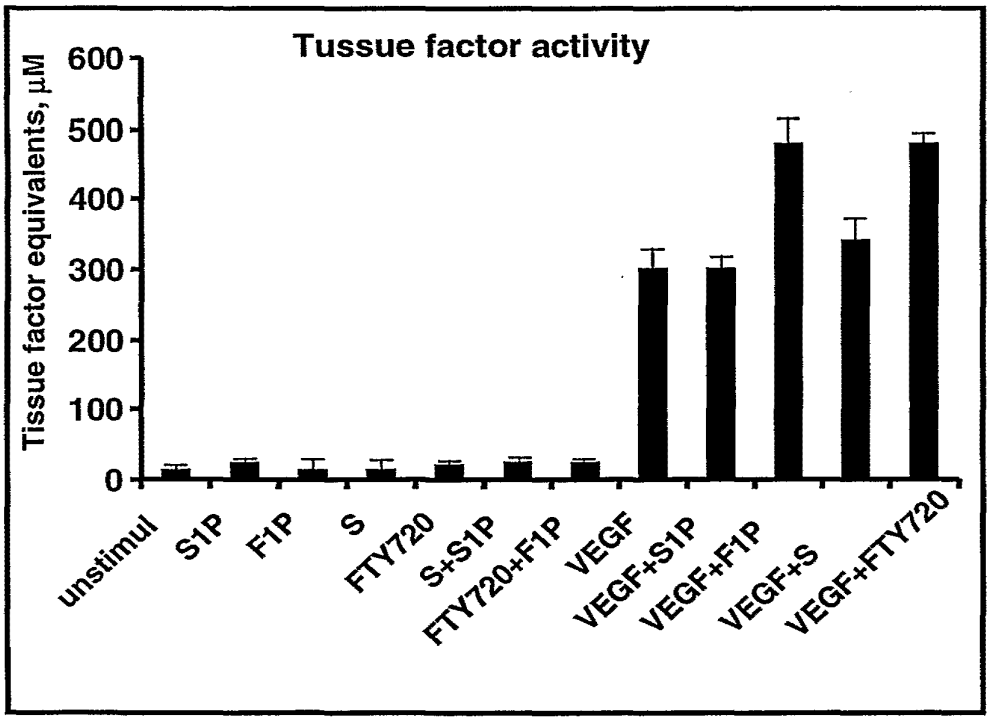
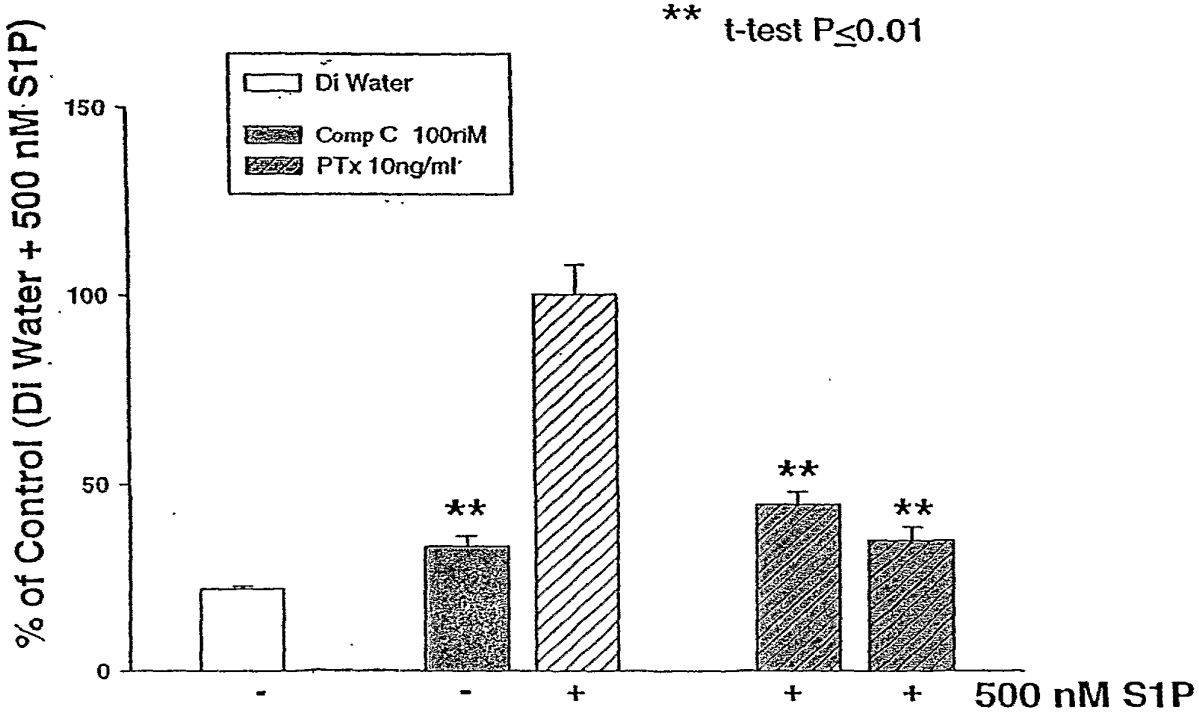

Tussue factor activity
Tissue factor equivalents, µM
600
500
400
300
200
100
0
unstimul
S1P
F1P
S
FTY720
S+S1P
FTY720+F1P
VEGF
VEGF+S1P
VEGF+F1P
VEGF+S
VEGF+FTY720

Fig 7


** t-test P≤0.01
Di Water
Comp C 100nM
PTx 10ng/ml
% of Control (Di Water + 500 nM S1P)
150
100
50
0
**
**
**
-
-
+
+
+
500 nM S1P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/05125

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/135 A61K31/381 A61P35/00

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 7 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)
EPO-Internal, PAJ, WPI Data, CHEM ABS Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STEPKOWSKI, STANISLAW M.: "Molecular targets for existing and novel immunosuppressive drugs" INTERNET, 'Online! 21 June 2000 (2000-06-21), XP002254088 Retrieved from the Internet: <URL:http://www-ermm.cbcu.cam.ac.uk/000017 69a.pdf> 'retrieved on 2003-09-09! ---	
A	EP 1 195 165 A (ONO PHARMACEUTICAL CO) 10 April 2002 (2002-04-10) ---	

-/--

[X] Further documents are listed in the continuation of box C.

[X] 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
15 September 2003	26/09/2003

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	Stienon, P

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	SOMPOL PERMPONGKOSOL ET ALL.: "Anticarcinogenic effect of FTY720 in human prostate carcinoma DU145 cells: modulation of mitogenic signaling, FAK, cell-cycle entry and apoptosis" INT.J.CANCER, vol. 98, 10 March 2002 (2002-03-10), pages 167-172, XP002254561 ---	
A	SUSAN PYNE ET ALL.: "SPHINGOSINE 1-PHOSPHATE SIGNALLING VIA THE ENDOTHELIAL DIFFERENTIATION GENE FAMILY OF G-PROTEIN-COUPLED RECEPTORS" PHARMACOLOGY & THERAPEUTICS, vol. 88, 2000, pages 115-131, XP002254562 ---	
A	HARUHITO AZUMA ET ALL.: "Marked Prevention of Tumor Growth and Metastasis by a Novel Immunosuppressive Agent, FTY720, in Mouse Breast" CANCER RESEARCH, vol. 62, 1 March 2002 (2002-03-01), pages 1410-1419, XP002254563 ------	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/05125

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1195165 A	10-04-2002	EP 1195165 A1	10-04-2002
		WO 0103739 A1	18-01-2001

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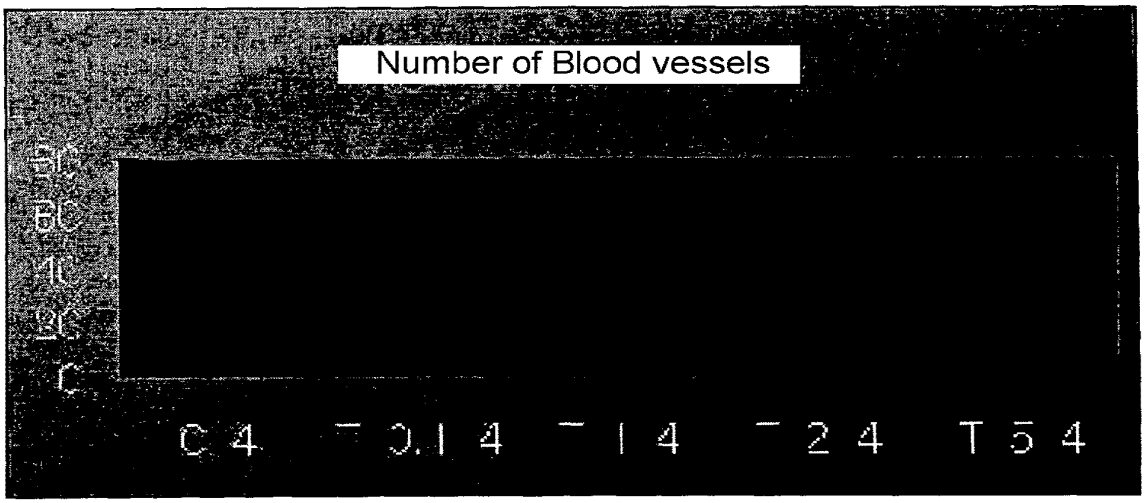
(54) Title: INHIBITOR OF ANGIOGENESIS AND KIT FOR TREATING CANCER COMPRISING THE INHIBITOR



(57) Abstract: The inhibitor of angiogenesis is characterized by containing tetraacetylphytosphingosine and the kit for inhibiting angiogenesis is characterized by the inhibitor. The inhibitor and kit are effective in treating and preventing disorders in connection with extremely increased angiogenesis such as angioma, tumor and psoriasis by effectively inhibiting angiogenesis. Furthermore, they are effective in inhibiting proliferation of cancer cells and metastasis of cancer without any adverse effect.

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INHIBITOR OF ANGIOGENESIS AND KIT FOR TREATING CANCER COMPRISING THE INHIBITOR

TECHNICAL FIELD

The present invention relates to the inhibitor of angiogenesis comprising tetraacethylphytosphingosine derivatives and the kit for treating cancer comprising the inhibitor.

BACKGROUND ART

A process called angiogenesis which was started to be researched with observation of new formation of blood vessels in placenta in 1935 was found in various fields such as nidation, developing child in a mother's womb, wound healing, menstruation for women, arthritis, diabetic retinopathy or the like. As accumulating evidences have been found that there are many blood vessels and frequent bleeding around cancer cells and angiogenesis plays key roles in development of cancer and the growth and metastasis of cancer cells, various researches on the substances which inhibit angiogenesis are under progression. As a results of full-scale researches on angiogenesis which was carried out from the 1960s, the fact that the cause of the rapid proliferation of cancer cells is angiogenesis was found, and angiogenic factors started to be found in 1980s. A variety of angiogenesis inhibition factors and angiogenesis inhibitors were found in 1990s, and their potentiality as an inhibitor of cancer cell proliferation was expanded drastically. Nowadays, many anti-angiogenic strategies are being evaluated in clinical trials. These approaches offer new hope for the successful treatment of cancer.

For develpomant and proliferation of cancer cells, first growth phase related to tumor growth factors is observed. In this phase, various tumor growth factors and blood vessels formation factors are expressed significantly, and cancer cells and new blood vessels are formed. Following the growth phase, a phase that infiltration of cancer cells occurs starts. An disequilibrium between proteinases which digest

extracellular matrix and basement membrane, and inhibitory substances of proteinases occurs in this phase, wherein proteinases such as MMP-2 (matrixmetalloproteinase-2), MMP-9 (matrixmetalloproteinase-9), uPA (urokinase type plasminogen activator) or the like increas, whereas PAI-1 (plasminogen activator inhibitor-1), TIMP (tissue inhibitor of metalloproteinase) or the like involved in inhibition of proteinases decrease. Finally, in a phase that metastasis of cancer cells occurs, activity of cell adhesion molecule increases, thereby increasing adhesiveness of cells and metastasis of cancer cells occurs completely. Since such changed biological activities occurred in development and metastasis of cancer can be regulated by each of specific inhibitors, various studies on biological treatments based on above concepts are set about actively, and improvements of treatment effect through selective treatment are expected.

First, infiltration of cancer cells and angiogenesis require proteinases. Cancer cells, fibroblasts and endothelial cells produce proteinases and decompose extracellular matrix and basement membrane to give rise to infiltration of cancer cells and angiogenesis. Proteinases involved in such process include serine protease and MMPs(metrixmetalloproteinase). In process that such proteases decompose extracellular matrix, uPA(urokinase type plasminogen activator) converts plasminogen into plasmin to destroy fibrin, fibronectin, proteoglycan, laminin around cancer cells, and activates collagenase to decompose collagen. However, since uPA is inhibited by PAI-1(plasminogen activator inhibitor-1), PAI-1 is expected to control abilities of angiogenesis and metastasis of cancer cells.

In cancer cells, MMP-2 and MMP-9 are primarily activated. MMP-2 is activated by MMP presented in cell membrane of cancer cells, whereas MMP-9 is inhibited by TIMP. Therefore, recently it is expected that controlling the disequilibrium of MMP and TIMP is used effectively to inhibit formation of blood vessels, metastasis and infiltration, and thus it is suggested as a novel treatment concept.

When cancer cells are not supplied with nutrients through the formation of blood vessels, their growth is limited. Also, newly formed blood vessels are major

network for metastasis in addition to channel for suppliment of nutrients. The relationship between the degree of tumor angiogenesis and metastasis was well known, and it is established in various cancer cells that capillaries density of in situ tumor plays a key role in expectation of prognosis and metastasis of cancer. Recently, since monoclonal antibodies directed to angiogenic factors were developed, the degree of angiogenesis could be determined by measuring directly of angiogenic factors such as bFGF, VEGF, TGF-b or the like.

The concepts of cancer treatment through inhibition of angiogenesis are that correction of biological changes which are disturbed due to cancer as compared cancer cell with normal cell is to prevent differentiation, proliferation and metastasis of cancer cell, and as a result to block growth of cancer. Based on such concepts, MMP inhibitors have been synthesized and used for treating cancer since 1980s. However, according to the results of clinical tests, the effect of MMP inhibitors is far below what are expected. This is because the clinical tests are carried out on the subjects of which cancer has been already metastasized.

Angiogenesis is essential for proliferation and metastasis of tumor cells. Also, newly formed blood vessels are major network of metastasis of cancer cells. A proliferation and a migration of endothelial cells are events that occurred frequently in only cancer cells, except for wound healing for adults and menstruation for women. Consequently, angiogenesis that occurs in cancer tissues rather than normal tissues is a very selective target of cancer treatment, and in theory such treatment is considered to have little adverse effect and can be used together with other cancer treatments, thereby increasing effect of the treatment. As a result, substances to inhibit effectively angiogenesis can be used effectively to treat disorders in connection with extremely increased angiogenesis such as angioma, tumor and psoriasis.

DISCLOSURE OF THE INVENTION

An object of the present invention is treating and preventing disorders in connection with extremely increased angiogenesis such as angioma, tumor and psoriasis by effectively inhibiting angiogenesis. Furthermore, other object of the present invention is providing a pharmaceutical composition to inhibit proliferation of and metastasis of cancer cells effectively without any adverse effect.

To achieve above objects, the inhibitor of angiogenesis of the present invention is characterized by containing tetraacetylphytosphingosine.

Also, the kit for treating cancer of the present invention is characterized by comprising the inhibitor of angiogenesis containing tetraacetylphytosphingosinethe.

The kit for treating cancer is characterized by further comprising anti-cancer drug and irradiator.

For the kit for treating cancer, the anti-cancer drug is characterized by spingolipid derivatives.

For the kit for treating cancer, the spingolipid derivatives are one or more spingolipid derivatives selected from the group consisting of phytosphingosine, enacethylphytosphingosine, C6 phytoceramide, C8 phytosphingosine, dimethylsphingosine, dimethylphytosphingosine and sphingosine.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

FIG. 1 is a graph representing the number of blood vessels determined on 4 days after treatment of 0.1 μM, 1 μM, 2 μM and 5 μM of a solution containing tetraacetylphytosphingosine according to the present invention respectively in comparison with negative control.

FIG. 2 is a graph representing the area of granulation tissue determined on 4

days after treatment of 0.1 μM, 1 μM, 2 μM and 5 μM of a solution containing tetraacetylphytosphingosine according to the present invention respectively in comparison with negative control.

FIG. 3 is a graph representing a result of toxicity test of a solution containing tetraacetylphytosphingosine according to the present invention on HUVEC cell.

FIG. 4 is a graph representing a result of angiognesis test of a solution containing tetraacetylphytosphingosine according to the present invention.

FIG. 5 is photographes representing a result of angiognesis test of a solution containing tetraacetylphytosphingosine according to the present invention.

FIG. 6 is photographes representing that a solution containing tetraacetylphytosphingosine according to the present invention inhibited migration of endothelial cells.

FIG. 7 is a graph representing that a solution containing tetraacetylphytosphingosine according to the present invention inhibited migration of endothelial cells.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention will now be described in more detail.

the inhibitor of angiogenesis and the kit for treating cancer comprising the same according to the present invention are described below in detail.

A composition according to the present invention comprises sphingolipid to inhibit angiogenesis very effectively. In particular, We found that acethylated derivatives of phytosphingosine, tetraacetylphytosphingosine inhibits angiogenesis and migration of HUVEC(Human Umbilical Vein Endothelial Cell) cell line strongly and it is effective in treating disorders in connection with extremely increased angiogenesis such as angioma, tumor and psoriasis by effectively inhibiting angiogenesis.

Angiogenesis in malignant tumor plays key roles that supply cancer cell with nutrients to allow cancer cell to grow rapidly, concurrently that function as migration

network for metastasizing cancer cell to another tissues and organs. Meanwhile, A number of newly formed blood vessels are observed in hyperkeratosis region of skin diseases such as psoriasis. In this respect, it is believed that an inhibitor of angiogenesis inhibits the growth of cancer cells and prevents the metastasis of cancer cells as well as has little adverse effect. Therefore, because it is expected that a inhibitor of angiogenesis increases the effect of cancer treatment significantly, many studies have been focused on a inhibitor of angiogenesis. Also, it is expected that when a inhibitor of angiogenesis is used in conjunction with various anticancer treatments in patients who have obvious goal of a treatment and is expected to take the effect of the treatment, the effect of treatment will be excellent.

Sphingolipid is well known as sunstances involved in signal transduction in cell and play an important role in proliferation, differentiation and apoptosis or programmed cell death of cell. Ceramide is a kind of sphingolipid, and is sphingosine-based signaling molecules that fatty acid is linked to sphingosine backbone. ceramide is known as 2nd messenger involved in stress signaling, senescence, cell death, etc. Ceramide is generated from hydrolysis of membrane sphingomyelin that enriched in the brain on receiving the signal from TNF-alpha, Fas, etc. and determine the destination of cell. Sphingomyelin is converted into ceramide by sphingomyelinase, and subsequently it is converted into sphingosine by removing the fatty acid from ceramide by ceramidase. Sphingosine is converted into sphingosine-1-phosphate by sphingosine kinase, and subsequently it is decomposed by lyase. It is known that ceramide and long chain base of sphingosine give rise to apoptosis, and sphingosine-1-phosphate that phosphate group is linked to sphingosine make a function to stimulate cell growth and cell proliferation. Since the balance of sphingosine and phosphated sphingosine within cell is involved in cell growth, cell proliferation and cell death, the change of sphingolipid concentration affects cell lethally. It is well known that most anticancer drugs have a mechanism that causes to apoptosis by increasing the amount of ceramide within cell. Also, It is known that since cancer cells have a different

metabolic pathway of ceramide as compared with normal cells, and thus the concentration of ceramide of normal cells is lower than that of cancer cell, apoptosis is not occur in cancer cells, and cancer cells can grow and proliferate rapidly. it has been found in various studied that ceramide and long chain bases of shhingosine cause cancer cells to apoptosis. Recently, It was found that various derivatives of phytosphingosine that is sphingolipid and is produced in yeast causes cancer cells to apoptosis.

Many studies have been performing which attempt to give rise to apoptosis of cancer cell by regulating the metabolism of ceramide. First, various chemotherapies and radition therapies which are carried out on cancer cells cause to formation of ceramide within cancer cells and as a result give rise to apoptosis of cancer cells. Also, other approach which gives rise to apoptosis of cancer cell is direct treatment of ceramide and long chain bases of sphingolipid to cancer cells. Further, still other approach which can be used effectively is to block the decomposition of ceramide by administering ceramidase inhibitor for preventing the decrease of ceramide contents, or to block conversion of sphingosine into sphingosine-1-phosphate by administering sphingosinekinase inhibitor such as DMS(dimethylsphingosine) for inhibiting the growth of cancer cells.

Sphingosine-1-phosphate is in large amount in blood and plays a role that transduces signals of various foreign responses. It is known that when blood vessels are injured due to wound, sphingosine-1-phosphate in blood that stimulates formation and migration of endothelial cells is secreted in large amount to heal wound rapidly. Also, it is a well known fact that SPC(sphingosinephosphorylcholine) which is shpingosine derivatives has a excellent effect on wound healing.

The effect of long chain bases of sphingosine to kill cancer cell, which were found in precedent various studies represents that sphingosine can be used effectively for treating cancer. However, substances that inhibit migration of endothelial cells and angiogenesis, and concurrently give rise to apoptosis are expected to have better effect

to inhibit growth of cencer cells and give rise to apoptosis.

Recently, it has been reported frequently that phytosphingosine produced in yeast like sphingosine give rise to apoptosis of cancer cell. It was found that derivatives of phytosphingosine(phytosphingosine, N-acethylsphingosine, tetraacethylsphingosine, C6 phytoceramide, C8 phytoceramide or the like) give rise to apoptosis in various cell lines including HaCat(keratinocyte), fibroblast, CHO(Chinese hamster ovarian cell), HL-60(Human leukemia), B16F10(Melanocyte cell line), U937(Monocyte) or the like and cancer cell lines(H460, A539 : lung cancer). Further, it is believed that phytosphingosine derivatives are involved in various inflammatory responses because of their inhibitory effect of protein kinase C and phospholipase D.

A compound of the present invention can be administered by any means that achieve its intended purpose. For example, administration can be by oral, parenteral, rectal, vaginal, topical, intravenous, intramuscular, intraperitoneal, subcutaneous or the like. The dosage administered of the active compound will be dependent upon the recipient, particular diseases or pathological states to be treated, the severity of diseases or pathological states, mode of administration and judgement of prescriber. A determination of the dosage based on the above factors is well known to those skilled in the art. Generally, the dosage is within range of about 0.01 mg/kg/day to about 2000 mg/kg/day, preferably 0.5 mg/kg/day to 2.5 mg/kg/day.

The compound of the present invention can be formulated into pharmaceutical compositions with suitable pharmaceutically acceptable carriers. The pharmaceutical compositions are manufactured in a manner that is, itself, known by using typical carriers(see, for example, E.W. Martin, Remington's Pharmaceutical Sciences, latest edition, Merck Publ. Co., Easton, PA). The compound of the present invention can be administered together with other anticancer drugs. Also, the pharmaceutical compositions can be administered in conjunction with other agents and treatments for treating diseases. For example, when the pharmaceutical compositions are administered, surgery, radiation therapy or chemotherapy can be also carried out

concurrently. According to the intended mode of administration, the pharmaceutical compositions can be in the forms of solid, semi-solid or liquid. The dosage forms include, but not limited to tablets, pills, capsules, suppositories, granules, small saccus, powders, creams, lotions, ointments, patches, liquid solutions, suspensions, dispersions, emulsions, syrups or the like. Also, the active ingredients can be encapsulated in liposome, microparticle or microcapsule.

