product containing residual acetic acid. The product is partitioned between ethyl acetate and water (pH = 7.1), the organic phase is dried ($MgSO_4$), and the solvent is concentrated and then triturated with ether to give N-hydroxy-2-[[4-methoxybenzenesulfonyl](2-[4-morpholino]ethyl)amino]acetamide, m.p. 108-112 °C.

The starting material is prepared as follows:

Ethyl 2-[[4-methoxybenzenesulfonyl]amino]acetate (13.7 g, 50.0 mmol) is dissolved in ethanol (500 mL), followed by the addition of sodium spheres (2.5 g, 109.0 mmol). To this solution is added N-(2-chloroethyl)-morpholine hydrochloride (10.0 g, 53.7 mmol), the reaction is stirred at room temperature for 2 hours, and then refluxed for 1.5 hours. The reaction is partitioned between ethyl acetate and brine. The aqueous phase is extracted well with ethyl acetate, the combined organic layers are dried ($MgSO_4$), and the solvent is evaporated to give ethyl 2-[[4-methoxybenzenesulfonyl](2-[4-morpholino]ethyl)amino]acetate.

Example 23: N-Hydroxy-2-[[4-aminobenzenesulfonyl](isobutyl)amino]acetamide, m.p. 50-55 °C, is obtained by hydrogenation of N-hydroxy-2-[[4-nitrobenzenesulfonyl](isobutyl)amino]acetamide (see example 17x), m.p. 128-130 °, using 10% palladium on carbon.

The starting material is prepared according to example 16 by coupling isobutylamine and 4-nitrobenzenesulfonyl chloride in the first step thereof.

Example 24: N-Hydroxy-2-[[4-dimethylaminobenzenesulfonyl](isobutyl)amino]acetamide, m.p. 127-129 °C, is obtained by methylation of N-hydroxy-2-[[4-aminobenzenesulfonyl](isobutyl)amino]acetamide using the procedure from Synthesis p. 709, 1987.

Example 25: Ethyl 2-[[4-hexyloxybenzenesulfonyl](isobutyl)amino]acetate (1.22 g, 3.05 mmol) is dissolved in methanol (15 mL). To this solution is added hydroxylamine hydrochloride (0.43 g, 6.11 mmol), followed by the addition of sodium methoxide, freshly prepared from sodium (0.35 g, 15.3 mmol) dissolved in methanol (5 mL). The reaction is stirred for 36 hours at room temperature. The reaction is worked up by partitioning between dilute hydrochloric acid (pH = ~3) and ethyl acetate. The aqueous phase is extracted well with ethyl acetate, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is crystallized from hexnae/ethyl acetate and collected by filtration to give N-hydroxy-2-[[4-hexyloxybenzenesulfonyl](isobutyl)amino]acetamide, m.p. 108-110 °C.

The starting material is prepared as follows:

A solution of ethanethiol (15 mL) and methylene chloride (15 mL) is cooled to 0 °C. Aluminum trichloride (9.62 g, 72.2 mmol) is added (the solution turns green), and the reaction is warmed to room temperature. Ethyl 2-[[4-methoxybenzenesulfonyl](isobutyl)amino]acetate (4.75 g, 14.44 mmol) is added in methylene chloride (5 mL), and the reactiom is stirred for 3.5 hours at room temperature. The reaction is then slowly quenched with water, and the crude reaction is partitioned between water and methylene chloride. The aqueous layer is extracted well with methylene chloride, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (25% to 50% ethyl acetate/hexane) to give ethyl 2-[[4-hydroxybenzenesulfonyl](isobutyl)amino]acetate.

Ethyl 2-[[4-hydroxybenzenesulfonyl](isobutyl)amino]acetate (1.0 g, 3.17 mmol) is dissolved in dimethylformamide (16 mL). Cesium carbonate (1.03 g, 3.17 mmol) is added, followed by 1-iodohexane (0.47 mL, 3.17 mmol), and the reaction is stirred overnight at room temperature. The reaction is then partitioned between water and ethyl acetate, the aqueous layer is extracted well with ethyl acetate, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (10% ethyl acetate/hexane) to give ethyl 2-[[4-hexyloxybenzenesulfonyl](isobutyl)amino]-acetate.

Example 26: The following compounds are prepared similarly to example 25:

(a) N-Hydroxy-2-[[4-ethoxybenzenesulfonyl](isobutyl)amino]acetamide,by using ethyl iodide in the cesium carbonate alkylation step, and carrying out the subsequent steps as described in example 25.

(b) N-Hydroxy-2-[[4-butyloxybenzenesulfonyl](isobutyl)amino]acetamide, m.p. 125-127 °C, by using iodobutane in the cesium carbonate alkylation step, and carrying out the subsequent steps as described in example 25.

(c) N-Hydroxy-2-[[4-(3-methyl)butyloxybenzenesulfonyl](isobutyl)amino]acetamide, m.p. 93-96 °C, by using 1-iodo-3-methylbutane in the cesium carbonate alkylation step, and carrying out the subsequent steps as described in example 25.

(d) N-Hydroxy-2-[[4-heptyloxybenzenesulfonyl](isobutyl)amino]acetamide, m.p. 120-123 °C, by using 1-iodoheptane in the cesium carbonate alkylation step, and carrying out the subsequent steps as described in example 25.

(e) N-Hydroxy-2-[[4-(cyclohexylmethoxy)benzenesulfonyl](isobutyl)amino]acetamide, m.p. 75-80 °C, by using cyclohexylmethyl bromide in the cesium carbonate alkylation step, and carrying out the subsequent steps as described in example 25.

(f) N-Hydroxy-2-[[4-isopropyloxybenzenesulfonyl](isobutyl)amino]acetamide, m.p. 65-66 °C, by using isopropyl bromide in the cesium carbonate alkylation step, and carrying out the subsequent steps as described in example 25.

(g) N-Hydroxy-2-[[4-ethoxyethoxybenzenesulfonyl](isobutyl)amino]acetamide, m.p. 111-114 °C, by using 2-bromoethyl ethyl ether in the cesium carbonate alkylation step, and carrying out the subsequent steps as described in example 25.

Example 27: (a) N-(t-butyloxy)-2-[[4-methoxybenzenesulfonyl](benzyl)amino]-2-[(2-methyl-5-tetrazolyl)-methyl]acetamide (0.77 g, 1.55 mmol) is dissolved in methylene chloride (2.0 mL) and ethanol (0.1 mL) in a glass sealed tube, and the reaction is cooled to 0 °C. Hydrochloric acid gas (from a lecture bottle) is bubbled through the solution for 20 minutes, and then the tube is sealed at room temperature for 3 days. After that time, the solvent is removed, and the reaction is partitioned between ethyl acetate and saturated sodium bicarbonate. The organic phase is dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