General nontoxic carriers include, but are not limited to mannitol, lactose, starch, magnesium stearate, sodium saccharine, talc, cellulose, glucose, sucrose, dextrose, glycerol, magnesium carbonate, triglyceride, oil, solvent, sterile water, isotonic saline(pharmaceutical grade) and the like. Solid composition such as tablets, pills, granules or the like can be coated conveniently.

Typically, composition for administrating intraveneously is a solution in sterile isotonic buffer and contains topical anesthetic for alleviating pain at injection site. If desired, drug can contain small amount of nontoxic auxillaries such as wetting agent, emulsifier, pH buffer and the like. Examples of such auxillaries include, but are not limited to sodium acetate, sorbitan monolaurate, triethanolamine and triethanolamine oleate. Also, the composition according to the present invention comprises excipients such as stabilizer, antioxidant, binder, colorant, flavoring agent and thickening agent.

The inhibitor of angiogenesis according to the present invention comprises tetraacetylphytosphingosine, preferably 0.001 percent by weight to 99 percent by weight of tetraacetylphytosphingosine based on total composition. Less than 0.001 percent by weight of tetraacetylphytosphingosine has little effect, and 99 percent by weight or less represents that there is other additives or impurities in the composition.

The present invention will now be illustrated by the following preferred embodiments, but not limiting the scope of the invention.

Examples

We established that tetraacetylphytosphingosine caused cancer cell to apoptosis, and concurrently inhibited angiogenesis by determining apoptosis and inhibitory effect of angiogenesis of tetraacetylphytosphingosine as described below, and thus it can be used effectively for treating cancer.

<Example 1: In vivo wound healing assay>

Female New Zealand White rabbits(body weight 2.0 kg) were used as experimental animals in this assay. PBS solution containing 0.1% BSA(BSA-PBS solution) was prepared as control solution for negative control group(comparative example 1). sphingosylphosphorylcholine(comparative example 2), phytosphingosine(comparative example 3), N-acethylphytosphingosine(comparative example 4) as positive control group, and tetraacetyl phytosphingosine(example 1) were dissolved in ethanol or methanol respectively. Each portion of the solutions was added to silicone glass tube. Then, it was charged with N_2 gas, and added 0.1% BSA-PBS solution. After coupling them with water sonicator and vortex, these solutions were spotted on and injected intradermally to wound region of experimental animals. At the same time, to study an effect of the angiogenesis as concentrations of tetraacethylsphingosine, the animal was treated with 0.1 μM, 1 μM, 2 μM and 5 μM of tetraacethylsphingosine, and campared with the results. Experimental animals were put into special stainless cages which was designed suitably to assay experimental animals, anesthetized by injecting ketamine(3-4 mg/kg) intramuscularly. The hair and corneous tissue of innerside of both ears were removed with shaving and washing, and then were disinfected with 70% ethanol. Four wound regions per ear were formed by using 6 mm punch for skin histological examination(Stiefel, Germany) under sterile condition if possible, and each wound region was spotted or injected intradermally 30 - 50 $\mu\ell$ of the control solution or each treatment material. Wound regions were sealed with cathereep(Nichiban Co., Tokyo Japan) which was cut in size which are greater than that of wound region to prevent contamination of the wound regions and

formation of crust. Then, the wound regions were protected with 2 x 2 gauze, and ears of rabbit were bandaged with elastopore(Nichiban Co., Tokyo Japan). Subsequently, the rabbits were bred in a cage per a rabbit. After 48 hrs, same procedure was repeated. On 4 days and 8 days after forming wound regions, sacrificing the rabbits and treating tissue for histological study. For histological study, wound tissues were fixed with 10% formalin, cut it in half longitudinally and made paraffin block. Then, approximately 5 ㎛ of segment was made, attached it to slide and stained with hematoxylin and eosin to observe a change of epidermis and dermis, and stained with Massons Trichrome to observe a degree of collagen formation of granulation tissue.

First, stained tissue specimen was calibrated with ocular micrometer of optical microscope for image analysis. Then, After photographing each histological change aspects with digital camera under 40X and 100X objective lens and saving them on computer(Pentium III), image analysis was carried out with Scion Image for Windows software which was provided by (C) 2000 Scion corporation as follows: that is, degree of epidermis migration could be determined by measuring the distance from left to right boundary of wound, and thickness of newly formed epidermis could be determined by measuring the thickness of three spots in 1 mm of pitch and averaging measured values. To compare with degree of granulation tissue formation of dermis, three methods were used: 1) A method of measuring total area of newly formed granulation tissue and comparing with it; 2) A method of counting the number of cells such as fibroblast presented in six spots of center region of wound under high magnification (100X) and comparing with it; and 3) A method of determining degree of angiogenesis by counting the number of capillaries in granulation tissue in a same way as described above. If there was eschar, it wasn't determined. The data obtained from the negative control group, the positive control group and the experimental group were analyzed statistically by carrying out paired student's t test. The results were shown in FIG. 1, FIG. 2 and Table 1.

FIG. 1 is a graph representing the number of blood vessels determined on 4

days after treatment of 0.1 μM, 1 μM, 2 μM and 5 μM of a solution containing tetraacetylphytosphingosine according to the present invention respectively in comparison with negative control. In FIG. 1, C-4 represents a result of a negative control group which elapsed 4 days after treatment. T-0.1-4 represents a result of a experimental group which elapsed 4 days after treatment of 0.1 μM of tetraacethylphytosphingosine(TAPS). T-1-4, T-2-4 and T-5-4 represent results of experimental groups which elapsed 4 days after treatment of 1 μM, 2 μM and 5 μM of TAPS respectively. As shown in FIG. 1, tetraacethylphytosphingosine inhibited angiogenesis greatly.

FIG. 2 is a graph representing the area of granulation tissue determined on 4 days after treatment of 0.1 μM, 1 μM, 2 μM and 5 μM of a solution containing tetraacetylphytosphingosine according to the present invention respectively in comparison with negative control. In FIG. 2, C-4 represents a result of a negative control group which elapsed 4 days after treatment, and T-0.1-4, T-1-4, T-2-4 and T-5-4 represent results of experimental groups which elapsed 4 days after treatment of 0.1 μM, 1 μM, 2 μM and 5 μM of TAPS respectively. As shown in FIG. 2, a solution containing tetraacetylphytosphingosine according to the present invention decreased the area of granulation tissue.

Meanwhile, same experiments were carried out with 5 μM of the negative control group(comparative example 1), sphingosylphosphorylcholine (SPC)(comparative example 2), phytosphingosine (PS)(comparative example 3), N-acethylphytosphingosine (NAPS)(comparative example 4) and tetraacetylphytosphingosine (TAPS) as example(example 1) respectively, then the number of blood vessels and the area of granulation tissue were determined. The results were as follows:

Table 1

	Comparative example 1 (negative control)	Comparative example 2:SPC (5 μM)	Comparative example 3:PS (5 μM)	Comparative example 4:NAPS (5 μM)	Example 1:TAPS (5 μM)
Area of granul-ation tissue	100%	151%	112%	212%	76%
Number of blood vessels	100%	148%	106%	96%	31%

As shown in the Table 1, sphingosinephosphorylcholine (SPC) prompted increment of the number of blood vessels and the area of granulation tissue significantly, and tetraacetylphytosphingosine (SPC) inhibited increment of the number of blood vessels and the area of granulation tissue significantly.

<Exemple 2: Toxicity test and angiogenesis test on HUVEC>

HUVEC (human umbilical vein endothelial cell) which was used in this study was cultured as follows: Umbilical cord soaked in cold PBS were cut in 15-20 cm, and washing it thoroughly. Then, cannular was inserted into veins of umbilical cord at its both ends, and sutured umbilical cord and cannular tightly. 2-way stopcock was connected to each cannular which was tightly inserted to umbilical cord, and then 0.45 ㎛ milipore filter was connected to one stopcock. After washing veins with PBS, collagenase solution was added, and incubated for 6 mins at 37℃. After 6 mins, collagenase solution was removed and harvested from veins by introducing heparin solution(10 ml) into the veins, and harvested cells were centrifuged at 1500 rpm for 5 mins. Precipitated endothelial cells were suspended in 5 ml of M199 medium that does

not contain FBS. Above suspension procedure was repeated twice. Then, endothelial cells obtained in this manner were suspended in 5 ml of a medium which is suitable for growing endothelial cells, transferred to T25 flask which coated with gelatin, and incubated in 5% CO_2 incubator at 37℃.

Meanwhile, human fibroblasts which were used in this study were incubated as follows: Skin tissues were obtained by circumcision sterilely, and washed three times with Hanks balanced solution to remove epidermis and subcutaneous fat. Dermis tissues were cut in suitable size and placed them on the bottom of 35 mm culture dish. For depositing dermis tissues to the culture dish thoroughly, the culture dish was placed in incubator(5% CO_2, 37℃, Forma Scientific, Inc., Ohio, U.S.A.) for 5 mins, and then culture broth was added. After about 1-2 weeks, grown fibroblasts were treated with 0.25% trypsin solution and 0.02% EDTA solution for 3-5 mins, separated and passaged.

Toxicity of cell and growing capacity were determined as follows:

Various cell suspensions were stained with 0.5 % Tryphan Blue to count cell numbers. Corresponding medium was loaded to column 1 of 96 multi well plate(blank). To all columns excluding column 1 was added dividedly 180 ㎕ of cells conditioned in corresponding medium, and incubated in incubator at 37℃ for 12 - 24 hrs. 20 ㎕ of a medicament that is 10% of culture broth was added to experimental group, and PBS in equal amount was added to control group. After further incubating for 2 days, 40 ㎕ of MTT solution that the concentration had adjusted to 5 mg/ml with PBS(pH 7.4) was added, and further incubated in incubator at 37℃ for 4 hrs. And then the plate was centrifuged at 1500 rpm for 10 mins to discard the supernatant. 150 ㎕ of 100% DMSO was added to the plate with multi-channel pipette, and the plate was shaked in plate shaker. Finally, the absorbance was determined with ELISA reader at 540 nm. Angiogenesis test of HUVEC was carried out as follows:

Matrigel was prepared as follows: First, collagen solution was prepared by mixing commercially available acid-soluble porcine type I collagen(3.0 mg/ml), 5x

DMEM and buffer(0.05 N NaOH, 2.2% $NaHC0_3$,200 mM HEPES; 7:2:1), loaded to 24 well or 96 well(100 - 300 ㎕/well) dividedly, and then incubated for approximately 10 mins to form a gel due to polymerization of collagen. Primary culture was carried to obtain HUVEC(those which were passaged five times), removed cells at 37℃, washed cells with PBS, detached cells with trypsin/EDTA, loaded cells to wells(4 - 6 x 10^4 cells/well), and then further incubated for 12 - 18 hrs. The results were shown in FIG. 3 and FIG. 4.

FIG. 3 is a graph representing a result of toxicity test of a solution containing tetraacetylphytosphingosine according to the present invention on HUVEC cell. As shown in FIG. 3, apoptosis occurred at the concentration of 5 μM or more of TAPS dramatically.

FIG. 4 is a graph representing a result of angiognesis test of a solution containing tetraacetylphytosphingosine according to the present invention.

Also, FIGs. 5(a), 5(b) and 5(c) are photographs representing results of angiogenesis test of a solution containing tetraacetylphytosphingosine according to the present invention.

As shown in FIG. 4 and FIG. 5, for tube formation test, TAPS was inhibited effectively a formation of blood vessels at the concentration of 0.1 μM, and inhibited nearly completely a formation of blood vessels at the concentration of 1 μM.

<Example 3 : Migration assay of endothelial cells>

Transwell membranes were coated with 0.2% gelatin, and then were left for 12 hrs at 4℃. The membrane for control group was treated with BSA-PBS as described in the above examples, whereas the membranes for experimental group were treated with 0.1 μM, 1 μM and 5 μM of tetraacetylphytosphingosine solution according to the present invention respectively as described in the above examples. After incubating these membranes for 2 hrs, staining them with Diff Quick solution, and then observing them on slide glass. The results were shown in FIG. 6 and FIG. 7.

FIG. 6 is a graph representing that a solution containing tetraacetylphytosphingosine according to the present invention inhibited migration of endothelial cells.

FIG. 7 is photographs representing that a solution containing tetraacetylphytosphingosine according to the present invention inhibited migration of endothelial cells. As shown in FIG. 6 and FIG. 7, migration of endothelial cells were inhibited by TAPS, since the number of endothelial cells migrating to the opposite side of the membrane was reduced as increasing the concentration of TAPS.

The inhibitor of the present invention and the kit comprising it are effective in treating and preventing disorders in connection with extremely increased angiogenesis such as angioma, tumor and psoriasis by effectively inhibiting angiogenesis. Furthermore, they are effective in inhibiting proliferation of cancer cells and metastasis of cancer without any adverse effect.

Although preferred embodiments of the present invention have been described for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

WHAT IS CLAIMED IS:

1. An inhibitor of angiogenesis containing tetraacetylphytosphingosine.

2. A kit for treating cancer, comprising an inhibitor of angiogenesis containing tetraacetylphytosphingosinethe.

3. The kit for treating cancer according to claim 2, wherein further comprising anti-cancer drug and irradiator.

4. The kit for treating cancer according to claim 3, wherein the anti-cancer drug is spingolipid derivatives.

5. The kit for treating cancer according to claim 4, wherein the spingolipid derivatives are one or more spingolipid derivatives selected from the group consisting of phytosphingosine, enacethylphytosphingosine, C6 phytoceramide, C8 phytosphingosine, dimethylsphingosine, dimethylphytosphingosine and sphingosine.A composition for inhibiting the activity of tyrosinase which comprises N-acetylphytosphingosine as an active ingredient.

Fig. 1

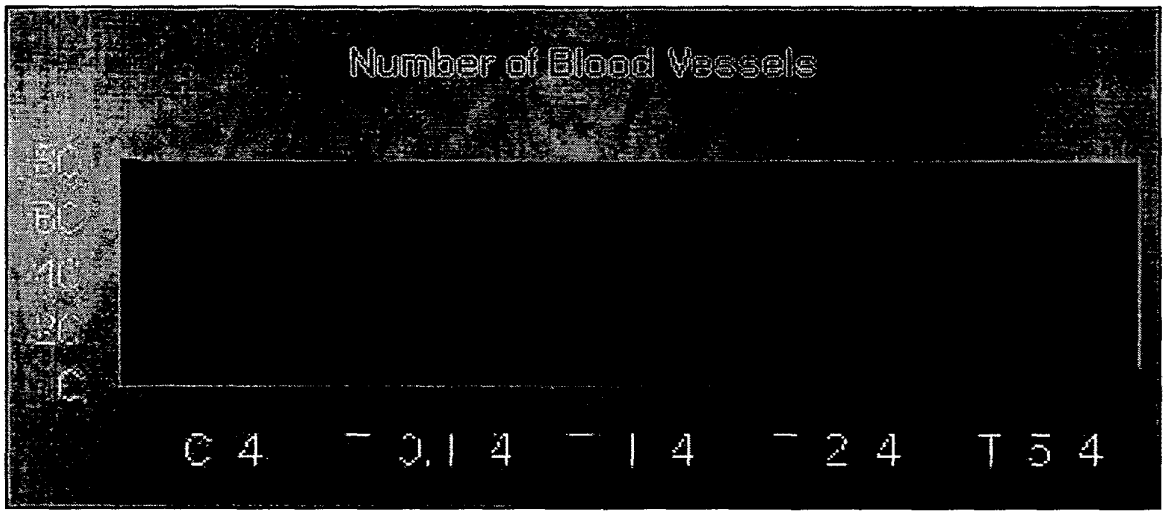

Number of Blood Vessels
C 4
T 0.1 4
T 1 4
T 2 4
T 5 4

Fig. 2

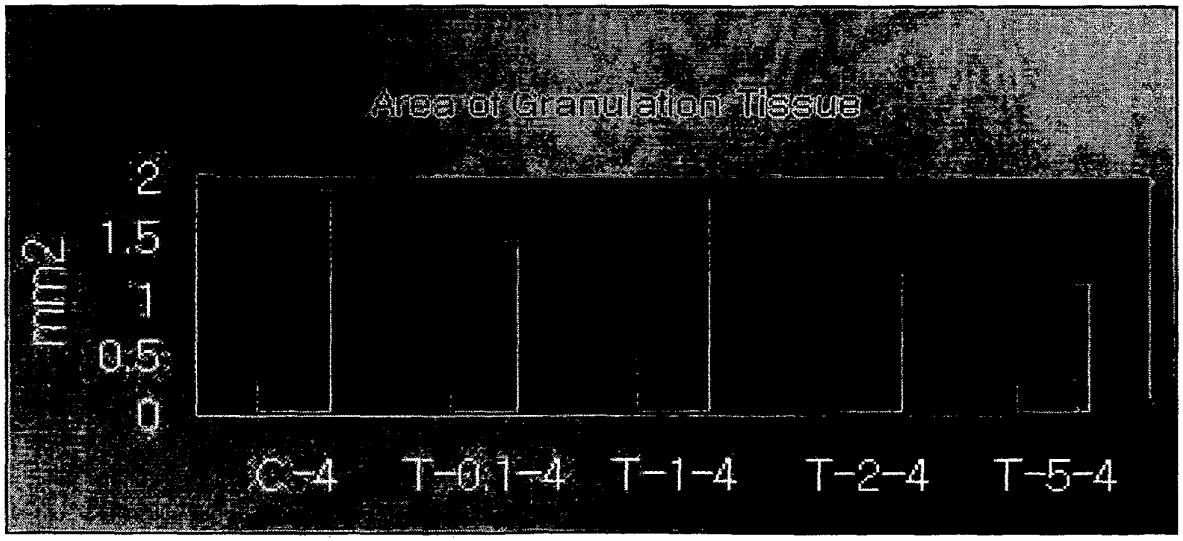

Area of Granulation Tissue
mm2
2
1.5
1
0.5
0
C-4
T-0.1-4
T-1-4
T-2-4
T-5-4

Fig. 3

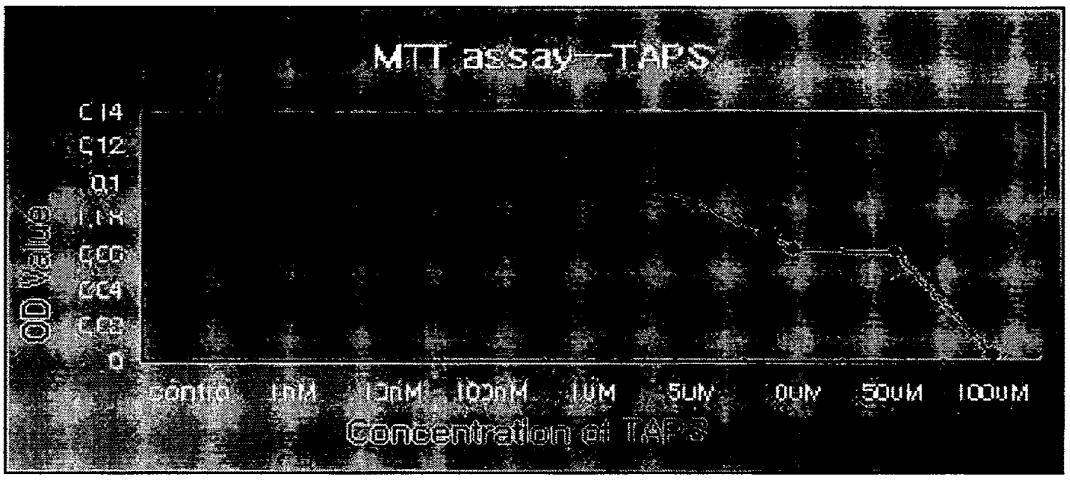

MTT assay--TAPS
OD Value
0
Concentration of TAPS

Fig. 4

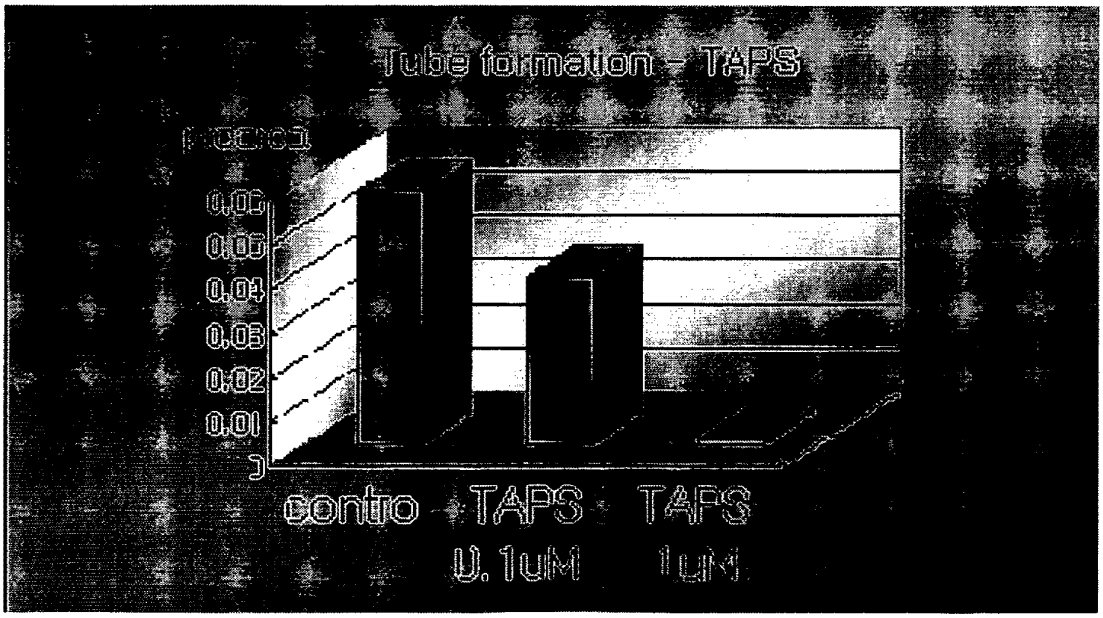

Tube formation - TAPS
0.06
0.05
0.04
0.03
0.02
0.01
0
conto
TAPS
0.1uM
TAPS
1uM

Fig. 5

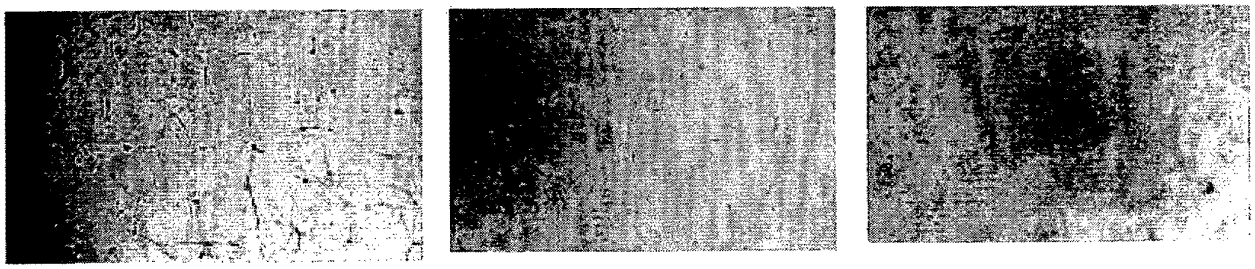

(a) Control
(b)TAPS 0.1uM
(c)TAPS 1uM

Fig. 6

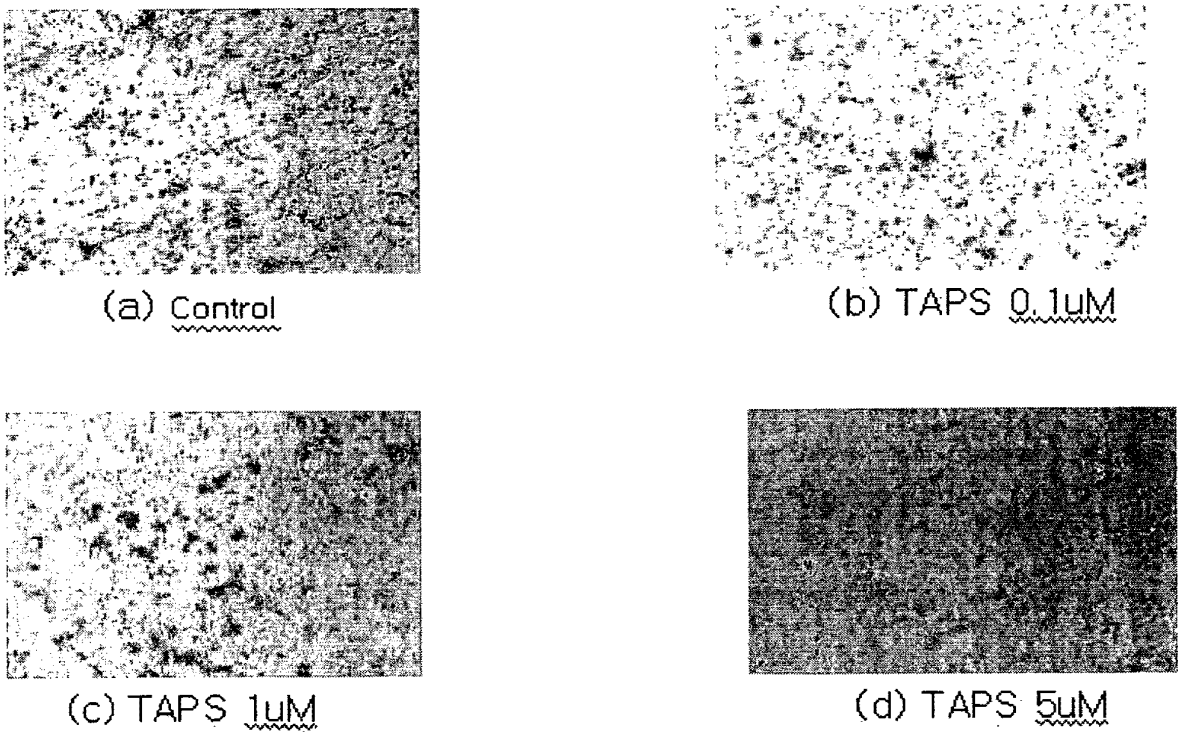
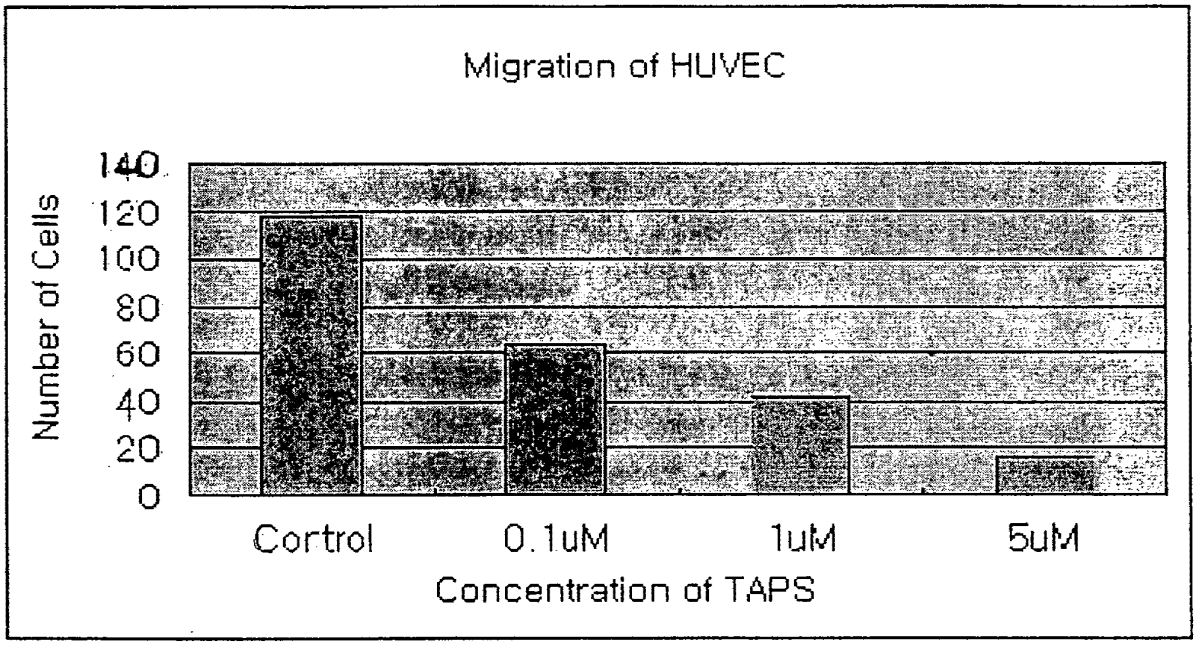

(a) Control
(b) TAPS 0.1uM
(c) TAPS 1uM
(d) TAPS 5uM

Fig. 7


Migration of HUVEC
Number of Cells
140
120
100
80
60
40
20
0
Cortrol
0.1uM
1uM
5uM
Concentration of TAPS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2003/002622

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 31/133

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 07 A61K 31/133

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
STN(CA, Medline)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, A	WO 03/92667 A1(DOOSAN CORP.) 13 NOVEMBER 2003 See the whole document	1-5
A	XIAODONG SHU, et al., "Sphingosine kinase mediates Vascular Endothelial Growth Factor-Induced Activation of Ras and Mitogen-Activated Protein Kinases", Molecular and Cellular Biology, November 2002, 22(22), pp.7758-7768 See the whole document	1-5
A	CHAN WOO KIM, et al., "Extracellular Membrane Vesicles from Tumor Cells Promote Angiogenesis via Sphingomyelin", Cancer Research, November 2002, 62(21), pp.6312-6317 See the whole document	1-5
P, A	US 6610534 B2(NOVARTIS AG.) 26 AUGUST 2003 See the Abstract	1-5

☐ 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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"O" document referring to an oral disclosure, use, exhibition or other means
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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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"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03/097631 A1	27.11.2003	KR 2042606 A	05.06.2002
US 6610534 B2	26.08.2003	WO 02/28406 A2	11.04.2002
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** METHOD OF ADMINISTERING AND USING VEGF INHIBITORS FOR THE TREATMENT OF HUMAN CANCER

(57) **Abstract:** A method of treating a human patient suffering from cancer, comprising administering an effective amount of a vascular endothelial growth factor (VEGF) trap antagonist to the human patient, the method comprising: (a) administering to the patient an initial use of at least approximately 0.3 mglkg of the VEGF antagonist; and (b) administering the patient a plurality of subsequent doses of the VEGF antagonist in an amount that approximately the same or less of the initial dose, wherein the subsequent doses are separated in time from each other by at least one day. The methods of the invention are useful for treating a human cancer selected from the group consisting of renal cell carcinoma, pancreatic carcinoma, breast cancer, prostate cancer, colorectal cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, and melanoma. The invention is further useful for treating a condition which benefits from the reduction of VEGFA and placental growth factor (PLGF).

WO 2005/123104 A2

METHOD OF ADMINISTERING AND USING VEGF INHIBITORS FOR THE TREATMENT OF HUMAN CANCER

Field of the Invention

[0001] The invention relates to methods of promoting regression of tumors and metastases by inhibiting vascular endothelial growth factor (VEGF) activity.

Description of Related Art

[0002] Vascular endothelial growth factor (VEGF) expression is nearly ubiquitous in human cancer, consistent with its role as a key mediator of tumor neoangiogenesis. Blockade of VEGF function, by binding to the molecule or its VEGFR-2 receptor, inhibits growth of implanted tumor cells in multiple different xenograft models (see, for example, Gerber et al. (2000) Cancer Res. 60:6253-6258). A soluble VEGF fusion protein antagonist, termed a "$VEGF_{R1R2}$ trap" or "VEGF trap" antagonist has been described (Kim et al. (2002) Proc. Natl. Acad. Sci. USA 99:11399-404; Holash et al. (2002) Proc. Natl. Acad. Sci. USA 99:11393-8.

Brief Summary of the Invention

[0003] In a first aspect, the invention features a method of treating a human patient suffering from cancer, comprising administering an effective amount of a vascular endothelial growth factor (VEGF) fusion protein trap antagonist to the human patient, the method comprising: (a) administering to the patient an initial dose of at least approximately 0.3 mg/kg of the VEGF trap antagonist; and (b) administering to the patient a plurality of subsequent doses of the VEGF trap antagonist in an amount that is approximately the same or less of the initial dose, wherein the subsequent doses are separated in time from each other by at least one day. The dosing regimen of the invention allows early attainment of an efficacious target trough serum concentration by providing an initial dose or doses of VEGF trap antagonist followed by subsequent doses of equal or smaller amounts of trap (greater front loading). The efficacious target trough serum concentration is reached in 4 weeks or less, preferably 3 weeks or less, more preferably 2 weeks or less, and most preferably 1 week or less, including 1 day or less. The target serum concentration is thereafter maintained by the administration of maintenance doses of equal or smaller amounts for the remainder of the treatment regimen or until suppression of disease symptoms is achieved.

[0004] In specific embodiments, the initial dose of the VEGF fusion protein antagonist is

in the range of approximately between 0.3 mg per kg body weight (mg/kg /kg) to 30 mg/kg. In a more specific embodiment, the initial dose is in the range of approximately 0.5 mg/kg to 10 mg/kg. In an even more specific embodiment, the initial dose is in the range of approximately 1 mg/kg to 6 mg/kg. Preferably, the cumulative weekly dose is in the range of 0.3 to 30 mg/kg.

[0005] In specific embodiments, at least one subsequent dose of the VEGF fusion protein antagonist is in the range of approximately between 0.3 mg/kg body weight to 30 mg/kg. In a more specific embodiment, at least one subsequent dose is in the range of approximately 0.5 mg/kg to 10 mg/kg. In an even more specific embodiment, at least one subsequent dose is in the range of approximately 1 mg/kg to 6 mg/kg.

[0006] In one embodiment, subsequent doses are separated in time from each other by at least 1 day, at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, or at least 3 months. According to the invention, the cycle of dosing is preferably repeated as necessary to achieve suppression of the disease symptoms.

[0007] The method of the invention may be used to treat primary and/or metastatic tumors arising in the brain and meninges, oropharynx, lung and bronchial tree, gastrointestinal tract, male and female reproductive tract, muscle, bone, skin and appendages, connective tissue, spleen, immune system, blood forming cells and bone marrow, liver and urinary tract, and special sensory organs such as the eye. More specifically, the human patient treated by the method of the invention is a patient diagnosed with one of the following cancers: renal cell carcinoma, pancreatic carcinoma, breast cancer, prostate cancer, colorectal cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, or melanoma. In a specific embodiment, the cancer being treated is renal cell carcinoma. In another embodiment, the cancer being treated is pancreatic carcinoma. In another embodiment, the cancer being treated is breast cancer. In another embodiment, the cancer being treated is colorectal cancer. In another embodiment, the cancer being treated is malignant mesothelioma. In another embodiment, the cancer being treated is multiple myeloma. In another embodiment, the cancer being treated is ovarian cancer. In another embodiment, the cancer being treated is melanoma. In another embodiment, the cancer being treated is non-small cell lung cancer. In another embodiment, the cancer being treated is prostate cancer.

[0008] The VEGF fusion protein trap antagonist is a dimer comprising two fusion proteins each composed of immunoglobulin (Ig)-like domains from two different VEGF receptors fused to a multimerizing component, wherein each fusion protein is capable of forming a higher order complex through interaction of multimerizing components on

different fusion proteins. The VEGF trap antagonist useful in the method of the present invention is a dimer capable of binding both vascular endothelial growth factor A (VEGFA) and placental growth factor (PLGF), and is selected from the group consisting of acetylated Flt-1(1-3)-Fc, Flt-1(1-$3_{R->N}$)-Fc, Flt-1(1-$3_{\Delta B}$)-Fc, Flt-1(2-$3_{\Delta B}$)-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a), and VEGFR1R2-FcΔC1(a). In a specific and preferred embodiment, the VEGF trap antagonist is VEGFR1R2-FcΔC1(a) (also termed VEGF $trap_{R1R2}$) having the nucleotide sequence set forth in SEQ ID NO: 1 and the amino acid sequence set forth in SEQ ID NO: 2. The invention encompasses the use of a VEGF trap that is at least 90%, 95%, 98%, or at least 99% homologous with the nucleotide sequence set forth in SEQ ID NO: 1 and/or the amino acid sequence set forth in SEQ ID NO:2.

[0009] Administration of the agent may be by any method known in the art, including subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous, intranasal, or oral routes of administration. In a preferred embodiment, the initial dose is administered by subcutaneous injection or intravenous injection. In further embodiments, the subsequent doses are administered by subcutaneous injection. In a preferred embodiment, the initial dose and at least one subsequent dose are administered by subcutaneous injection.

[0010] In a second aspect, the invention features a method of treating a human patient susceptible to or diagnosed with a disorder which is inhibited by an agent capable of blocking or inhibiting vascular endothelial growth factor A (VEGF A), wherein the agent capable of blocking or inhibiting VEGFA is a VEGF trap antagonist, the method comprising: (a) administering to the patient an initial dose of at least approximately 0.3 mg/kg of the VEGF trap; and (b) administering to the patient a plurality of subsequent doses of the VEGF trap in an amount that is approximately the same or less of the initial dose, wherein the subsequent doses are separated in time from each other by at least one day. In a specific and preferred embodiment, the VEGF trap antagonist is VEGFR1R2-FcΔC1(a) (also termed VEGF $trap_{R1R2}$) having the nucleotide sequence set forth in SEQ ID NO: 1 and the amino acid sequence set forth in SEQ ID NO: 2.

[0011] In a third embodiment, the invention features a therapeutic method of the invention optionally combined with a second chemotherapeutic agent. Chemotherapeutic agents combinable with administration of VEGF trap include, for example, anti-VEGF antibodies, anthracycline derivatives, such as doxorubicin or epirubicin taxol, and taxoid derivatives such as paclitaxel (Taxol®) and related derivatives.

[0012] Other objects and advantages will become apparent from a review of the ensuing detailed description.

Detailed Description

[0013] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only the appended claims.

[0014] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0015] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

General Description

[0016] Vascular endothelial growth factor/vascular permeability factor (VEGF) was initially identified as a tumor-derived factor capable of increasing vascular permeability. It was subsequently found to be a proliferative factor for endothelial cells. In the embryo, VEGF is absolutely essential for the development of the vasculature. In the adult, VEGF is up-regulated in a variety of normal and pathological processes associated with increased vascular permeability and angiogenesis.

[0017] The family of VEGF-related angiogenic growth factors is comprised of VEGF itself (VEGF-A) and the related proteins VEGF-B, -C, -D and E, and placental growth factor (PLGF). In addition, there are at least four different isoforms of VEGF-A. However, as some members of the family have only recently been identified, their biological importance is still poorly understood. The actions of VEGF and its related factors are mediated by a group of three receptor tyrosine kinases, VEGFR1, VEGFR2 and VEGFR3.

[0018] The importance of VEGF in tumor angiogenesis has been demonstrated in a

number of animal models, where blocking VEGF signaling by a variety of strategies has proven effective at decreasing angiogenesis and inhibiting tumor growth (Gourley and Williamson (2000) Curr. Pharm. Des. 6:417-39). The permeability-inducing properties of VEGF are also of pathological importance, for example in edema formation, ascites and pleural effusions related to cancer. The degree of vascularization and of VEGF production have been proposed as prognostic factors for many types of solid and hematological malignancies (reviewed by Poon et al (2001) J. Clin. Oncol. 19:1207-1225).

[0019] Consistent with predictions from animal studies, blockade of VEGF using a humanized monoclonal antibody has emerged reporting promising results in cancer patients, based on preliminary reports from early clinical trials (Bergsland et al. (2000) ASCO Abstract #939). The VEGF fusion protein trap antagonist, because of its greater affinity for VEGF and its ability to bind other VEGF family members such as the PlGFs, is a potent and useful anti-cancer therapeutic agent.

Definitions

[0020] By the term "therapeutically effective dose" is meant a dose that produces the desired effect for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding). Efficacy can be measured in conventional ways, depending on the condition to be treated. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP), or determining the response rates (RR). Therapeutically effective amount also refers to a target serum concentration, such as a trough serum concentration, that has been shown to be effective in suppressing disease symptoms when maintained for a period of time.

[0021] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, prostate cancer, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

More Specifically, the methods of the invention are useful for treating any condition or disease which is ameliorated or inhibited with a VEGF inhibitor. Accordingly, when the disease or condition is cancer, the cancer treated by the method of the invention is one which is ameliorated or inhibited by administration of a VEGF inhibitor.

[0022] By the term "blocker", "inhibitor", or "antagonist" is meant a substance that retards or prevents a chemical or physiological reaction or response. Common blockers or inhibitors include but are not limited to antisense molecules, antibodies, antagonists and their derivatives. More specifically, an example of a VEGF blocker or inhibitor is a VEGF receptor-based antagonist including, for example, an anti-VEGF antibody, or a VEGF trap antagonist such as VEGFR1R2-FcΔC1(a) (SEQ ID NOs:1-2). For a complete description of VEGF-receptor based antagonists including VEGFR1R2-FcΔC1(a), see PCT publication WO/00/75319.

[0023] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

[0024] The term "serum concentration," "serum drug concentration," or "serum VEGF trap concentration" refers to the concentration of a drug, such as the VEGF fusion protein trap antagonist, in the blood serum or plasma of an animal or human patient being treated with the drug. Serum concentration is preferably determined by immunoassay. Preferably, the immunoassay is an ELISA according to the procedure disclosed herein.

[0025] The term "peak serum concentration" refers to the maximal serum drug concentration shortly after delivery of the drug into the animal or human patient, after the drug has been distributed throughout the blood system, but before significant tissue distribution, metabolism or excretion of drug by the body has occurred.

[0026] The term "trough serum concentration" refers to the serum drug concentration at a time after delivery of a previous dose and immediately prior to delivery of the next subsequent dose of drug in a series of doses. Generally, the trough serum concentration is a minimum sustained efficacious drug concentration in the series of drug administrations. Also, the trough serum concentration is frequently targeted as a minimum serum concentration for efficacy because it represents the serum concentration at which another dose of drug is to be administered as part of the treatment regimen. If the delivery of drug is by intravenous administration, the trough serum concentration is most preferably attained within 1 day of a front loading initial

drug delivery. If the delivery of drug is by subcutaneous administration, the peak serum concentration is preferably attained in 3 days or less. According to the invention, the trough serum concentration is preferably attained in 4 weeks or less, preferably 3 weeks or less, more preferably 2 weeks or less, most preferably in 1 week or less, including 1 day or less using any of the drug delivery methods disclosed herein.

[0027] The term "intravenous infusion" refers to introduction of a drug into the vein of an animal or human patient over a period of time greater than approximately 5 minutes, preferably between approximately 30 to 90 minutes, although, according to the invention, intravenous infusion is alternatively administered for 10 hours or less.

[0028] The term "subcutaneous administration" refers to introduction of a drug under the skin of an animal or human patient, preferable within a pocket between the skin and underlying tissue, by relatively slow, sustained delivery from a drug receptacle. The pocket may be created by pinching or drawing the skin up and away from underlying tissue.

[0029] The term "front loading" when referring to drug administration is meant to describe an initially higher dose followed by the same or lower doses at intervals. The initial higher dose or doses are meant to more rapidly increase the animal or human patient's serum drug concentration to an efficacious target serum concentration. According to the present invention, front loading is achieved by an initial dose or doses delivered over three weeks or less that causes the animal's or patient's serum concentration to reach a target serum trough concentration. Preferably, the initial front loading dose or series of doses is administered in two weeks or less, more preferably in 1 week or less, including 1 day or less. Most preferably, where the initial dose is a single dose and is not followed by a subsequent maintenance dose for at least 1 week, the initial dose is administered in 1 day or less. Where the initial dose is a series of doses, each dose is separated by at least 3 hours, but not more than 3 weeks or less, preferably 2 weeks or less, more preferably 1 week or less, most preferably 1 day or less.

The VEGF Fusion Protein Trap Antagonist

[0030] In a preferred embodiment, the VEGF trap is a receptor-Fc fusion protein consisting of the principal ligand-binding portions of the human VEGFR1 and VEGFR2 receptor extracellular domains fused to the Fc portion of human IgG1. Specifically, the VEGF trap antagonist consists essentially of Ig domain 2 from VEGFR1, which is fused to Ig domain 3 from VEGFR2, which in turn is fused to the Fc domain of IgG1 (SEQ ID

[0031] In a preferred embodiment, an expression plasmid encoding the VEGF trap is transfected into CHO cells, which secrete VEGF trap into the culture medium. The resulting VEGF trap is a dimeric glycoprotein with a protein molecular weight of 97 kDa and contains ~15% glycosylation to give a total molecular weight of 115 kDa.

[0032] Since the VEGF trap binds its ligands using the binding domains of high-affinity receptors, it has a greater affinity for VEGF than do monoclonal antibodies. The VEGF trap binds VEGF-A (K_D = 0.5 pM), PLGF1 (K_D = 1.3 nM), and PLGF2 (K_D = 50 pM); binding to other VEGF family members has not yet been fully characterized.

Treatment Population

[0033] The method of the invention may be used to treat tumors arising in the brain and meninges, oral pharynx, lung and bronchial tree, gastrointestinal tract, male and female reproductive tract, muscle, bone, skin, connective tissue, immune system, blood forming cells and bone marrow, liver and urinary tract, and special sensory organs such as the eye. More specifically, human patients suffering from renal cell carcinoma, pancreatic carcinoma, breast cancer, prostate cancer, colorectal cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, or melanoma may be treated with the VEGF trap as described below.

Combination Therapies

[0034] In numerous embodiments, a VEGF fusion protein trap antagonist may be administered in combination with one or more additional compounds or therapies, including a second VEGF trap molecule. Combination therapy includes administration of a single pharmaceutical dosage formulation which contains a VEGF trap and one or more additional agents; as well as administration of a VEGF trap and one or more additional agent(s) in its own separate pharmaceutical dosage formulation. For example, a VEGF trap and a cytotoxic agent, a chemotherapeutic agent or a growth inhibitory agent can be administered to the patient together in a single dosage composition such as a combined formulation, or each agent can be administered in a separate dosage formulation. Where separate dosage formulations are used, the VEGF-specific fusion protein of the invention and one or more additional agents can be administered concurrently, or at separately staggered times, i.e., sequentially.

[0035] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to

include radioactive isotopes (e.g. I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

[0036] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (Cytoxan®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (Taxol®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (Taxotere®; Aventis

Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0037] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially a cancer cell either *in vitro* or *in vivo*. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), Taxol ®, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C.

Pharmaceutical Compositions

[0038] Pharmaceutical compositions useful in the practice of the method of the invention include a therapeutically effective amount of an active agent, and a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium

chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[0039] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, or intramuscular administration to human beings. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0040] The active agents of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

Articles of Manufacture

[0041] In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container, a label and a package insert. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the

composition is a VEGF fusion protein trap antagonist. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes. In addition, the article of manufacture may comprise a package inserts with instructions for use, including, e.g., a warning that the composition is not to be used in combination with anthacycline-type chemotherapeutic agent, e.g. doxorubicin or epirubicin.

[0042] Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

Examples

[0043] The following example is put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Pharmacokinetics and Safety of the VEGF Fusion Protein Trap Antagonist in Primates

[0044] Preclinical toxicology studies were conducted with the VEGF trap (SEQ ID NO:2) in primates and rodents. Four- and thirteen week toxicology studies in cynomolgus monkeys showed that the VEGF trap was well tolerated when administered subcutaneously three times per week at doses of 1.5, 5, and 15 mg/kg (four week study), or twice a week at 1.5, 5, 15 or 30 mg/kg in the thirteen-week study. The VEGF trap was not highly immunogenic after four weeks in monkeys; only one mid-dose animal developed low titer antibodies.

Example 2: Treatment of Solid Tumors or Non-Hodgkin's Lymphoma.

[0045] Patients with refractory solid tumors or non-Hodgkin's lymphoma receiving no concurrent treatment for their cancer are treated with the VEGF trap as follows. The dose levels range from 0.3 mg/kg to 30 mg/kg given subcutaneously. Each patient receives a single initial dose of the VEGF trap followed by four weeks of observation and pharmacokinetic blood sampling. Beginning in the fifth week of the study, patients receive a series of 6 weekly injections at the assigned dose level. Plasma levels of the VEGF trap, and VEGF, both free and bound together as a complex, are monitored. Tumor burden is assessed at the beginning and end of the weekly dosing period and periodically during treatment; patients with stable disease, partial or complete responses may continue dosing for up to an additional 6 months in a continuation study. At higher dose levels where efficacy might be anticipated, patients undergo Dynamic Contrast Enhanced MRI scans to assess effects of VEGF trap administration on tumor perfusion.

We claim,

1. Use of a vascular endothelial growth factor (VEGF) antagonist comprising a dimer of two fusion polypeptides, each fusion polypeptide comprising (a) an immunoglobulin (Ig)-like domain 2 of a Flt-1 and Ig domain 3 of an Flk-1 or Flt-4, and (b) a multimerizing component, in the preparation of a medicament for treating a human patient suffering from cancer, by a method comprising:

(a) administering to the patient an initial dose of at least approximately 0.3 mg/kg of the VEGF antagonist; and

(b) administering to the patient a plurality of subsequent doses of the VEGF antagonist that are approximately the same or less than the initial dose, wherein the subsequent doses are separated in time from each other by at least one day.

2. Use according to claim 1, wherein the initial dose is from approximately 0.3 mg/kg to approximately 30 mg/kg; preferably from approximately 0.5 mg/kg to approximately 10 mg/kg.

3. Use according to claim 2, wherein the initial dose is approximately 1 mg/kg to approximately 6 mg/kg.

4. Use according to claim any one of the preceding claims, wherein the initial dose is approximately 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg and 6 mg/kg.

5. Use according to any one of the preceding claims, wherein the subsequent doses are the same or different and are from approximately 0.3 mg/kg to approximately 30 mg/kg; preferably from approximately 0.5 mg/kg to approximately 10 mg/kg.

6. Use according to claim 5, wherein the subsequent doses are from approximately 1 mg/kg, approximately 2 mg/kg, approximately 3 mg/kg, approximately 4 mg/kg, approximately 5 mg/kg and approximately 6 mg/kg.

7. Use according to any one of the preceding claims, wherein subsequent doses are separated in time from each other by at least 1 week.

8. Use according to claim 7, wherein subsequent doses are separated in time from each other by at least 1 month.

9. Use according to any one of the preceding claims, wherein the cancer is selected from renal cell carcinoma, pancreatic carcinoma, breast cancer, prostate cancer, colorectal cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, and melanoma.

10. Use according to any one of the preceding claims, wherein the cancer is one that is inhibited by a vascular endothelial growth factor (VEGF) antagonist and a placental growth factor (PLGF) antagonist.

11. Use according to any one of the preceding claims, wherein the VEGF antagonist is selected from acetylated Flt-1(1-3)-Fc, Flt-1(1-$3_{R->N}$)-Fc, Flt-1(1-$3_{\Delta B}$)-Fc, Flt-1(2-$3_{\Delta B}$)-Fc, Flt-1(2-3)-Fc, Flt-1D2-VEGFR3D3-FcΔC1(a), Flt-1D2-Flk-1D3-FcΔC1(a), and VEGFR1R2-FcΔC1(a).

12. Use according to claim 11, wherein the VEGF antagonist is VEGFR1R2-FcΔC1 comprising the amino acid sequence of SEQ ID NO:2.

13. Use according to any one of the preceding claims, wherein the medicament is formulated for administration of the initial dose and subsequent doses by subcutaneous injection or intravenous injection.

14. A method of treating a human patient diagnosed with a cancer comprising administering an effective amount of a VEGF antagonist as defined in claim 1, 11 or 12, to the human patient, the method comprising:

(a) administering to the patient an initial dose of at least approximately 0.3 mg/kg of the VEGF antagonist; and

(b) administering to the patient a plurality of subsequent doses of the VEGF antagonist that are approximately the same as or less than the initial dose, wherein the subsequent doses are separated in time from each other by at least one week.

15. A method according to claim 14, wherein the initial dose and/or the subsequent doses are as defined in any one of claims 2 to 6.

16. A method according to claim 14 or 15, wherein the cancer is as defined in claim 9 or 10.

17. A method according to any one of claims 14 to 16, wherein subsequent doses are separated in time from each other by at least three weeks.

18. A method according to any one of claims 14 to 17 wherein the initial dose and/or the subsequent doses are administered by subcutaneous injection or intravenous injection.

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(54) Title: ORGANIC COMPOUNDS

(57) Abstract: Disclosed are pharmaceutical combinations comprising at least one S1P receptor agonist, as well as a method for treating demyelinating diseases, e.g. multiple sclerosis or disorders associated therewith or Guillain-Barré syndrome, comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a) an S1P receptor agonist, and b) at least one co-agent shown to have clinical activity against at least one symptom of a demyelinating disease.

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Organic Compounds

The present invention relates to pharmaceutical combinations comprising at least one S1P receptor agonist and their uses in treating demyelinating diseases, e.g. multiple sclerosis and disorders associated therewith.

Multiple sclerosis is an immune-mediated disease of the central nervous system white matter with chronic inflammatory demyelination leading to progressive decline of motor and sensory functions and permanent disability. Manifestations of clinical disease usually begin in early adulthood, with women outnumbering men 2:1. The therapy of multiple sclerosis is only partially effective, and in most cases only offers a delay in disease progression despite anti-inflammatory and immunosuppressive treatment. Clinicians usually categorize patients into four types of disease patterns:

- *Relapsing-remitting* (RR-MS): Discrete motor, sensory, cerebellar or visual attacks that occur over 1-2 weeks and often resolve over 1-2 months, with or without treatment. Some patients accrue disability with each episode, yet remain clinically stable between relapses. About 85% of patients initially experience the RR form of MS, but within 10 years about half will develop the secondary progressive form.
- *Secondary-progressive* (SP-MS): Initially RR followed by gradually increasing disability, with or without relapses. Major irreversible disabilities appear most often during SP.
- *Primary-progressive* (PP-MS): Progression disease course from onset without any relapses or remissions, affecting about 15% of MS patients.
- *Progressive-relapsing* (PR-MS): Progressive disease from onset with clear acute relapses; periods between relapses characterized by continuing progression.

Accordingly, there is a need for agents which are effective in the treatment of demyelinating diseases, e.g. multiple sclerosis or Guillain-Barré syndrome, e.g. including reduction of, alleviation of, stabilization of or relief from the symptoms or illness which affect the organism.

It has now been found that a combination comprising at least one S1P receptor agonist and a co-agent, e.g. as defined below, has a beneficial effect on demyelinating diseases, e.g. multiple sclerosis and the disorders associated therewith.

In accordance with the particular findings of the present invention, there is provided

1. A pharmaceutical combination comprising:

a) an S1P receptor agonist, and

b) at least one co-agent shown to have clinical activity against at least one demyelinating disease symptom, e.g. a multiple sclerosis symptom or a symptom of Guillain-Barré syndrome.

2. 1 A method for treating a demyelinating disease, e.g. multiple sclerosis or disorders associated therewith or Guillain-Barré syndrome, comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of an S1P receptor agonist, e.g. a compound of formulae I to VII as defined hereinafter, and at least one co-agent, e.g. as indicated hereinafter.

2.2 A method for alleviating or delaying progression of the symptoms of a demyelinating disease, e.g. multiple sclerosis or Guillain-Barré syndrome, comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of an S1P receptor agonist, e.g. a compound of formulae I to VII as defined herein after, and at least one co-agent, e.g. as indicated hereinafter.

An early symptom of multiple sclerosis is optic neuritis. Accordingly, the present invention also provides

2.3 A method for treating, alleviating or delaying progression of optic neuritis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an S1P receptor agonist, e.g. a compound of formulae I to VII as specified herein after, e.g. Compound A or B or a pharmaceutically acceptable salt thereof.

3. A pharmaceutical combination as disclosed herein for use in any one of the methods 2.1 to 2.3.

4.1 A pharmaceutical composition for treating, alleviating or delaying progression of optic neuritis comprising an S1P receptor agonist, e.g. a compound of formulae I to VII as defined herein after, e.g. Compound A or B, together with one or more pharmaceutically acceptable diluents or carriers therefor.

4.2 A compound of formulae I to VII as defined herein after, e.g. Compound A or B, for use in the treatment, alleviating or delay of progression of optic neuritis.

4.3 An S1P receptor agonist, e.g. a compound of formulae I to VII as defined herein after, e.g. Compound A or B, for use in the preparation of a medicament for use in the treatment, alleviating or delay of progression of optic neuritis.

5.1 Use of an S1P receptor agonist, e.g. a compound of formulae I to VII as defined herein after, e.g. Compound A or B, for the preparation of a medicament for treating, alleviating or delaying the progression of optic neuritis.

5.2 Use of a) a sphingosine-1-phosphate (S1P) receptor agonist, and b) at least one co-agent shown to have clinical activity against at least one symptom of a demyelinating disease, for the preparation of a pharmaceutical combination for treating, alleviating or delaying progression of the symptoms of a demyelinating disease, e.g. for the preparation of a pharmaceutical combination for separate, simultaneous or sequential use in such a method.

5.3 A pharmaceutical composition as disclosed herein for separate, simultaneous or sequential use in medicine, e.g. in a method as disclosed at 2.1 to 2.3.

The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients.

The term "fixed combination" as that term is used herein means that the active ingredients, e.g. the S1P receptor agonist and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. As an example, a fixed combination would be one capsule containing two active ingredients.

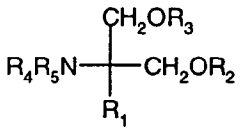
The term "non-fixed combination" as that term is used herein means that the active ingredients, e.g. the S1P receptor agonist and a co-agent, are both administered to a

patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the two compounds in the body, preferably at the same time. As an example, a non-fixed combination would be two capsules each containing one active ingredient where the purpose is to have the patient achieve treatment with both active ingredients together in the body.

An S1P receptor agonist is an immunomodulating compound which elicits a lymphopenia resulting from a re-distribution, preferably reversible, of lymphocytes from circulation to secondary lymphatic tissue, without evoking a generalized immunosuppression. Naïve cells are sequestered; CD4 and CD8 T-cells and B-cells from the blood are stimulated to migrate into lymph nodes (LN) and Peyer's patches (PP), and thus for example infiltration of cells into transplanted organs is inhibited.

Examples of appropriate S1P receptor agonists are, for example:

- Compounds as disclosed in EP627406A1, e.g. a compound of formula I



I

wherein R_1 is a straight- or branched (C_{12-22})carbon chain

- which may have in the chain a bond or a hetero atom selected from a double bond, a triple bond, O, S, NR_6, wherein R_6 is H, alkyl, aralkyl, acyl or alkoxycarbonyl, and carbonyl, and/or
- which may have as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxyimino, hydroxy or carboxy; or

R_1 is

- a phenylalkyl wherein alkyl is a straight- or branched (C_{6-20})carbon chain; or
- a phenylalkyl wherein alkyl is a straight- or branched (C_{1-30})carbon chain wherein said phenylalkyl is substituted by
- a straight- or branched (C_{6-20})carbon chain optionally substituted by halogen,
- a straight- or branched (C_{6-20})alkoxy chain optionally substitued by halogen,

- a straight- or branched (C_{6-20})alkenyloxy,
- phenylalkoxy, halophenylalkoxy, phenylalkoxyalkyl, phenoxyalkoxy or phenoxyalkyl,
- cycloalkylalkyl substituted by C_{6-20}alkyl,
- heteroarylalkyl substituted by C_{6-20}alkyl,
- heterocyclic C_{6-20}alkyl or
- heterocyclic alkyl substituted by C_{2-20}alkyl,

and wherein

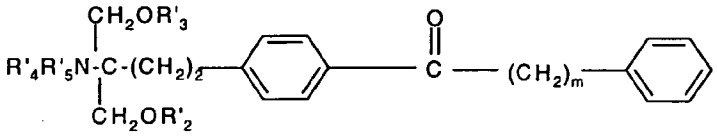
the alkyl moiety may have

- in the carbon chain, a bond or a heteroatom selected from a double bond, a triple bond, O, S, sulfinyl, sulfonyl, or NR_6, wherein R_6 is as defined above, and
- as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxy or carboxy, and

each of R_2, R_3, R_4 and R_5, independently, is H, C_{1-4} alkyl or acyl

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in EP 1002792A1, e.g. a compound of formula II

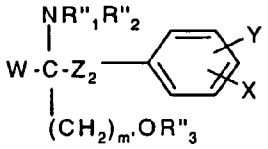


II

wherein m is 1 to 9 and each of R'_2, R'_3, R'_4 and R'_5, independently, is H, alkyl or acyl,

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in EP0778263 A1, e.g. a compound of formula III



III

wherein W is H; C_{1-6}alkyl, C_{2-6}alkenyl or C_{2-6}alkynyl; unsubstituted or by OH substituted phenyl; $R''_4O(CH_2)_n$; or C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH;

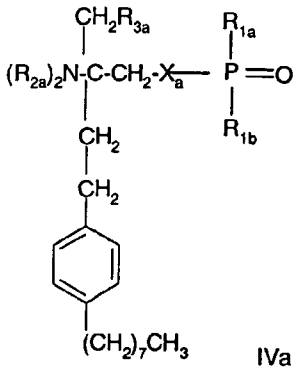
X is H or unsubstituted or substituted straight chain alkyl having a number p of carbon atoms or unsubstituted or substituted straight chain alkoxy having a number (p-1) of carbon atoms, e.g. substituted by 1 to 3 substitutents selected from the group consisting of C_{1-6} alkyl, OH, C_{1-6}alkoxy, acyloxy, amino, C_{1-6}alkylamino, acylamino, oxo, haloC_{1-6}alkyl, halogen, unsubstituted phenyl and phenyl substituted by 1 to 3 substituents selected from the group consisting of C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl and halogen; Y is H, C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl or halogen, Z_2 is a single bond or a straight chain alkylene having a number or carbon atoms of q,

each of p and q, independently, is an integer of 1 to 20, with the proviso of $6 \leq p+q \leq 23$, m' is 1, 2 or 3, n is 2 or 3,

each of R''_1, R''_2, R''_3 and R''_4, independently, is H, C_{1-4}alkyl or acyl,

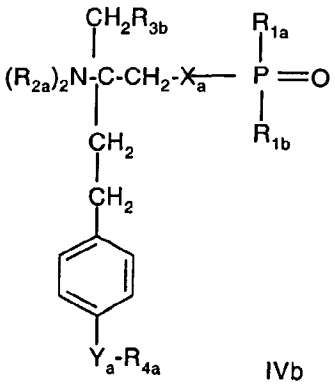
or a pharmaceutically acceptable salt thereof,

- Compounds as disclosed in WO02/18395, e.g. a compound of formula IVa or IVb



IVa

or



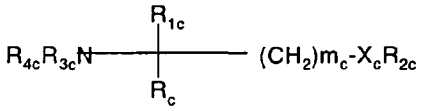
IVb

wherein X_a is O, S, NR_{1s} or a group $-(CH_2)_{na}-$, which group is unsubstituted or substituted by 1 to 4 halogen; n_a is 1 or 2, R_{1s} is H or (C_{1-4})alkyl, which alkyl is

unsubstituted or substituted by halogen; R_{1a} is H, OH, (C_{1-4})alkyl or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by 1 to 3 halogen; R_{1b} is H, OH or (C_{1-4})alkyl, wherein alkyl is unsubstituted or substituted by halogen; each R_{2a} is independently selected from H or (C_{1-4})alkyl, which alkyl is unsubstituted or substitued by halogen; R_{3a} is H, OH, halogen or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; and R_{3b} is H, OH, halogen, (C_{1-4})alkyl wherein alkyl is unsubstituted or substituted by hydroxy, or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; Y_a is $-CH_2-$, -C(O)-, -CH(OH)-, -C(=NOH)-, O or S, and R_{4a} is (C_{4-14})alkyl or (C_{4-14})alkenyl;

or a pharmaceutically acceptable salt or hydrate thereof;

- Compounds as disclosed in WO 02/076995, e.g. a compound of formula V



V

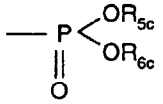
wherein

m_c is 1, 2 or 3;

X_c is O or a direct bond;

R_{1c} is H; C_{1-6} alkyl optionally substituted by OH, acyl, halogen, C_{3-10}cycloalkyl, phenyl or hydroxy-phenylene; C_{2-6}alkenyl; C_{2-6}alkynyl; or phenyl optionally substituted by OH;

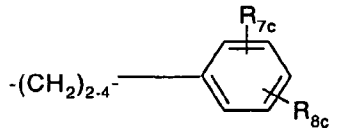
R_{2c} is



wherein R_{5c} is H or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms, and R_{6c} is H or C_{1-4}alkyl optionally substituted by halogen;

each of R_{3c} and R_{4c}, independently, is H, C_{1-4}alkyl optionally substituted by halogen, or acyl, and

R_c is C_{13-20}alkyl which may optionally have in the chain an oxygen atom and which may optionally be substituted by nitro, halogen, amino, hydroxy or carboxy; or a residue of formula (a)



(a)

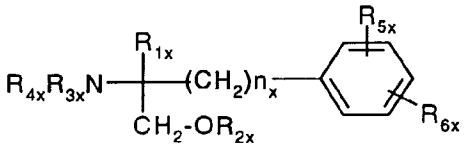
wherein R_{7c} is H, C_{1-4}alkyl or C_{1-4}alkoxy, and R_{8c} is substituted C_{1-20}alkanoyl, phenylC_{1-14}alkyl wherein the C_{1-14}alkyl is optionally substituted by halogen or OH, cycloalkylC_{1-14}alkoxy or phenylC_{1-14}alkoxy wherein the cycloalkyl or phenyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy, phenylC_{1-14}alkoxy-

C_{1-14}alkyl, phenoxyC_{1-14}alkoxy or phenoxyC_{1-14}alkyl,

R_c being also a residue of formula (a) wherein R_{8c} is C_{1-14}alkoxy when R_{1c} is C_{1-4}alkyl,

C_{2-6}alkenyl or C_{2-6}alkynyl,

or a compound of formula VI



VI

wherein

n_x is 2, 3 or 4

R_{1x} is H; C_{1-6}alkyl optionally substituted by OH, acyl, halogen, cycloalkyl, phenyl or hydroxy-phenylene; C_{2-6}alkenyl; C_{2-6}alkynyl; or phenyl optionally substituted by OH;

R_{2x} is H, C_{1-4} alkyl or acyl

each of R_{3x} and R_{4x}, independently is H, C_{1-4}alkyl optionally substituted by halogen or acyl,

R_{5x} is H, C_{1-4}alkyl or C_{1-4}alkoxy, and

R_{6x} is C_{1-20} alkanoyl substituted by cycloalkyl; cyloalkylC_{1-14}alkoxy wherein the cycloalkyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy;

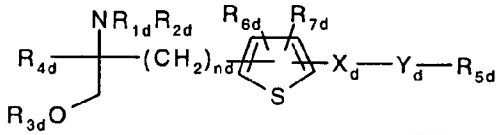
phenylC_{1-14}alkoxy wherein the phenyl ring is optionally substituted by halogen, C_{1-4}alkyl and/or C_{1-4}alkoxy,

R_{6x} being also C_{4-14}alkoxy when R_{1x} is C_{2-4}alkyl substituted by OH, or pentyloxy or hexyloxy when R_{1x} is C_{1-4}akyl,

provided that R_{6x} is other than phenyl-butylenoxy when either R_{5x} is H or R_{1x} is methyl,

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in WO02/06268Al, e.g. a compound of formula VII



VII

wherein each of R_{1d} and R_{2d}, independently, is H or an amino-protecting group;

R_{3d} is hydrogen or a hydroxy-protecting group;

R_{4d} is lower alkyl;

n_d is an integer of 1 to 6;

X_d is ethylene, vinylene, ethynylene, a group having a formula – D-CH_2- (wherein D is carbonyl, – CH(OH)-, O, S or N), aryl or aryl substituted by up to three substitutents selected from group a as defined hereinafter;

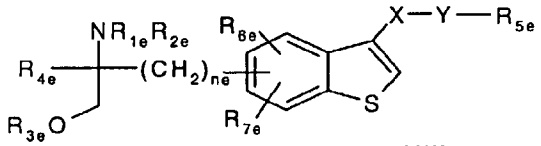
Y_d is single bond, C_{1-10}alkylene, C_{1-10}alkylene which is substituted by up to three substitutents selected from groups a and b, C_{1-10}alkylene having O or S in the middle or end of the carbon chain, or C_{1-10}alkylene having O or S in the middle or end of the carbon chain which is substituted by up to three substituents selected from groups a and b;

R_{5d} is hydrogen, cycloalkyl, aryl, heterocycle, cycloalkyl substituted by up to three substituents selected from groups a and b, aryl substituted by up to three substituents selected from groups a and b, or heterocycle substituted by up to three substituents selected from groups a and b; and

each of R_{6d} and R_{7d}, independently, is H or a substituent selected from group a;

<group a > is halogen, lower alkyl, halogeno lower alkyl, lower alkoxy, lower alkylthio, carboxyl, lower alkoxycarbonyl, hydroxy, lower aliphatic acyl, amino, mono-lower alkylamino, di-lower alkylamino, lower aliphatic acylamino, cyano or nitro;
<group b > is cycloalkyl, aryl, heterocycle, each being optionally substituted by up to three substituents selected from group a;
with the proviso that when R_{5d} is hydrogen, Y_d is a either a single bond or linear C_{1-10} alkylene, or a pharmacologically acceptable salt or ester thereof.

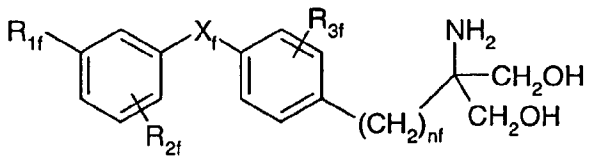
-Compounds as disclosed in JP-14316985 (JP2002316985), e.g. a compound of formula VIII:



VIII

wherein R_{1e},R_{2e},R_{3e},R_{4e},R_{5e},R_{6e},R_{7e}, n_e, X_e and Y_e are as disclosed in JP-14316985; or a pharmacologically acceptable salt or ester thereof.

-Compounds as disclosed in WO 03/29184 and WO 03/29205, e.g. compounds of formula IX



IX

wherein X_f is O or S, and R_{1f}, R_{2f}, R_{3f} and n_f are as disclosed in WO 03/29184 and O3/29205, e.g. 2-amino-2-[4-(3-benzyloxyphenoxy)-2-chlorophenyl]propyl-1,3-propane-diol or 2-amino-2-[4-(benzyloxyphenylthio)-2- chlorophenyl]propyl-1,3-propane-diol.

In each case where citations of patent applications are given, the subject matter relating to the compounds is hereby incorporated into the present application by reference.

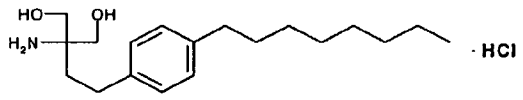
Acyl may be a residue R_y-CO- wherein R_y is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenyl-C_{1-4}alkyl. Unless otherwise stated, alkyl, alkoxy, alkenyl or alkynyl may be straight or branched.

When in the compounds of formula I the carbon chain as R_1 is substituted, it is preferably substituted by halogen, nitro, amino, hydroxy or carboxy. When the carbon chain is interrupted by an optionally substituted phenylene, the carbon chain is preferably unsubstituted. When the phenylene moiety is substituted, it is preferably substituted by halogen, nitro, amino, methoxy, hydroxy or carboxy.

Preferred compounds of formula I are those wherein R_1 is C_{13-20}alkyl, optionally substituted by nitro, halogen, amino, hydroxy or carboxy, and, more preferably those wherein R_1 is phenylalkyl substituted by C_{6-14}-alkyl chain optionally substituted by halogen and the alkyl moiety is a C_{1-6}alkyl optionally substituted by hydroxy. More preferably, R_1 is phenyl-C_{1-6}alkyl substituted on the phenyl by a straight or branched, preferably straight, C_{6-14}alkyl chain. The C_{6-14}alkyl chain may be in ortho, meta or para, preferably in para.

Preferably each of R_2 to R_5 is H.

A preferred compound of formula I is 2-amino-2-tetradecyl-1,3-propanediol. A particularly preferred S1P receptor agonist of formula I is FTY720, i.e. 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol in free form or in a pharmaceutically acceptable salt form (referred to hereinafter as Compound A), e.g. the hydrochloride, as shown:



A preferred compound of formula II is the one wherein each of R'_2 to R'_5 is H and m is 4, i.e. 2-amino-2-{2-[4-(1-oxo-5-phenylpentyl)phenyl]ethyl}propane-1,3-diol, in free form or in pharmaceutically acceptable salt form (referred to hereinafter as Compound B), e.g the hydrochloride.

A preferred compound of formula III is the one wherein W is CH_3, each of R''_1 to R''_3 is H, Z_2 is ethylene, X is heptyloxy and Y is H, i.e. 2-amino-4-(4-heptyloxyphenyl)-2-methyl-butanol, in free form or in pharmaceutically acceptable salt form (referred to

hereinafter as Compound C), e.g. the hydrochloride. The R-enantiomer is particularly preferred.

A preferred compound of formula IVa is the FTY720-phosphate (R_{2a} is H, R_{3a} is OH, X_a is O, R_{1a} and R_{1b} are OH). A preferred compound of formula IVb is the Compound C-phosphate (R_{2a} is H, R_{3b} is OH, X_a is O, R_{1a} and R_{1b} are OH, Y_a is O and R_{4a} is heptyl). A preferred compound of formula V is Compound B-phosphate.

A preferred compound of formula V is phosphoric acid mono-[(R)-2-amino-2-methyl-4-(4-pentyloxy-phenyl)-butyl]ester.

A preferred compound of formula VIII is (2R)-2-amino-4-[3-(4-cyclohexyloxybutyl)benzo[b]thien-6-yl]-2-methylbutan-1-ol.

When the compounds of formulae I to IX have one or more asymmetric centers in the molecule, the present invention is to be understood as embracing the various optical isomers, as well as racemates, diastereoisomers and mixtures thereof are embraced. Compounds of formula III or IVb, when the carbon atom bearing the amino group is asymmetric, have preferably the R-configuration at this carbon atom.

Examples of pharmaceutically acceptable salts of the compounds of the formulae I to IX include salts with inorganic acids, such as hydrochloride, hydrobromide and sulfate, salts with organic acids, such as acetate, fumarate, maleate, benzoate, citrate, malate, methanesulfonate and benzenesulfonate salts, or, when appropriate, salts with metals such as sodium, potassium, calcium and aluminium, salts with amines, such as triethylamine and salts with dibasic amino acids, such as lysine. The compounds and salts of the methods of the present invention encompass hydrate and solvate forms.

The co-agent b) may be selected from the following groups of compounds:

i) Interferons, e.g. pegylated or non-pegylated α-interferons, or β-interferons or τ–interferons, e.g. administered by subcutaneous, intramuscular or oral routes, preferably β-interferons;

ii) An altered peptide ligand such as Glatiramer, e.g. in the acetate form;

iii) Immunosuppressants with optionally antiproliferative/antineoplastic activity, e.g. mitoxantrone, methotrexate, azathioprine, cyclophosphamide, or steroids, e.g. methylprednisolone, prednisone or dexamethasone, or steroid-secreting agents, e.g. ACTH;

iv) Adenosine deaminase inhibitors, e.g. cladribine;

v) IV immunoglobulin G (e.g. as disclosed in Neurology, 1998, May 50(5):1273-81

vi) Monoclonal antibodies to various T-cell surface markers, e.g. natalizumab (ANTEGREN®) or alemtuzumab;

vii) TH2 promoting cytokines, e.g. IL-4, IL-10, or compounds which inhibit expression of TH1 promoting cytokines, e.g. phosphodiesterase inhibitors, e.g. pentoxifylline;

viii) Antispasticity agents including baclofen, diazepam, piracetam, dantrolene, lamotrigine, rifluzole, tizanidine, clonidine, beta blockers, cyproheptadine, orphenadrine or cannabinoids;

ix) AMPA glutamate receptor antagonists, e.g. 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline, [1,2,3,4,-tetrahydro-7-morpholin-yl-2,3-dioxo-6-(trifluoromethyl)quinoxalin-1-yl]methylphosphonate, 1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5H-2,3-benzodiazepine, or (-)1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-4,5-dihydro-3-methylcarbamoyl-2,3-benzodiazepine;

x) Inhibitors of VCAM-1 expression or antagonists of its ligand, e.g. antagonists of the $\alpha 4\beta 1$ integrin VLA-4 and/or alpha-4-beta-7 integrins, e.g. natalizumab (ANTEGREN®);

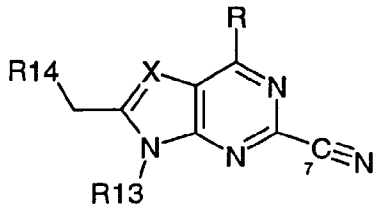
xi) Anti-Macrophage migration inhibitory factor (Anti-MIF);

xii) Cathepsin S inhibitors;

xiii) mTor inhibitors.

Cathepsin S inhibitors include e.g.:

a) a compound as disclosed in WO 03/20721, e.g. a compound of formula:



I

wherein

R is H, -R2, -OR2 or NR1R2,

wherein R1 is H, lower alkyl or C_3 to C_{10} cycloalkyl, and

R2 is lower alkyl or C_3 to C_{10} cycloalkyl, and

wherein each of R1 and R2 independently, is optionally substituted by halo, hydroxy, lower alkoxy, CN, NO_2, or optionally mono- or di-lower alkyl substituted amino;

X is =N- or =C(Z)-,

wherein Z is H, –C(O)-NR3R4, -NH-C(O)-R3, -CH_2-NH-C(O)-R3, -C(O)-R3, -S(O)-R3, -$S(O)_2$-R3,-CH_2-C(O)-R3, -CH_2-NR3R4, -R4, -C≡C-CH_2-R5, N-heterocyclyl, N-heterocyclyl-carbonyl, or –C(P)=C(Q)-R4

wherein

each of P and Q, independently, is H, lower alkyl or aryl,

R3 is aryl, aryl–lower alkyl, C_3-C_{10}cycloalkyl, C_3-C_{10}cycloalkyl-lower alkyl, heterocyclyl or heterocyclyl-lower alkyl,

R4 is H, aryl, aryl–lower alkyl, aryl-lower-alkenyl, C_3-C_{10}cycloalkyl, C_3-C_{10}cycloalkyl-lower alkyl, heterocyclyl or heterocyclyl-lower alkyl, or

wherein R3 and R4 together with the nitrogen atom to which they are joined to form an N-heterocyclyl group,

wherein N-heterocyclyl denotes a saturated, partially unsaturated or aromatic nitrogen containing heterocyclic moiety attached via a nitrogen atom thereof having from 3 to 8 ring atoms optionally containing a further 1, 2 or 3 heteroatoms selected from N, NR6, O, S, S(O) or $S(O)_2$ wherein R6 is H or optionally substituted (lower alkyl, carboxy, acyl (including both lower alkyl acyl, e.g. formyl, acetyl or propionyl, or aryl acyl, e.g. benzoyl), amido, aryl, S(O) or $S(O)_2$), and wherein the N-heterocyclyl is optionally fused in a bicyclic structure, e.g. with a benzene or pyridine ring, and wherein the N-heterocyclyl is optionally linked in a spiro structure with a 3 to 8

membered cycloalkyl or heterocyclic ring wherein the heterocyclic ring has from 3 to 10 ring members and contains from 1 to 3 heteroatoms selected from N, NR6, O, S, S(O) or $S(O)_2$ wherein R6 is as defined above), and

wherein heterocyclyl denotes a ring having from 3 to 10 ring members and containing from 1 to 3 heteroatoms selected from N, NR6, O, S, S(O) or $S(O)_2$ wherein R6 is as defined above), and

wherein each of R3 and R4, independently, is optionally substituted by one or more groups, e.g. 1-3 groups, selected from halo, hydroxy, oxo, lower alkoxy, CN or NO_2, or optionally substituted (optionally mono- or di-lower alkyl substituted amino, aryl, aryl-lower alkyl, N-heterocyclyl or N-heterocyclyl-lower alkyl (wherein the optional substitution comprises from 1 to 3 substituents selected from halo, hydroxy, lower alkoxy, CN, NO_2, or optionally mono- or di-lower alkyl substituted amino)), and

wherein

R5 is aryl, aryl-lower alkyl, aryloxy, aroyl or N-heterocyclyl as defined above, and

wherein R5 is optionally substituted by R7 which represents from 1 to 5 substitutents selected from halo, hydroxy, CN, NO_2 or oxo, or optionally substituted (lower-alkoxy, lower-alkyl, aryl, aryloxy, aroyl, lower-alkylsulphonyl, arylsulphonyl, optionally mono- or di-lower alkyl substituted amino, or N-heterocyclyl, or N-heterocyclyl-lower alkyl (wherein N-heterocyclyl is as defined above), and

wherein R7 is optionally substituted by from 1 to 3 substitutents selected from halo, hydroxy, optionally mono- or di- lower-alkyl substituted amino, lower-alkyl carbonyl, lower-alkoxy or lower-alkylamido;

R13 is lower alkyl, C3 to C10 cycloalkyl or C3-C10cycloalkyl-lower alkyl, all of which are independently optionally substituted by halo, hydroxy, CN, NO2 or optionally mono- or di-lower alkyl-substituted amino; and

R14 is H or optionally substituted (aryl, aryl-W-, aryl-lower alkyl-W-, C3 to C10 cycloalkyl, C3 to C10 cycloalkyl-W-, N-heterocyclyl or N-heterocyclyl-W- (wherein N-heterocyclyl is as defined above), phthalimide, hydantoin, oxazolidinone, or 2,6-dioxo-piperazine),

wherein –W- is –O-, -C(O)-, -NH(R6)-, -NH(R6)-C(O)-, -NH(R6)-C(O)-O-, (where R6 is as defined above),-S(O)-, $-S(O)_2-$ or –S-,

wherein R14 is optionally substituted by R18 which represents from 1 to 10 substitutents selected from halo, hydroxy, CN, NO_2, oxo, amido, carbonyl, sulphonamido, lower-alkyldioxymethylene, or optionally substituted (lower-alkoxy, lower-alkyl, lower-alkenyl, lower alkynyl, lower alkoxy carbonyl, optionally mono- or di-lower alkyl substituted amino, aryl, aryl-lower alkyl, aryl-lower alkenyl, aryloxy, aroyl, lower-alkylsulphonyl, arylsulphonyl, N-heterocyclyl, N-heterocyclyl-lower alkyl (wherein N-heterocyclyl is as defined above), heterocyclyl or R14 comprising aryl has aryl fused with a hetero-atom containing ring, and
wherein R18 is optionally substituted by R19 which represents from 1 to 4 substitutents selected from halo, hydroxy, CN, NO_2 or oxo, or optionally substituted (lower-alkoxy, lower-alkyl, lower-alkoxy-lower-alkyl, C_3-C_{10}cycloalkyl, lower-alkoxy carbonyl, halo-lower alkyl, optionally mono- or di-lower alkyl substituted amino, aryl, aryloxy, aroyl (e.g. benzoyl), acyl (e.g. lower-alkyl carbonyl), lower-alkylsulphonyl, arylsulphonyl or N-heterocyclyl, or N-heterocyclyl-lower alkyl (wherein N-heterocyclyl is as defined above)),

wherein R19 is optionally substituted by from 1 to 4 substitutents selected from halo, hydroxy, CN, NO_2, oxo, optionally mono- or di-lower alkyl substituted amino, lower-alkyl, or lower-alkoxy;

b) a compound as disclosed in WO 00/69855, e.g. N2-(3-furanylcarbonyl)-L-norleucine-2(S)-methyl-4-oxotetrahydrofuran-3(R)-yl amide;

c) a compound as disclosed in WO 01/19796, WO 01/19808, WO 02/51983, WO 03/24923, WO 03/24924, WO 03/41649 or WO 03/42197, e.g. N-(2-(1-cyanocyclopropylamino)-1(R)-(2-benzylsulfonylmethyl)-2-oxoethyl)morpholine-4-carboxamide, N-(2-(cyanomethylamino)-1-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)pyridine-4-carboxamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)-3,4-difluorobenzamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)-3-methylbenzamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)-1H-indole-5-carboxamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)-5-methylthiophene-2-carboxamide, N-(2-(4-cyano-1-methylpiperidin-4-ylamino)-1(R)-

(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)morpholine-4-carboxamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)-4-fluorobenzamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)thiophene-3-carboxamide, N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)thiophene-2-carboxamide or N-(2-(cyanomethylamino)-1(R)-(2-(difluoromethoxy)benzylsulfonylmethyl)-2-oxoethyl)morpholine-4-carboxamide;

d) a compound as disclosed in WO 00/51998, WO 03/29200 or WO 03/37892, e.g. N-(1(S)-(N-(2-(benzyloxy)-1(R)-cyanoethyl)carbamoyl)-2-cyclohexylethyl)morpholine-4-carboxamide;

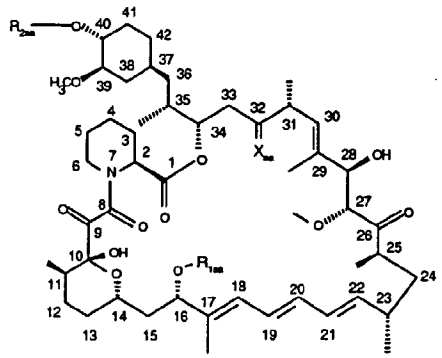
e) a compound as disclosed in WO 02/14314, WO 02/14315 or WO 02/14317, e.g. N1-(3-chloro-2-(4-(2-hydroxy-3-(5-(methylsulfonyl)-3-(4-(trifluoromethyl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo(4,3-pyridin-1-yl)propyl)piperazin-1-yl)phenyl)-N3-methylurea, 1-(1-(3-(3-(4-bromophenyl)-5-(methylsulfonyl)-4,5,6,7-tetrahydro-1H-pyrazolo(4,3-c)pyridine-1-yl)-2-hydroxypropyl)piperidin-4-yl)-6-chloro-1,2,3,4-tetrahydroquinolin-2-one, or 1-(5-(methylsulfonyl)-3-(4-(trifluoromethyl)phenyl-4,5,6,7-tetrahydro-1H-pyrazolo(4,3-c)pyridine-1-yl)-3-(4-(6-(4-morpholinyl)-1H-pyrrolo(3,2-c)pyridine-3-yl)piperidin-1-yl)propan-2-ol;

f) a compound as disclosed in WO 01/89451, e.g. 5-(2-morpholin-4ylethoxy)benzofuran-2-carboxylic acid ((S)-3-methyl-1-((S)-3-oxo-1-(2-(3-pyridin-2-ylphenyl)-acetyl)azepan-4-ylcarbamoyl)butylamide;

g) a compound as disclosed in WO 02/32879, WO 01/09169 or WO 00/59881A1, e.g. N-(1-benzothien-2-ylcarbonyl)-N-(2-(2-fluorophenyl)-4-oxo-1,2,3,4-tetrahydropyrimidin-5-yl)-L-leucinamide;

h) a compound as disclosed in WO 00/48992, WO 00/49007 or WO 00/49008.

The term "mTOR inhibitor" as used herein includes, but is not limited to rapamycin (sirolimus) or a derivative thereof. Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus. Suitable derivatives of rapamycin include e.g. compounds of formula A



A

wherein

R_{1aa} is CH_3 or C_{3-6}alkynyl,

R_{2aa} is H or $-CH_2-CH_2-OH$, 3-hydroxy-2-(hydroxymethyl)-2-methyl-propanoyl or tetrazolyl, and

X_{aa} is =O, (H,H) or (H,OH)

provided that R_{2aa} is other than H when X_{aa} is =O and R_{1aa} is CH_3.

or a prodrug thereof when R_{2aa} is $-CH_2-CH_2-OH$, e.g. a physiologically hydrolysable ether thereof.

Compounds of formula A are disclosed e.g. in WO 94/09010, WO 95/16691, WO 96/41807, USP 5,362,718 or WO 99/15530 which are incorporated herein by reference. They may be prepared as diclosed or by analogy to the procedures described in these references.

Preferred rapamycin derivatives are 32-deoxorapamycin, 16-pent-2-ynyloxy-32-deoxorapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin and, more preferably, 40-0-(2-hydroxyethyl)-rapamycin. Further examples of rapamycin derivatives include e.g. CCI779 or 40- [3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]-rapamycin or a pharmaceutically acceptable salt thereof, as disclosed in USP 5,362,718, ABT578 or 40-(tetrazolyl)-rapamycin, particularly 40-epi-(tetrazolyl)-rapamycin, e.g. as disclosed in WO 99/15530, or rapalogs as disclosed e.g. in WO 98/02441 and WO01/14387, e.g. AP23573 or TAFA-93.

In each case where citations of patent applications or scientific publications are given, the subject-matter relating to the compounds is hereby incorporated into the present application by reference. Comprised are likewise the pharmaceutically acceptable salts thereof, the corresponding racemates, diastereoisomers, enantiomers, tautomers as well as the corresponding crystal modifications of above disclosed compounds where present, e.g. solvates, hydrates and polymorphs, which are disclosed therein. The compounds used as active ingredients in the combinations of the invention can be prepared and administered as described in the cited documents, respectively. Also within the scope of this invention is the combination of more than two separate active ingredients as set forth above, i.e. a pharmaceutical combination within the scope of this invention could include three active ingredients or more. Further both the first agent and the co-agent are not the identical ingredient.

Utility of the compounds of formula I in treating demyelinating diseases, e.g. multiple sclerosis or Guillain-Barré syndrome as hereinabove specified, may be demonstrated in animal test methods as well as in clinic, for example in accordance with the methods hereinafter described. The most widely used animal model for multiple sclerosis is experimental autoimmune encephalomyelitis (EAE), based on shared histopathological and clinical features with the human disease.

A.1 In vivo: SJL/J Mouse model of chronic progressive EAE

The disease course in this animal model shares some common features with SP- and PR-MS. Immunization: On day 0 female SJL/J mice are immunized (subcutaneous flank injection) with 200 µl inoculum containing 500 µg bovine myelin basic protein (MBP) emulsified in complete Freund's adjuvant (CFA). On day 9 mice are boosted by a second MBP injection and an additional intravenous adjuvant injection consisting of 200 ng *B. pertussis* toxin. A final Pertussis injection is given on day 11.

Most of the MBP-immunized mice exhibit a severe bout of EAE by day 21. This is followed by a recovery phase starting around day 25, during which time mice remain symptom-free for about 20 days. Subsequently, by days 45-47, approximately 50% of the animals go into the progressive phase of the disease. Therefore, therapeutic

treatment with test compounds starts on day 21 when the disease is fully established and continues until day 70, unless stated otherwise. Recombinant mouse interferon β (INFβ Calbiochem/Biosciences) is dissolved in saline and given by intraperitoneal injection 3x per week. Compound (a), e.g. Compound A or B, is diluted in water and given p.o. 5x per week by gavage. Mice in the vehicle control group are MBP-immunized and treated with water.

Each experimental group consists of 10 mice, which are examined daily for clinical EAE symptoms. Disease incidence and the day of EAE onset also are recorded. Clinical grades of EAE are assessed using a scale from 0 to 3. Any disease-related mortality which occurs after starting drug treatment is recorded with a maximum score of 3.

Compound (a), e.g. Compound A or B at 0.6 mg/kg p.o. in combination with INFβ (10 000 IU) prevents disease progression for one month (days 45-75), compared to the vehicle-treated controls. In contrast, administration of INFβ alone (10 000 IU 3x/week) only marginally inhibits disease progression for about 1 week, after which the mice went on to develop a full EAE response that is indistinguishable from the disease course in vehicle-treated controls by day 68 onwards.

A.2 In vivo: Optic Neuritis in the DA rat model of chronic-protracted EAE

Ocular pathologic manifestations such as optic neuritis (neuromyelitis optica) are frequent in multiple sclerosis and often precede or accompany plaque formation in the brain white matter. Ocular areas, especially the optic chiasma, also are important targets in demyelinating forms of EAE. In such EAE models, functional disability caused by demyelination of the optic nerve can be assessed by electrophysiological methods, such as visual evoked cortical potentials and electroretinogram, in conjunction with morphological analysis of the ocular tissue.

Immunization: On day 0, female DA rats are immunized by a single intradermal injection at the tail base with 100-200 µl inoculum containing a recombinant encephalitogenic peptide, e.g. myelin oligodendrocyte glycoprotein, or a homogenate of syngeneic central nervous system tissue emulsified in one part CFA (volume:volume). Neurologic symptoms develop by 10 days post-immunization, and

clinical grades of EAE are assessed using a scale of 0 to 4. Therapeutic treatment with the test compound starts when the disease is fully established, usually on day 12, and continues for 2 weeks. Compound (a), e.g. Compound A or B at 0.3 mg/kg p.o. given once a day for 2 weeks, prevents disease progression for at least 2 months, compared to the vehicle-treated controls. Using combination treatment, suboptimal doses of Compound A or B (<0.1 mg/kg p.o.) and a mTOR inhibitor (<1 mg/kg p.o.) also curtail development of EAE symptoms and prevent disease-related weight loss after therapeutic dosing in the DA rat model. In a prophylactic treatment regimen, similar combinations of Compound A or B and a mTOR inhibitor prevent disease onset in the Lewis rat model of EAE, induced by an intradermal injection of guinea pig neuroantigen.

B Clinical Trial

Suitable clinical studies are, for example, open label, dose escalation studies in patients with multiple sclerosis. Such studies prove in particular the synergism of the active ingredients of the combination of the invention. The beneficial effects on multiple sclerosis can be determined directly through the results of these studies which are known as such to a person skilled in the art. Such studies are, in particular, suitable to compare the effects of a monotherapy using the active ingredients and a combination of the invention. Preferably, the dose of agent (a) is escalated until the Maximum Tolerated Dosage is reached, and the co-agent (b) is administered with a fixed dose. Alternatively, the agent (a) is administered in a fixed dose and the dose of co-agent (b) is escalated. Each patient receives doses of the agent (a) either daily or intermittent. The efficacy of the treatment can be determined in such studies, e.g., after 12, 18 or 24 weeks by evaluation of symptom scores every 6 weeks.

Alternatively, a placebo-controlled, double blind study can be used in order to prove the benefits of the combination of the invention mentioned herein.

The administration of a pharmaceutical combination of the invention results not only in a beneficial effect, e.g. a synergistic therapeutic effect, e.g. with regard to alleviating, delaying progression of or inhibiting the symptoms, but also in further surprising beneficial effects, e.g. fewer side-effects, an improved quality of life or a

decreased morbidity, compared with a monotherapy applying only one of the pharmaceutically active ingredients used in the combination of the invention.

A further benefit is that lower doses of the active ingredients of the combination of the invention can be used, for example, that the dosages need not only often be smaller but are also applied less frequently, which may diminish the incidence or severity of side-effects. This is in accordance with the desires and requirements of the patients to be treated.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

It is one objective of this invention to provide a pharmaceutical composition comprising a quantity, which is jointly therapeutically effective against multiple sclerosis or disorders associated therewith comprising a combination of the invention. In this composition, the first agent a) and co-agent (b) may be administered together, one after the other or separately in one combined unit dosage form or in two separate unit dosage forms. The unit dosage form may also be a fixed combination.

The pharmaceutical compositions for separate administration of the first agent a) and co-agent b) or for the administration in a fixed combination, i.e. a single galenical composition comprising at least two combination partners a) and b), according to the invention may be prepared in a manner known per se and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including humans, comprising a therapeutically effective amount of at least one pharmacologically active combination partner alone, e.g. as indicated above, or in combination with one or more pharmaceutically acceptable carriers or diluents, especially suitable for enteral or parenteral application.

Suitable pharmaceutical compositions contain, for example, from about 0.1 % to about 99.9%, preferably from about 1 % to about 60 %, of the active ingredient(s).

Pharmaceutical preparations for the combination therapy for enteral or parenteral administration are, for example, those in unit dosage forms, such as sugar-coated tablets, tablets, capsules or suppositories, or ampoules. If not indicated otherwise, these are prepared in a manner known per se, for example by means of conventional mixing, granulating, sugar-coating, dissolving or lyophilizing processes. It will be appreciated that the unit content of a combination partner contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached by administration of a plurality of dosage units.

In particular, a therapeutically effective amount of each of the combination partner of the combination of the invention may be administered simultaneously or sequentially and in any order, and the components may be administered separately or as a fixed combination. For example, the method of delay of progression or treatment of multiple sclerosis or disorders associated therewith according to the invention may comprise (i) administration of the first agent a) in free or pharmaceutically acceptable salt form and (ii) administration of a co-agent b) in free or pharmaceutically acceptable salt form, simultaneously or sequentially in any order, in jointly therapeutically effective amounts, preferably in synergistically effective amounts, e.g. in daily or intermittently dosages corresponding to the amounts described herein. The individual combination partners of the combination of the invention may be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. Furthermore, the term administering also encompasses the use of a pro-drug of a combination partner that convert *in vivo* to the combination partner as such. The instant invention is therefore to be understood as embracing all such regimens of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

The effective dosage of each of the combination partners employed in the combination of the invention may vary depending on the particular compound or pharmaceutical composition employed, the mode of administration, the condition being treated, the severity of the condition being treated. Thus, the dosage regimen of the combination of the invention is selected in accordance with a variety of factors

including the route of administration and the renal and hepatic function of the patient. A physician, clinician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the single active ingredients required to alleviate, counter or arrest the progress of the condition. Optimal precision in achieving concentration of the active ingredients within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the active ingredients' availability to target sites, particularly when co-agent b) is a small molecule.

Daily dosages for the first agent a) will, of course, vary depending on a variety of factors, for example the compound chosen, the particular condition to be treated and the desired effect. In general, however, satisfactory results are achieved on administration of agent a) at daily dosage rates of the order of ca. 0.03 to 2.5 mg/kg per day, particularly 0.1 to 2.5 mg/kg per day, e.g. 0.5 to 2.5 mg/kg per day, as a single dose or in divided doses. The S1P receptor agonist, e.g. a compound of formulae I to VII, e.g. Compound A or B, may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise from ca. 0.02 to 50 mg active ingredient, usually 0.1 to 30 mg, e.g. Compound A or B, together with one or more pharmaceutically acceptable diluents or carriers therefor. These dosages are also indicated when the S1P receptor agonist is used alone in the treatment of optic neuritis.

Interferons may be administered to a human in the following dosage ranges: interferon β–1b: up to 0.25 mg sc; interferon β–1a: up to 30 μg im; interferon α-2a: up to 10 million I.U. (MIU) sc or up to 1 MIU orally; interferon α-2b: up to 10 MIU sc or up to 1 MIU orally; pegylated interferon α-2a: up to 270 μg sc; pegylated interferon α-2b: up to 2.0 μg/kg sc.

Glatiramer may be administered to a human in a dosage range up to 20 mg sc, or up to 50 mg po.

Antineoplastic/antiproliferative immunosuppressants may be administered to a human in the forllowing dosage ranges: cyclophosphamide 500-1500 mg/m^2 IV; methotrexate up to 20 mg po; mitoxantrone 12 mg/m^2 IV, or azathioprine 2 mg/kg po.

Steroids may be administered to a human in the following dosage ranges: methylprednisolone 1-2-mg IV, or 24-48 mg po; prednisone 1 mg/kg po, or ACTH up to 100 MIU.

ADA inhibitors such as cladribine may be administered to a human in a dosage range up to 0.07 mg/kg/day.

IV Immunoglobulin G may be administered in a human in a dosage range up to 400 mg/kg IV.

Monoclonal antibodies to various T-cell surface markers may be administered in a human in the following dosage ranges: natalizumab up to 3mg/kg IV, alemtuzumab up to 30 mg sc or IV.

TH2 promoting cytokines may be administered to a human in the following dosage ranges: IL-4 up to 3μg/kg sc, or IL-10 up to 20μg/kg sc. Compounds which inhibit expression of TH1 promoting cytokines such as the phosphodiesterase inhibitor pentoxifylline may be administered in a human in a dosage range up to 4 mg po.

Antispasticity agents may be administered in a human in the following doage ranges: baclofen up to 100 mg po, diazepam up to 20 mg po, piracetam up to 24 mg po, dantrolene up to 100 mg po, lamotrigine up to 100 mg/day, riluzole up to 100 mg po, tizanidine up to 12 mg po, clonidine up to 0.1 mg po, β blockers (e.g. propanolol) up to 160 mg po, cyproheptadine up to 8 mg po, orphenadrine up to 100 mg po and cannabinoids (e.g. dronabinol) up to 5 mg po.

Cathepsin S inhibitors, e.g. a compound as disclosed in WO 03/20721, may be administered to a human in the dosage range 0.1 to 100 mg/kg/day.

mTor inhibitors, e.g. rapamycin or a derivative thereof, e.g. 40-O-(2-hydroxyethyl)-rapamycin, may be administered in a dosage range varying from about 0.1 to 25 mg/kg/day.

When used in treating, alleviating or delaying progression of optic neuritis, the S1P receptor agonist, e.g. a compound of formula I to VII, e.g. a compound A or B, may be administered systematically or topically, by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, topically, e.g. in the form of a topical ophthalmic composition, e.g. comprising an ophthalmic carrier. Pharmaceutical compositions comprising an S1P receptor agonist in association with at least one pharmaceutically acceptable carrier or diluent may be manufactured in conventional manner, e.g. by mixing the ingredients.

Compounds of formulae I to VII are well tolerated at dosages required for use in accordance with the present invention. For example, the acute LD_{50} is >10 mg/kg p.o. in rats and monkeys for Compound A.

CLAIMS

1. A pharmaceutical combination comprising:

a) a sphingosine-1-phosphate (S1P) receptor agonist, and

b) at least one co-agent shown to have clinical activity against at least one symptom of a demyelinating disease.

2. A pharmaceutical composition for treating, alleviating or delaying progression of optic neuritis comprising an S1P receptor agonist together with one or more pharmaceutically acceptable diluents or carriers therefor.

3. A combination or composition according to claim 1 or claim 2 wherein the S1P receptor agonist is selected from the compounds of formulae I to III, IVa, IVb, and V to VII substantially as described and defined herein.

4. A combination according to claim 1 or claim 3, wherein the co-agent b) is selected from the group consisting of interferons, altered peptide ligands, immunosuppressants, adenosine deaminase inhibitors, IV immunoglobulin G, monoclonal antibodies to T-cell surface markers, TH2 promoting cytokines, compounds which inhibit expression of TH1 promoting cytokines, antispasticity agents, AMPA glutamate receptor antagonists, inhibitors of VCAM-1 expression or antagonists of its ligand, anti-macrophage migration inhibitory factor, cathepsin S inhibitors and mTOR inhibitors.

5. A combination or composition according to any preceding claim, wherein the S1P receptor agonist is selected from 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, 2-amino-2-{2-[4-(1-oxo-5-phenylpentyl)phenyl]ethyl}propane-1,3-diol and their respective phosphate, in free form or in a pharmaceutically acceptable salt form.

6. A method for treating, alleviating or delaying progression of the symptoms of a demyelinating disease comprising co-administration of a therapeutically effective amount of a) an S1P receptor agonist, and b) at least one co-agent shown to have clinical activity against at least one symptom of a demyelinating disease.

7. A method for treating, alleviating or delaying progression of optic neuritis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an S1P receptor agonist.

8. A method according to claim 6 or 7 wherein the S1P receptor agonist is selected from a compound of formulae I to VII substantially as described and defined herein.

9. A method according to claim 6, 7 or 8 wherein the S1P receptor agonist is selected from 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, 2-amino-2-{2-[4-(1-oxo-5-phenylpentyl)phenyl]ethyl}propane-1,3-diol and their respective phosphate, in free form or in a pharmaceutically acceptable salt form.

10. A method according to claim 6, wherein the co-agent b) is selected from the group consisting of interferons, altered peptide ligands, immunosuppressants, adenosine deaminase inhibitors, IV immunoglobulin G, monoclonal antibodies to T-cell surface markers, TH2 promoting cytokines, compounds which inhibit expression of TH1 promoting cytokines, antispasticity agents, AMPA glutamate receptor antagonists, inhibitors of VCAM-1 expression or antagonists of its ligand, anti-macrophage migration inhibitory factor, cathepsin S inhibitors and mTOR inhibitors.

11. A combination or composition according to any of claims 1 to 5, for treating, alleviating or delaying progression of the symptoms of a demyelinating disease.

12. Use of a) a sphingosine-1-phosphate (S1P) receptor agonist, and b) at least one co-agent shown to have clinical activity against at least one symptom of a demyelinating disease, for the preparation of a pharmaceutical combination for treating, alleviating or delaying progression of the symptoms of a demyelinating disease.

13. Use of an S1P receptor agonist for the preparation of a medicament for treating, alleviating or delaying the progression of optic neuritis.

Electronic Acknowledgement Receipt

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First Named Inventor/Applicant Name:	Peter C. Hiestand
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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		50279_CNT_IDS.pdf	187681 e51219ae72e6bab935f8843029a7d76fdf1c6e3a	yes	3

	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Transmittal Letter		1	1	
	Information Disclosure Statement (IDS) Form (SB08)		2	3	
Warnings:					
Information:					
2	Foreign Reference	WO03099192.pdf	1892887 6e8701da61ce0c497d932370412163ebcbd223ac	no	45
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3	Foreign Reference	WO03097028.pdf	2444945 f9b23b57d40b545fe0e843317fb7709c0d3c3f03	no	49
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5	Foreign Reference	WO05123104.pdf	1096874 deb5c789fb5712e1e2ac5f334d19ac1d531c9b2e	no	19
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6	Non Patent Literature	LaMontagne.pdf	21746088 fbf1bed67337d01daf1e32c89ebfce924149795e	no	11
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7	Non Patent Literature	Ho.pdf	1299531 cb10de653d6061c98ef4a1865c0a39a3f08acfbb	no	10
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8	Non Patent Literature	Virely.pdf	158514 9034b9d785d0b1ded247548c811e64f2d5ad5984	no	12
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11	Foreign Reference	WO04028521.pdf	3033930 3f3459886dc5c388f4f45bf0449f5e5146f44839	no	29
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO: 13/149468
FILED: May 31, 2011
FOR: DOSAGE REGIMEN OF AN S1P RECEPTOR AGONIST

Art Unit: 1629
Examiner:

MS: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

This paper is being filed:

☒ supplemental to the Information Disclosure Statement filed .

☒ before the mailing date of a first Office Action on the merits, and so under 37 C.F.R. §1.97(b)(3) no fees are required.

If a fee is deemed to be required, the Commissioner is hereby authorized to charge such fee to Deposit Account No. 19-0134 in the name of Novartis.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

☒ Copies of the references are enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Respectfully submitted,

Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
+1 862 7783785
Date: 9/29/11

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/149,468

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	330
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	540
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	220
TOTAL CLAIMS (37 CFR 1.16(i))	11 minus 20 =	*			OR	x 52 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*				x 220 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							390
			TOTAL			TOTAL	1480

* If the difference in column 1 is less than zero, enter "0" in column 2.

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
13149468	

Applicant(s) Peter Hiestand

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1			1			
2			1			
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Total Indep	0		3		0	
Total Depend	0	↵	8	↵	0	↵
Total Claims	0		11		0	

	*		*		*	
	Indep	Depend	Indep	Depend	Indep	Depend
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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/149,468	05/31/2011	1629	1480	PAT050279-US-CNT	10	3

CONFIRMATION NO. 1536

1095
NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 101/2
EAST HANOVER, NJ 07936-1080

FILING RECEIPT

OC000000048231974

Date Mailed: 06/20/2011

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

Peter C. Hiestand, Allschwil, SWITZERLAND;
Christian Schnell, Hesingue, FRANCE;

Power of Attorney: The patent practitioners associated with Customer Number 001095

Domestic Priority data as claimed by applicant

This application is a CON of 12/303,765 12/08/2008 ABN
which is a 371 of PCT/EP2007/005597 06/25/2007

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)
UNITED KINGDOM 0612721.1 06/27/2006

Request to Retrieve - This application either claims priority to one or more applications filed in an intellectual property Office that participates in the Priority Document Exchange (PDX) program or contains a proper **Request to Retrieve Electronic Priority Application(s)** (PTO/SB/38 or its equivalent). Consequently, the USPTO will attempt to electronically retrieve these priority documents.

If Required, Foreign Filing License Granted: 06/15/2011

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/149,468**

Projected Publication Date: 09/29/2011

Non-Publication Request: No

Early Publication Request: No

Title

S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS

Preliminary Class

514

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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NOT GRANTED

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PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	1	of	2

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
		WO 2006/058316	06-01-2006			☐
		WO 2004/113330	12-29-2004			☐
						☐
						☐
						☐
						☐

Examiner Signature		**Date Considered**	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw a line through citation if not in conformance and not considered. Include copy of this form with the next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kind Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08b (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet	2	of	2

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	Hiestand, Peter C. et al.
Art unit	
Examiner Name	
Attorney Docket Number	PAT050279-US-CNT

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		Brinkmann, Volker et al., "The Immune Modulator FTY720 Targets Sphingosine 1-Phosphate Receptors", The Journal of Biological Chemistry, Vol. 277, No. 24, Issue of June 14, pp. 21453-21457, (2002).	☐
		Miller et al., Neurol, & Neurosci. Reports, (September, 2010), 1095), pp. 397-406	☐
		Hla, T., FASEB Journal, (March 6, 2006), 20(4), part 1, A20.	☐
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Examiner Signature		**Date Considered**	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw a line through citation if not in conformance and not considered. Include copy of this form with the next communication to applicant.

[1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Filer:	Karen DeBenedictis/Denise Cooper
Attorney Docket Number:	PAT050279-US-CNT

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	330	330
Utility Search Fee	1111	1	540	540
Utility Examination Fee	1311	1	220	220
Pages:				
Claims:				
Multiple dependent claims	1203	1	390	390
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	1480

Electronic Acknowledgement Receipt

EFS ID:	10199863
Application Number:	13149468
International Application Number:	
Confirmation Number:	1536
Title of Invention:	S1P RECEPTOR MODULATORS FOR TREATING MUTIPLE SCLEROSIS
First Named Inventor/Applicant Name:	Peter C. Hiestand
Customer Number:	01095
Filer:	Karen DeBenedictis/Denise Cooper
Filer Authorized By:	Karen DeBenedictis
Attorney Docket Number:	PAT050279-US-CNT
Receipt Date:	31-MAY-2011
Filing Date:	
Time Stamp:	17:48:14
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1480
RAM confirmation Number	5139
Deposit Account	190134
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		50279_CNT_PCTAPPLN.pdf	730979 4e4ab92ca95a3ecdd01f27f624483de675c7bcff	yes	17

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	15
Claims	16	17

Warnings:

Information:

2		50279_CNT_Appln.pdf	628946 500ee6065f08db80cf63315c2088588988f6a804	yes	17

Multipart Description/PDF files in .zip description

Document Description	Start	End
Transmittal of New Application	1	2
Application Data Sheet	3	5
Oath or Declaration filed	6	8
Preliminary Amendment	9	14
Transmittal Letter	15	15
Information Disclosure Statement (IDS) Filed (SB/08)	16	17

Warnings:

Information:

3	Fee Worksheet (PTO-875)	fee-info.pdf	36539 6e00ebf527b026a0d669945735d9d1765f044e1b	no	2

Warnings:

Information:

Total Files Size (in bytes):	1396464

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

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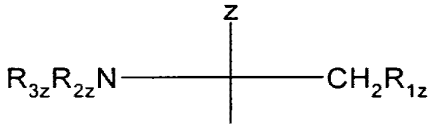
Organic Compounds

The present invention relates to the use of an S1P receptor modulator in the treatment or prevention of neo-angiogenesis associated with a demyelinating disease, e.g. multiple sclerosis.

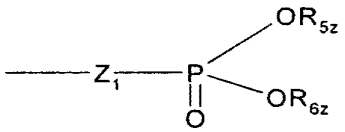
S1 P receptor modulators are typically sphingosine analogues, such as 2-substituted 2-amino- propane-1,3-diol or 2-amino-propanol derivatives, e. g. a compound comprising a group of formula X .

Sphingosine-1 phosphate (hereinafter "S1P") is a natural serum lipid. Presently there are eight known S1P receptors, namely S1P1 to S1P8. S1 P receptor modulators are typically sphingosine analogues, such as 2-substituted 2-amino- propane-1,3-diol or 2-amino-propanol derivatives, e. g. a compound comprising a group of formula X



(X)

wherein Z is H, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, phenyl, phenyl substituted by OH, C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH, or CH_2-R_{4z} wherein R_{4z} is OH, acyloxy or a residue of formula (a)



(a)

wherein Z_1 is a direct bond or O, preferably O;

each of R_{5z} and R_{6z}, independently, is H, or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms;

R_{1z} is OH, acyloxy or a residue of formula (a); and each of R_{2z} and R_{3z} independently, is H, C_{1-4}alkyl or acyl.

Group of formula X is a functional group attached as a terminal group to a moiety which may be hydrophilic or lipophilic and comprise one or more aliphatic, alicyclic, aromatic and/or heterocyclic residues, to the extent that the resulting molecule wherein at least one of Z and

R_{1z} is or comprises a residue of formula (a), signals as an agonist at one of more sphingosine-1-phosphate receptor.

S1P receptor modulators are compounds which signal as agonists at one or more sphingosine-1 phosphate receptors, e.g. S1P1 to S1P8. Agonist binding to a S1P receptor may e.g. result in dissociation of intracellular heterotrimeric G-proteins into Gα-GTP and Gβγ-GTP, and/or increased phosphorylation of the agonist-occupied receptor and activation of downstream signaling pathways/kinases.

The binding affinity of S1P receptor modulators to individual human S1P receptors may be determined in following assay:
S1P receptor modulator activities of compounds are tested on the human S1P receptors $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$ and $S1P_5$. Functional receptor activation is assessed by quantifying compound induced GTP [γ-^{35}S] binding to membrane protein prepared from transfected CHO or RH7777 cells stably expressing the appropriate human S1P receptor. The assay technology used is SPA (scintillation proximity based assay). Briefly, DMSO dissolved compounds are serially diluted and added to SPA- bead (Amersham-Pharmacia) immobilised S1P receptor expressing membrane protein (10-20μg/well) in the presence of 50 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$, 10 μM GDP, 0.1% fat free BSA and 0.2 nM GTP [γ-^{35}S] (1200 Ci/mmol). After incubation in 96 well microtiterplates at RT for 120 min, unbound GTP [γ-^{35}S] is separated by a centrifugation step. Luminescence of SPA beads triggered by membrane bound GTP [γ-^{35}S] is quantified with a TOPcount plate reader (Packard). EC_{50}s are calculated using standard curve fitting software. In this assay, the S1P receptor modulators preferably have a binding affinity to S1P receptor <50 nM.

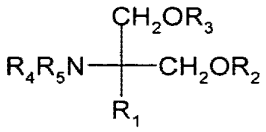
Preferred S1P receptor modulators are e.g. compounds which in addition to their S1P binding properties also have accelerating lymphocyte homing properties, e.g. compounds which elicit a lymphopenia resulting from a re-distribution, preferably reversible, of lymphocytes from circulation to secondary lymphatic tissue, without evoking a generalized immunosuppression. Naïve cells are sequestered; CD4 and CD8 T-cells and B-cells from the blood are stimulated to migrate into lymph nodes (LN) and Peyer's patches (PP).

The lymphocyte homing property may be measured in following Blood Lymphocyte Depletion assay:
A S1P receptor modulator or the vehicle is administered orally by gavage to rats. Tail blood for hematological monitoring is obtained on day –1 to give the baseline individual values, and at 2, 6, 24, 48 and 72 hours after application. In this assay, the S1P receptor agonist or

modulator depletes peripheral blood lymphocytes, e.g. by 50%, when administered at a dose of e.g. < 20 mg/kg.

Examples of appropriate S1P receptor modulators are, for example:

- Compounds as disclosed in EP627406A1, e.g. a compound of formula I



I

wherein R_1 is a straight- or branched (C_{12-22})chain

- which may have in the chain a bond or a hetero atom selected from a double bond, a triple bond, O, S, NR_6, wherein R_6 is H, C_{1-4}alkyl, aryl-C_{1-4}alkyl, acyl or (C_{1-4}alkoxy)carbonyl, and carbonyl, and/or
    - which may have as a substituent C_{1-4}alkoxy, C_{2-4}alkenyloxy, C_{2-4}alkynyloxy, arylC_{1-4}alkyl-oxy, acyl, C_{1-4}alkylamino, C_{1-4}alkylthio, acylamino, (C_{1-4}alkoxy)carbonyl, (C_{1-4}alkoxy)-carbonylamino, acyloxy, (C_{1-4}alkyl)carbamoyl, nitro, halogen, amino, hydroxyimino, hydroxy or carboxy; or

R_1 is

- a phenylalkyl wherein alkyl is a straight- or branched (C_{6-20})carbon chain; or
- a phenylalkyl wherein alkyl is a straight- or branched (C_{1-30})carbon chain wherein said phenylalkyl is substituted by
- a straight- or branched (C_{6-20})carbon chain optionally substituted by halogen,
- a straight- or branched (C_{6-20})alkoxy chain optionally substitued by halogen,
- a straight- or branched (C_{6-20})alkenyloxy,
- phenyl-C_{1-14}alkoxy, halophenyl-C_{1-4}alkoxy, phenyl-C_{1-14}alkoxy-C_{1-14}alkyl, phenoxy-C_{1-4}alkoxy or phenoxy-C_{1-4}alkyl,
- cycloalkylalkyl substituted by C_{6-20}alkyl,
- heteroarylalkyl substituted by C_{6-20}alkyl,
- heterocyclic C_{6-20}alkyl or
- heterocyclic alkyl substituted by C_{2-20}alkyl,

and wherein

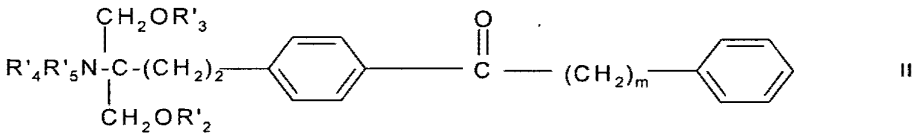
the alkyl moiety may have

- in the carbon chain, a bond or a heteroatom selected from a double bond, a triple bond, O, S, sulfinyl, sulfonyl, or NR_6, wherein R_6 is as defined above, and
- as a substituent C_{1-4}alkoxy, C_{2-4}alkenyloxy, C_{2-4}alkynyloxy, arylC_{1-4}alkyloxy, acyl, C_{1-4}alkylamino, C_{1-4}alkylthio, acylamino, (C_{1-4}alkoxy)carbonyl, (C_{1-4}alkoxy)carbonylamino, acyloxy, (C_{1-4}alkyl)carbamoyl, nitro, halogen, amino, hydroxy or carboxy, and

each of R_2, R_3, R_4 and R_5, independently, is H, C_{1-4} alkyl or acyl

or a pharmaceutically acceptable salt or hydrate thereof;

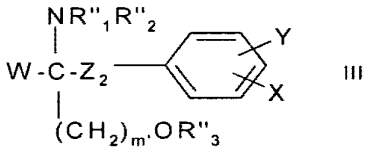
- Compounds as disclosed in EP 1002792A1, e.g. a compound of formula II



II

wherein m is 1 to 9 and each of R'_2, R'_3, R'_4 and R'_5, independently, is H, C_{1-6}alkyl or acyl, or a pharmaceutically acceptable salt or hydrate thereof;

- Compounds as disclosed in EP0778263 A1, e.g. a compound of formula III



III

wherein W is H; C_{1-6}alkyl, C_{2-6}alkenyl or C_{2-6}alkynyl; unsubstituted or by OH substituted phenyl; $R''_4O(CH_2)_n$; or C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH;

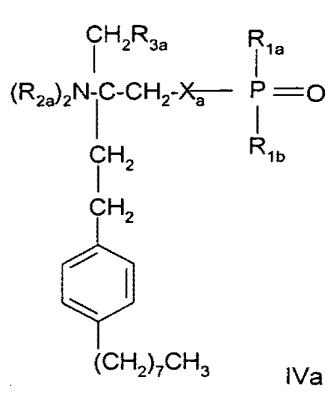
X is H or unsubstituted or substituted straight chain alkyl having a number p of carbon atoms or unsubstituted or substituted straight chain alkoxy having a number (p-1) of carbon atoms, e.g. substituted by 1 to 3 substitutents selected from the group consisting of C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyloxy, amino, C_{1-6}alkylamino, acylamino, oxo, haloC_{1-6}alkyl, halogen, unsubstituted phenyl and phenyl substituted by 1 to 3 substituents selected from the group consisting of C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl and halogen; Y is H, C_{1-6}alkyl, OH, C_{1-6}alkoxy, acyl, acyloxy, amino, C_{1-6}alkylamino, acylamino, haloC_{1-6}alkyl or halogen, Z_2 is a single bond or a straight chain alkylene having a number or carbon atoms of q,

each of p and q, independently, is an integer of 1 to 20, with the proviso of $6 \leq p+q \leq 23$, m' is 1, 2 or 3, n is 2 or 3,

each of R''_1, R''_2, R''_3 and R''_4, independently, is H, C_{1-4}alkyl or acyl,

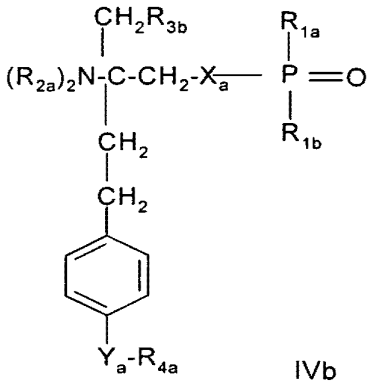
or a pharmaceutically acceptable salt or hydrate thereof,

- Compounds as disclosed in WO02/18395, e.g. a compound of formula IVa or IVb



IVa

or

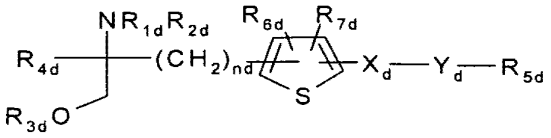


IVb

wherein X_a is O, S, NR_{1s} or a group $-(CH_2)_{na}-$, which group is unsubstituted or substituted by 1 to 4 halogen; n_a is 1 or 2, R_{1s} is H or (C_{1-4})alkyl, which alkyl is unsubstituted or substituted by halogen; R_{1a} is H, OH, (C_{1-4})alkyl or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by 1 to 3 halogen; R_{1b} is H, OH or (C_{1-4})alkyl, wherein alkyl is unsubstituted or substituted by halogen; each R_{2a} is independently selected from H or (C_{1-4})alkyl, which alkyl is unsubstituted or substitued by halogen; R_{3a} is H, OH, halogen or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; and R_{3b} is H, OH, halogen, (C_{1-4})alkyl wherein alkyl is unsubstituted or substituted by hydroxy, or $O(C_{1-4})$alkyl wherein alkyl is unsubstituted or substituted by halogen; Y_a is $-CH_2-$, -C(O)-, -CH(OH)-, -C(=NOH)-, O or S, and R_{4a} is (C_{4-14})alkyl or (C_{4-14})alkenyl;

or a pharmaceutically acceptable salt or hydrate thereof;

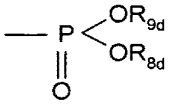
- Compounds as disclosed in WO02/06268Al, e.g. a compound of formula V



V

wherein each of R_{1d} and R_{2d}, independently, is H or an amino-protecting group;

R_{3d} is hydrogen, a hydroxy-protecting group or a residue of formula



R_{4d} is C_{1-4}alkyl;

n_d is an integer of 1 to 6;

X_d is ethylene, vinylene, ethynylene, a group having a formula – D-CH_2- (wherein D is carbonyl, – CH(OH)-, O, S or N), aryl or aryl substituted by up to three substitutents selected from group a as defined hereinafter;

Y_d is single bond, C_{1-10}alkylene, C_{1-10}alkylene which is substituted by up to three substitutents selected from groups a and b, C_{1-10}alkylene having O or S in the middle or end of the carbon chain, or C_{1-10}alkylene having O or S in the middle or end of the carbon chain which is substituted by up to three substituents selected from groups a and b;

R_{5d} is hydrogen, C_{3-6}cycloalkyl, aryl, heterocyclic group, C_{3-6}cycloalkyl substituted by up to three substituents selected from groups a and b, aryl substituted by up to three substituents selected from groups a and b, or heterocyclic group substituted by up to three substituents selected from groups a and b;

each of R_{6d} and R_{7d}, independently, is H or a substituent selected from group a;

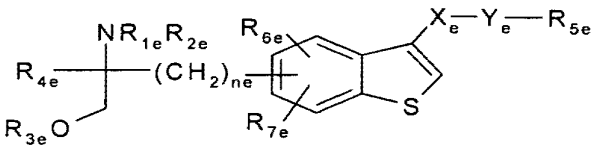
each of R_{8d} and R_{9d}, independently, is H or C_{1-4}alkyl optionally substituted by halogen;

<group a > is halogen, lower alkyl, halogeno lower alkyl, lower alkoxy, lower alkylthio, carboxyl, lower alkoxycarbonyl, hydroxy, lower aliphatic acyl, amino, mono-lower alkylamino, di-C_{1-4}alkylamino, acylamino, cyano or nitro; and

<group b > is C_{3-6}cycloalkyl, aryl or heterocyclic group, each being optionally substituted by up to three substituents selected from group a;

with the proviso that when R_{5d} is hydrogen, Y_d is a either a single bond or linear C_{1-10} alkylene, or a pharmacologically acceptable salt, ester or hydrate thereof;

-Compounds as disclosed in JP-14316985 (JP2002316985), e.g. a compound of formula VI

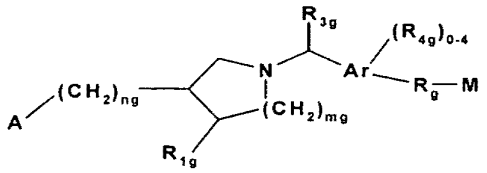


VI

wherein R_{1e},R_{2e},R_{3e},R_{4e},R_{5e},R_{6e},R_{7e}, n_e, X_e and Y_e are as disclosed in JP-14316985;

or a pharmacologically acceptable salt, ester or hydrate thereof;

-Compounds as disclosed in WO03/062252A1, e.g. a compound of formula VII

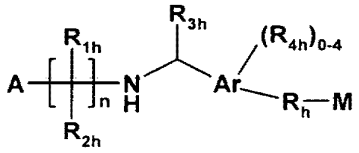


VII

wherein

Ar is phenyl or naphthyl; each of m_g and n_g independently is 0 or 1; A is selected from COOH, PO_3H_2, PO_2H, SO_3H, $PO(C_{1-3}alkyl)OH$ and 1*H*-tetrazol-5-yl; each of R_{1g} and R_{2g} independently is H, halogen, OH, COOH or C_{1-4}alkyl optionally substituted by halogen; R_{3g} is H or C_{1-4}alkyl optionally substituted by halogen or OH; each R_{4g} independently is halogen, or optionally halogen substituted C_{1-4}alkyl or C_{1-3}alkoxy; and each of R_g and M has one of the significances as indicated for B and C, respectively, in WO03/062252A1;
or a pharmacologically acceptable salt, solvate or hydrate thereof;

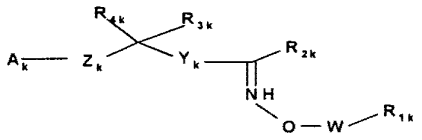
-Compounds as disclosed in WO 03/062248A2, e.g. a compound of formula VIII



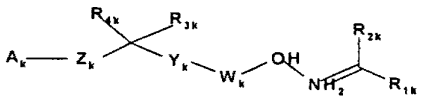
VIII

wherein Ar is phenyl or naphthyl; n is 2,3 or 4; A is COOH, 1*H*-tetrazol-5-yl, PO_3H_2, PO_2H_2, -SO_3H or $PO(R_{5h})OH$ wherein R_{5h} is selected from C_{1-4}alkyl, hydroxyC_{1-4}alkyl, phenyl, -CO-C_{1-3}alkoxy and –CH(OH)-phenyl wherein said phenyl or phenyl moiety is optionally substituted; each of R_{1h} and R_{2h} independently is H, halogen, OH, COOH, or optionally halogeno substituted C_{1-6}alkyl or phenyl; R_{3h} is H or C_{1-4}alkyl optionally substituted by halogen and/ OH; each R_{4h} independently is halogeno, OH, COOH, C_{1-4}alkyl, $S(O)_{0,1\ or2}C_{1-3}$alkyl, C_{1-3}alkoxy, C_{3-6}cycloalkoxy, aryl or aralkoxy, wherein the alkyl portions may optionally be substituted by 1-3 halogens; and each of R_h and M has one of the significances as indicated for B and C, respectively, in WO03/062248A2
or a pharmacologically acceptable salt, solvate or hydrate thereof.

- Compounds as disclosed in WO 04/103306A, WO 05/000833, WO 05/103309 or WO 05/113330, e.g. compounds of formula IXa or IXb



IXa



IXb

wherein

A_k is $COOR_{5k}$, $OPO(OR_{5k})_2$, $PO(OR_{5k})_2$, SO_2OR_{5k}, $POR_{5k}OR_{5k}$ or 1*H*-tetrazol-5-yl, R_{5k} being H or C_{1-6}alkyl;

W_k is a bond, C_{1-3}alkylene or C_{2-3}alkenylene;

Y_k is C_{6-10}aryl or C_{3-9}heteroaryl, optionally substituted by 1 to 3 radicals selected from halogene, OH, NO_2, C_{1-6}alkyl, C_{1-6}alkoxy; halo-substituted C_{1-6}alkyl and halo-substituted C_{1-6}alkoxy;

Z_k is a heterocyclic group as indicated in WO 04/103306A, e.g. azetidine;

R_{1k} is C_{6-10}aryl or C_{3-9}heteroaryl, optionally substituted by C_{1-6}alkyl, C_{6-10}aryl, C_{6-10}arylC_{1-4}alkyl, C_{3-9}heteroaryl, C_{3-9}heteroarylC_{1-4}alkyl, C_{3-8}cycloalkyl, C_{3-8}cycloalkylC_{1-4}alkyl, C_{3-8}heterocycloalkyl or C_{3-8}heterocycloalkylC_{1-4}alkyl; wherein any aryl, heteroaryl, cycloalkyl or heterocycloalkyl of R_{1k} may be substituted by 1 to 5 groups selected from halogen, C_{1-6}alkyl, C_{1-6}alkoxy and halo substituted-C_{1-6}alkyl or -C_{1-6}alkoxy;

R_{2k} is H, C_{1-6}alkyl, halo substituted C_{1-6}alkyl, C_{2-6}alkenyl or C_{2-6}alkynyl: and

each of R_{3k} or R_{4k}, independently, is H, halogen, OH, C_{1-6}alkyl, C_{1-6}alkoxy or halo substituted C_{1-6}alkyl or C_{1-6}alkoxy;

and the N-oxide derivatives thereof or prodrugs thereof,

or a pharmacologically acceptable salt, solvate or hydrate thereof.

The compounds of formulae I to IXb may exist in free or salt form. Examples of pharmaceutically acceptable salts of the compounds of the formulae I to VI include salts with inorganic acids, such as hydrochloride, hydrobromide and sulfate, salts with organic acids, such as acetate, fumarate, maleate, benzoate, citrate, malate, methanesulfonate and benzenesulfonate salts, or, when appropriate, salts with metals such as sodium, potassium, calcium and aluminium, salts with amines, such as triethylamine and salts with dibasic amino acids, such as lysine. The compounds and salts of the combination of the present invention encompass hydrate and solvate forms.

Acyl as indicated above may be a residue R_y-CO- wherein R_y is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenyl-C_{1-4}alkyl. Unless otherwise stated, alkyl, alkoxy, alkenyl or alkynyl may be straight or branched.

Aryl may be phenyl or naphthyl, preferably phenyl.

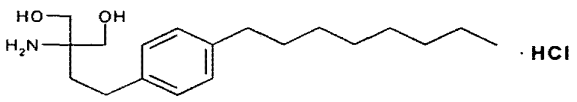
When in the compounds of formula I the carbon chain as R_1 is substituted, it is preferably substituted by halogen, nitro, amino, hydroxy or carboxy. When the carbon chain is interrupted by an optionally substituted phenylene, the carbon chain is preferably unsubstituted. When the phenylene moiety is substituted, it is preferably substituted by halogen, nitro, amino, methoxy, hydroxy or carboxy.

Preferred compounds of formula I are those wherein R_1 is C_{13-20}alkyl, optionally substituted by nitro, halogen, amino, hydroxy or carboxy, and, more preferably those wherein R_1 is phenylalkyl substituted by C_{6-14}-alkyl chain optionally substituted by halogen and the alkyl moiety is a C_{1-6}alkyl optionally substituted by hydroxy. More preferably, R_1 is phenyl-C_{1-6}alkyl substituted on the phenyl by a straight or branched, preferably straight, C_{6-14}alkyl chain. The C_{6-14}alkyl chain may be in ortho, meta or para, preferably in para.

Preferably each of R_2 to R_5 is H.

In the above formula of V "heterocyclic group" represents a 5- to 7 membered heterocyclic group having 1 to 3 heteroatoms selected from S, O and N. Examples of such heterocyclic groups include the heteroaryl groups indicated above, and heterocyclic compounds corresponding to partially or completely hydrogenated heteroaryl groups, e.g. furyl, thienyl, pyrrolyl, azepinyl, pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, 1,2,3-oxadiazolyl, triazolyl, tetrazolyl, thiadiazolyl, pyranyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, tetrahydropyranyl, morpholinyl, thiomorpholinyl, pyrrolidinyl, pyrrolyl, imidazolidinyl, pyrazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl or pyrazolidinyl. Preferred heterocyclic groups are 5-or 6-membered heteroaryl groups and the most preferred heteocyclic group is a morpholinyl, thiomorpholinyl or piperidinyl group.

A preferred compound of formula I is 2-amino-2-tetradecyl-1,3-propanediol. A particularly preferred S1P receptor agonist of formula I is FTY720, i.e. 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol in free form or in a pharmaceutically acceptable salt form (referred to hereinafter as Compound A), e.g. the hydrochloride salt, as shown:



A preferred compound of formula II is the one wherein each of R'_2 to R'_5 is H and m is 4, i.e. 2-amino-2-{2-[4-(1-oxo-5-phenylpentyl)phenyl]ethyl}propane-1,3-diol, in free form or in pharmaceutically acceptable salt form (referred to hereinafter as Compound B), e.g the hydrochloride.

A preferred compound of formula III is the one wherein W is CH_3, each of R''_1 to R''_3 is H, Z_2 is ethylene, X is heptyloxy and Y is H, i.e. 2-amino-4-(4-heptyloxyphenyl)-2-methyl-butanol, in free form or in pharmaceutically acceptable salt form (referred to hereinafter as Compound C), e.g. the hydrochloride. The R-enantiomer is particularly preferred.

Compounds may e in phosphorylated form. A preferred compound of formula IVa is the FTY720-phosphate (R_{2a} is H, R_{3a} is OH, X_a is O, R_{1a} and R_{1b} are OH). A preferred compound of formula IVb is the Compound C-phosphate (R_{2a} is H, R_{3b} is OH, X_a is O, R_{1a} and R_{1b} are OH, Y_a is O and R_{4a} is heptyl). A preferred compound of formula V is Compound B-phosphate.

A preferred compound of formula VI is (2R)-2-amino-4-[3-(4-cyclohexyloxybutyl)-benzo[b]thien-6-yl]-2-methylbutan-1-ol.

A preferred compound of formula IXa is e.g. 1-{4-[1-(4-cyclohexyl-3-trifluoromethyl-benzyloxyimino)-ethyl]-2-ethyl-benzyl}-azetidine-3-carboxylic acid, or a prodrug thereof.

S1P receptor agonists or modulators are known as having immunosuppressive properties or anti-angiogenic properties in the treatment of tumors, e.g. as disclosed in EP627406A1, WO 04/103306, WO 05/000833, WO 05/103309, WO 05/113330 or WO 03/097028.

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system with chronic inflammatory demyelination leading to progressive decline of motor and sensory functions and permanent disability. The therapy of multiple sclerosis is only partially effective, and in most cases only offers a short delay in disease progression despite anti-inflammatory and immunosuppressive treatment. Accordingly, there is a need for agents which are effective in the inhibition or treatment of demyelinating diseases, e.g. multiple sclerosis or Guillain-Barré syndrome, including reduction of, alleviation of, stabilization of or relief from the symptoms which affect the organism.

Characteristic pathological features of demyelinating diseases include inflammation, demyelination and axonal and oligodendrocyte loss. In addition lesions can also have a significant vascular component. A firm link has recently been established between chronic inflammation and angiogenesis and neovascularization seems to have a significant role in the progression of disease.

It has now been found that S1P receptor modulators have an inhibitory effect on neo-angiogenesis associated with demyelinating diseases, e.g. MS.

In a series of further specific or alternative embodiments, the present invention provides:

1.1 A method for preventing, inhibiting or treating neo-angiogenesis associated with a demyelinating disease, e.g. MS, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to IXb.

1.2 A method for alleviating or delaying progression of the symptoms of a demyelinating disease, e.g. multiple sclerosis or Guillain-Barré syndrome, in a subject in need thereof, in which method neo-angiogenesis associated with said disease is prevented or inhibited, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to IXb.

1.3 A method for reducing or preventing or alleviating relapses in a demyelinating disease, e.g. multiple sclerosis or Guillain-Barré syndrome, in a subject in need thereof, in which method neo-angiogenesis associated with said disease is prevented or inhibited, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to IXb.

1.4 A method for slowing progression of a demyelinating disease, e.g. multiple sclerosis or Guillain-Barré syndrome, in a subject being in a relapsing-remitting phase of the disease, in which method neo-angiogenesis associated with said disease is prevented or inhibited, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to XIb.

1.5 A method as indicated above, wherein the S1P receptor modulator is administered intermittently.

For example, the S1P receptor modulator may be administered to the subject every 2nd or 3rd day or once a week.

2. A pharmaceutical composition for use in any one of the methods 1.1 to 1.5, comprising an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined hereinabove, together with one or more pharmaceutically acceptable diluents or carriers therefor.

3. An S1P receptor modulator, e.g a compound of formula I to IXb as defined herein above, for use in any one of the methods 1.1 to 1.5.

4 An S1P receptor modulator, e.g. a compound of formulae I to IXb as defined herein above, for use in the preparation of a medicament for use in any one of the methods 1.1 to 1.5.

Clinicians usually categorize patients having MS into four types of disease patterns:

- *Relapsing-remitting* (RR-MS): Discrete motor, sensory, cerebellar or visual attacks that occur over 1-2 weeks and often resolve over 1-2 months. Some patients accrue disability with each episode, yet remain clinically stable between relapses. About 85% of patients initially experience the RR form of MS, but within 10 years about half will develop the secondary progressive form.
- *Secondary-progressive* (SP-MS): Initially RR followed by gradually increasing disability, with or without relapses. Major irreversible disabilities appear most often during SP.
- *Primary-progressive* (PP-MS): Progression disease course from onset without any relapses or remissions, affecting about 15% of MS patients.
- *Progressive-relapsing* (PR-MS): Progressive disease from onset with clear acute relapses; periods between relapses characterized by continuing progression.

Accordingly, the S1P receptor modulators, e.g. a compound of formulae I to IXb as defined hereinabove, may be useful in the treatment of one or more of *Relapsing-remitting* (RR-MS), *Secondary-progressive* (SP-MS), *Primary-progressive* (PP-MS) and *Progressive-relapsing* (PR-MS).

In particular, the S1P receptor modulators as described herein, e.g. FTY720, i.e. 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-dio, are useful for treating PP-MS.

Utility of the S1P receptor modulators, e.g. the S1P receptor modulators comprising a group of formula X, in preventing or treating neo-angiogenesis associated with a demyelinating disease as hereinabove specified, may be demonstrated in animal test methods as well as in clinic, for example in accordance with the methods hereinafter described.

In vivo: Relapsing Experimental Autoimmune Encephalomyelitis (EAE)

Disease is induced in female Lewis rats by immunization with guinea pig spinal cord tissue emulsified in complete Freund's adjuvant. This results in an acute disease within 11 days, followed by an almost complete remission around day 16 and a relapse at around days 26.

On day 26 rats are thoracectomized after having been deeply anesthetized with Isoflurane (3%, 20 L / min) and perfused through the left ventricle of the heart. The left ventricle is punctured with a 19 gauge needle from a winged infusion set (SV-19BLK; Termudo, Elkton, MD), which is connected to an airtight pressurized syringe containing the rinsing solution (NaCl 0.9% with 250,000 U/l heparin at 35°C). The right atrium is punctured to provide outflow, and the perfusate is infused under a precise controlled pressure of 120 mm Hg. The perfusion is continued for 5 min (at a constant rate of 20 ml/min) followed by a pre-fixation solution (2% performaldehyde in PBS at 35°C). Finally, up to 30 ml of polyurethane resin (PUI14; Vasqtec, Zürich, Switzerland) is infused at the same rate. After 48 h, the resin-filled brain and spinal cord are excised from the animal and the soft tissue removed by maceration in 7.5% KOH during 24 hr at 50°C. The casts are then thoroughly cleaned with and stored in distilled water before drying by lyophilization. These vascular casts are quantitated using micro computer tomography.

In this assay, a S1P1 receptor modulator, e.g. Compound A significantly blocks disease-associated neo-angiogenesis when administered to the animals at a dose of from 0.1 to 20 mg/kg p.o. For example, Compound A, in the hydrochloride salt form, fully blocks disease-associated angiogenesis and completely inhibits the relapse phases when administered daily at a dose of 0.3 mg/kg p.o. The same effect is obtained when Compound A, in the hydrochloride salt form, is administered p.o. at 0.3 mg/kg every 2nd or 3rd day or once a week.

C. Clinical Trial

Investigation of clinical benefit of a S1P receptor agonist, e.g. a compound of formula I, e.g. Compound A.

20 patients with relapsing-remitting MS receive said compound at a daily dosage of 0.5, 1.25 or 2.5 mg p.o. The general clinical state of the patient is investigated weekly by physical and laboratory examination. Disease state and changes in disease progression are assessed every 2 months by radiological examination (MRI) and physical examination. Initially patients receive treatment for 2 to 6 months. Thereafter, they remain on treatment for as long as their disease does not progress and the drug is satisfactorily tolerated.

Main variables for evaluation: Safety (adverse events), standard serum biochemistry and hematology, magnetic resonance imaging (MRI).

Daily dosages required in practicing the method of the present invention when a S1P receptor modulator alone is used will vary depending upon, for example, the compound used, the host, the mode of administration and the severity of the condition to be treated. A preferred daily dosage range is about from 0.1 to 100 mg as a single dose or in divided

doses. Suitable daily dosages for patients are on the order of from e.g. 0.1 to 50 mg p.o. The S1P receptor modulator may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, nasally, pulmonary (by inhalation) or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise from ca. 0.1 to 30 mg, usually 0.25 to 30 mg S1P receptor modulator, together with one or more pharmaceutically acceptable diluents or carriers therefore. As already mentioned, the S1Preceptor modulator, e.g. Compound A, may alternatively be administered intermittently, e.g. at a dose of 0.5 to 30 mg every other day or once a week.

According to another embodiment of the invention, the S1P receptor modulator may be administered as the sole active ingredient or in conjunction with, e.g. as an adjuvant to, a VEGF-receptor antagonist.

Examples of suitable VEGF-receptor antagonist include e.g. compounds, proteins or antibodies which inhibit the VEGF receptor tyrosine kinase, inhibit a VEGF receptor or bind to VEGF, and are e.g. in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 98/35958, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or a pharmaceutically acceptable salt thereof, e.g. the succinate, in WO 00/27820, e.g. a N-aryl(thio) anthranilic acid amide derivative e.g. 2-[(4-pyridyl)methyl]amino-N-[3-methoxy-5-(trifluoromethyl)phenyl]benzamide or 2-[(1-oxido-4-pyridyl)methyl]amino-N-[3-trifluoromethylphenyl]benzamide, or in WO 00/09495, WO 00/59509, WO 98/11223, WO 00/27819, WO 01/55114, WO 01/58899 and EP 0 769 947; those as described by M. Prewett et al in Cancer Research 59 (1999) 5209-5218, by F. Yuan et al in Proc. Natl. Acad. Sci. USA, vol. 93, pp. 14765-14770, Dec. 1996, by Z. Zhu et al in Cancer Res. 58, 1998, 3209-3214, and by J. Mordenti et al in Toxicologic Pathology, Vol. 27, no. 1, pp 14-21, 1999; in WO 00/37502 and WO 94/10202; Angiostatin™, described by M. S. O'Reilly et al, Cell 79, 1994, 315-328; Endostatin™, described by M. S. O'Reilly et al, Cell 88, 1997, 277-285; anthranilic acid amides; ZD4190; ZD6474; SU5416; SU6668; or anti-VEGF antibodies or anti-VEGF receptor antibodies,e.g. RhuMab.

4-Pyridylmethyl-phthalazine derivatives are e.g. preferred inhibitors of VEGF receptor tyrosine kinase. Such derivatives and their preparation, pharmaceutical formulations thereof and methods of making such compounds are described in WO00/59509, EP02/04892, WO01/10859 and, in particular, in U.S. Patent No. 6,258,812, which are here incorporated by reference.

Where the S1P receptor modulator is administered in conjunction with a VEGF-receptor antagonist, dosages of the co-administered VEGF-receptor agonist will of course vary depending on the type of co-drug employed, e.g. whether it is a steroid or a calcineurin inhibitor, on the specific drug employed, on the condition being treated and so forth. In accordance with the foregoing the present invention provides in a yet further aspect:

5. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective non-toxic amount of a S1P receptor modulator and a VEGF-receptor antagonist, e.g. as indicated above.

6. A pharmaceutical combination, e.g. a kit, comprising a) a first agent which is a S1P receptor modulator as disclosed herein, in free form or in pharmaceutically acceptable salt form, and b) a VEGF-receptor antagonist, e.g. as indicated above. The kit may comprise instructions for its administration.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

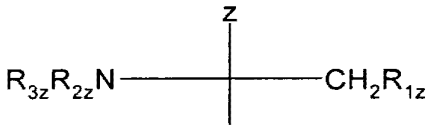
The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a S1P receptor modulator and a VEGF-receptor antagonist, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a S1P receptor modulator and a VEGF-receptor antagonist, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the 2 compounds in the body of the patient.

CLAIMS

1. Use of an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined herein above, in the preparation of a medicament for preventing, inhibiting or treating neo-angiogenesis associated with a demyelinating disease, e.g. multiple sclerosis.

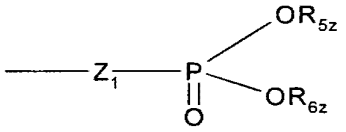
2. Use of an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined herein above, in the preparation of a medicament for preventing, inhibiting or treating PP-MS.

3. Use of claim 1 or 2, wherein the S1P receptor modulator comprises a group of formula X :



(X)

wherein Z is H, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, phenyl, phenyl substituted by OH, C_{1-6}alkyl substituted by 1 to 3 substituents selected from the group consisting of halogen, C_{3-8}cycloalkyl, phenyl and phenyl substituted by OH, or CH_2-R_{4z} wherein R_{4z} is OH, acyloxy or a residue of formula (a)



(a)

wherein Z_1 is a direct bond or O, preferably O;
each of R_{5z} and R_{6z}, independently, is H, or C_{1-4}alkyl optionally substituted by 1, 2 or 3 halogen atoms;
R_{1z} is OH, acyloxy or a residue of formula (a); and each of R_{2z} and R_{3z} independently, is H, C_{1-4}alkyl or acyl.

4. Use of any preceding claim, wherein the medicament is co-administered, e.g. concomitantly or in sequence, with a VEGF-receptor antagonist, e.g. as defined hereinabove.

5. A pharmaceutical composition for use of any preceding claim, comprising an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined hereinabove, together with one or more pharmaceutically acceptable diluents or carriers therefor.

6. A pharmaceutical combination, e.g. a kit, comprising a) a first agent which is a S1P receptor modulator e.g. a compound of formulae I to XIb as defined herein above, in free form or in pharmaceutically acceptable salt form, and b) a VEGF-receptor antagonist, e.g. as defined hereinabove.

7. A method for preventing, inhibiting or treating neo-angiogenesis associated with a demyelinating disease, e.g. multiple sclerosis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined hereinabove.

8. A method of preventing, inhibiting or treating PP-MS in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an S1P receptor modulator, e.g. a compound of formulae I to IXb as defined hereinabove.

9. A method according to claim 8, wherein the S1P receptor modulator is administered intermittently.

10. A method, use, pharmaceutical composition or pharmaceutical combination of any preceding claim, wherein the S1P receptor modulator or agonist is 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, 2-amino-2-[4-(3-benzyloxyphenoxy)-2-chlorophenyl]ethyl-1,3-propane-diol, or 1-{4-[1-(4-cyclohexyl-3-trifluoromethyl-benzyloxyimino)-ethyl]-2-ethyl-benzyl}-azetidine-3-carboxylic acid, in free form or in a pharmaceutically acceptable salt form.

11. A method, use, pharmaceutical composition or pharmaceutical combination according to any one of the preceding claims, wherein the S1P receptor modulator is 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol in free form or in a pharmaceutically acceptable salt form.

Docket Number	PAT050279-US-CNT

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

UTILITY PATENT APPLICATION TRANSMITTAL AND FEE SHEET

Transmitted herewith for filing under 37 CFR § 1.53(b)(1) is a **continuation** of prior Application No. 12/303765, filed December 8, 2008.

Applicant (or identifier): Hiestand, Peter C. et al.

Title: S1P Receptor Modulators for Treating Multiple Sclerosis

Enclosed are:

1. ☒ Specification (Including Claims and Abstract) - 18 pages
2. ☐ Drawings - sheets
3. Declaration and Power of Attorney
   a. ☐ Newly executed (original or copy)
   b. ☒ Copy from a prior application (signed or with indication that original was signed)
      i. ☐ Deletion of Inventors
      Signed statement attached deleting inventor(s) named in the prior application
4. ☒ Incorporation By Reference
   The entire disclosure of the prior application, from which a copy of the Declaration and Power of Attorney is supplied under Box 3b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
5. ☐ Microfiche Computer Program (appendix)
6. Nucleotide and/or Amino Acid Sequence Submission
   ☐ Computer Readable Copy
   ☐ Paper Copy
   ☐ Statement Verifying Identity of Above Copies
7. ☒ Preliminary Amendment
8. ☐ Assignment Papers (Cover Sheet & Document(s))
9. ☐ English Translation of
10. ☒ Information Disclosure Statement
11. ☐ Certified Copy of Priority Document(s)
12. ☐ Return Receipt Postcard
13. ☒ Application Data Sheet
14. ☐ Other:

☒ The right to elect an invention or species that is different from that elected in parent Application No. 12/303765 in the event of a restriction or election of species requirement that is identical or substantially similar to that made in said parent application is hereby reserved.

Filing fee calculation:

☐ Before calculating the filing fee, please enter the enclosed Preliminary Amendment.
☐ Before calculating the filing fee, please cancel claims .

Basic Filing Fee									$ 330
Search Fee									$ 540
Examination Fee									$ 220
Multiple Dependent Claim Fee ($ 390)									$ 390
Foreign Language Surcharge ($)									$ 0
	For	Number Filed		Number Extra		Rate			
Extra Claims	Total Claims	10	-20	0	x	$ 52	=	$ 0	
	Independent Claims	3	-3	0	x	$ 220	=	$ 0	
Application Size Fee									
Total Sheets		Extra Sheets		Number of each additional 50 or fraction thereof (rounded up to a whole number)		Rate			
18	- 100	0	/50	0	x	$ 270	=	$ 0	
TOTAL FILING FEE								$ 1480	

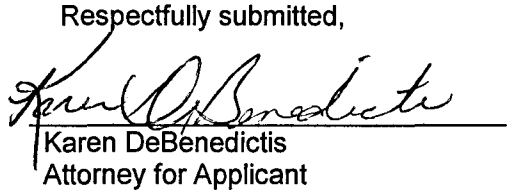
☒ Please charge Deposit Account No. 19-0134 in the name of Novartis in the amount of $1480. An additional copy of this paper is enclosed. The Commissioner is hereby authorized to charge any additional fees under 37 CFR §1.16 and §1.17 which may be required in connection with this application, or credit any overpayment, to Deposit Account No. 19-0134 in the name of Novartis.

Please address all correspondence to the address associated with Customer No. 001095, which is currently:

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936

Please direct all telephone calls to the undersigned at the number given below, and all telefaxes to (973) 781-8064.

Respectfully submitted,



Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
(862) 778-3785

Date: 5/31/11

PTO/SB/14 (07-07)
Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	PAT050279-US-CNT
	Application Number	
Title of Invention	S1P RECEPTOR MODULATORS FOR TREATING MULTIPLE SCLEROSIS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Applicant Information:

Applicant 1

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Peter	C.	HIESTAND	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

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Citizenship under 37 CFR 1.41(b)	AU		

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Address 2			
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Postal Code	4123	Country	CH

Applicant 2

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Christian		SCHNELL	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Hésingue	Country Of Residence	FR
Citizenship under 37 CFR 1.41(b)	FR		

Mailing Address of Applicant:

Address 1	Rue de Buschwiller 9		
Address 2			
City	Hésingue	State/Province	
Postal Code	68220	Country	FR

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

PTO/SB/14 (07-07)
Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	PAT050279-US-CNT
	Application Number	
Title of Invention	S1P RECEPTOR MODULATORS FOR TREATING MULTIPLE SCLEROSIS	

Customer Number	01095		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	S1P RECEPTOR MODULATORS FOR TREATING MULTIPLE SCLEROSIS		
Attorney Docket Number	PAT050279-US-CNT	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		Sub Class (if any)	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Enter either Customer Number or complete the Representative Name section below. If both sections are completed the Customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	01095		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78(a)(2) or CFR 1.78(a)(4), and need not otherwise be made part of the specification.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	12/303765	2008-12-08
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
12/303765	a 371 of international	PCT/EP07/05597	2007-06-25

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	PAT050279-US-CNT
	Application Number	
Title of Invention	S1P RECEPTOR MODULATORS FOR TREATING MULTIPLE SCLEROSIS	

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(a).

Remove

Application Number	Country	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
0612721.1	GB	2006-06-27	(•) Yes ( ) No

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Assignee Information:

Providing this information in the application data sheet does not substitute for compliance with any requirement of part 3 of Title 37 of the CFR to have an assignment recorded in the Office.

Assignee 1

If the Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information:

Address 1			
Address 2			
City		**State/Province**	
Country		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee Data may be generated within this form by selecting the **Add** button.

Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.

Signature	Karen D. Benedictis			Date (YYYY-MM-DD)	2011-05-31
First Name	Karen	Last Name	DeBenedictis	Registration Number	32977

US Case **50279-WO-PCT**

DECLARATION AND POWER OF ATTORNEY FOR UNITED STATES PATENT APPLICATION

☒Original ☐ Supplemental ☐ Substitute

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a United States patent is sought on the invention entitled

ORGANIC COMPOUNDS

the specification of which:

☐ is attached hereto.

☐ was filed on _____ (day/month/year) as Application No. _____

and, if this box (☐) contains an *

☐ was amended on _____ (day/month/year)

☒ was filed as Patent Cooperation Treaty international Application No.

PCT/EP2007/005597 on **25/June/2007** (day/month/year)

and, if this box (☐) contains an *

☐ entered the national stage in the United States and was accorded Application No.

_____

and, if this box (☐) contains an *

☐ was amended, subsequent to entry into the national stage, on _____ (day/month/year)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) specifically referred to above and, if this application was filed as a Patent Cooperation Treaty international application, by any amendments made during the international stage (including any made under Patent Cooperation Treaty Rule 91, Article 19 and Article 34).

I acknowledge my duty to disclose information which is material to patentability as defined in 37 C.F.R. 1.56, including, for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or Patent Cooperation Treaty international filing date of the continuation-in-part application.

I hereby claim the benefit under 35 U.S.C. 119(a)-(d) or (f) or 365(b) of any foreign application(s) for patent, inventor's certificate or plant breeder's right certificate listed below and under 35 U.S.C. 365(a) of any Patent Cooperation Treaty international application(s) designating at least one country other than the United States listed below and have also listed below any foreign application(s) for patent, inventor's certificate or plant breeder's right certificate and Patent Cooperation Treaty international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application the priority of which is claimed for that subject matter:

COUNTRY/REGION (OR P.C.T.)	APPLICATION No.	FILING DATE (day/month/year)	PRIORITY CLAIMED	
Great Britain	**0612721.1**	**27/June/2006**	☒ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below:

APPLICATION NO.	FILING DATE (day/month/year)

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s) listed below and under 35 U.S.C. 365(c) of any Patent Cooperation Treaty international application(s) designating the United States listed below:

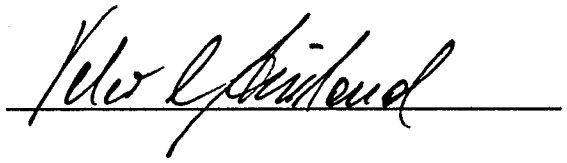
United States Application No.	United States Filing Date (day/month/year)	Status (Pending, Abandoned or U.S. Patent No.)	International Application No. and Filing Date (day/month/year)

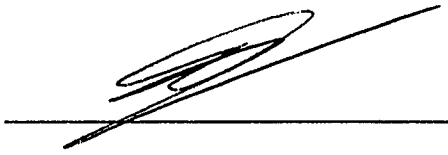
I hereby appoint all of the registered practitioners associated with Customer No. 001095, respectively and individually, as my attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

If this box (☐) contains an x ☒, I hereby authorize the registered practitioners associated with Customer No. 001095 and any others acting on my behalf to take any action relating to this application based on communications from Corporate Intellectual Property of Novartis International AG, Basle, Switzerland, or an affiliate thereof or a successor thereto, without direct communication from me.

Please send all correspondence relating to this application to the address associated with Customer No. 001095.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full name of sole or first joint inventor	**Peter C. HIESTAND**
Inventor's signature	 Date 2/07/2007 (day/month/year)
Residence	**4123 Allschwil, CH**
Citizenship	**Austria**
Post Office Address	**Schönenbuchstrasse 13a, 4123 Allschwil, CH**

Full name of second joint inventor, if any	**Christian SCHNELL**
Inventor's signature	 Date 16/7/2007 (day/month/year)
Residence	**68220 Hésingue, FR**
Citizenship	**France**
Post Office Address	**Rue de Buschwiller 9, 68220 Hésingue, FR**

IMPORTANT: Before this declaration is signed, the patent application (the specification, the claims and this declaration) must be read and understood by each person signing it, and no changes may be made in the application after this declaration has been signed.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO: Not Yet Known
FILED: Herewith
FOR: S1P Receptor Modulators for Treating Multiple Sclerosis

Art Unit:
Examiner:

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir:

Prior to the examination of the above-referenced patent application, please enter the following preliminary amendments.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of the claims which begins on page 4 of this paper.

Remarks/Arguments begin on page 6 of this paper.

Amendments to the Specification:

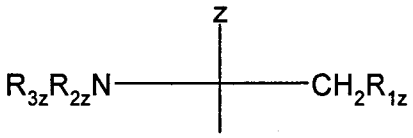
Please insert the following as the first paragraph beneath the title on page 1:

This application is a Continuation of U.S. Application No. 12/303765 filed December 8, 2008 which is a 371 of PCT/EP2007/005597 filed on June 25, 2007, which claims benefit of Great Britain Application No. 0612721.1 filed on June 27, 2006, which in their entirety are herein incorporated by reference.—

A copy of the abstract is herein provided on the following separate sheet.

Abstract

The present invention relates uses of an S1P receptor modulator such as 2-substituted 2-amino-propane-1,3-diol or 2-amino-propanol derivatives, e. g. a compound comprising a group of formula X



(X)

for the treatment or prevention of neo-angiogenesis associated with a demyelinating disease, e.g. multiple sclerosis.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1 – 11. (Cancelled).

Claim 12. (New) A method for inhibiting or treating neo-angiogenesis associated with multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 13. (New) A method for reducing or alleviating relapses in multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 14. (New) A method according to claim 12 for inhibiting or treating neo-angiogenesis associated with relapsing-remitting multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 15. (New) A method according to claim 13 for reducing or alleviating relapses in relapsing-remitting multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 16. (New) A method according to claim 12 or 13 wherein 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride is administered.

Claim 17. (New) A method for slowing progression of multiple sclerosis in a subject in need thereof, comprising administering to said subject 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol, in free form or in a pharmaceutically acceptable salt form, at a daily dosage of 0.5 mg.

Claim 18. (New) A method according to claim 17 wherein the disease is relapsing-remitting multiple sclerosis.

Claim 19. (New) A method according to claim 17 wherein the subject is in a relapsing-remitting phase of the disease.

Claim 20. (New) A method according to claim 17 wherein the disease is primary-progressive multiple sclerosis (PP-MS).

Claim 21. (New) A method according to claim 17, wherein 2-amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride is administered.

REMARKS/ARGUMENTS

The foregoing amendments to the specification are to insert the cross-reference beneath the title and to place the Abstract on a separate sheet. The amendments to the claims are to place the claims in better form and remove multiple dependencies. No new matter has been added. Should the Examiner have any questions, please contact the undersigned attorney.

Respectfully submitted,

Karen DeBenedictis
Attorney for Applicant
Reg. No. 32,977

Novartis Pharmaceuticals Corporation
One Health Plaza, Bldg. 101
East Hanover, NJ 07936
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Date: 5/31/11

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Hiestand, Peter C. et al.
APPLICATION NO:
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Art Unit:
Examiner:

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

This paper is being filed:

☒ within three months of the filing date of the application. Therefore, no fees are required.

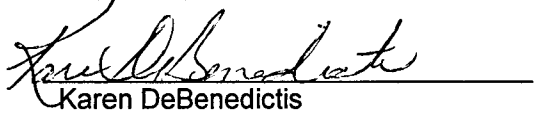
If a fee is deemed to be required, the Commissioner is hereby authorized to charge such fee to Deposit Account No. 19-0134 in the name of Novartis.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO/SB/08A/B.

☒ The references are of record in parent Application No. 12/303765 filed December 8, 2008, and copies are available therein.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO/SB/08A/B form(s).

Respectfully submitted,



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Date: 5/31/11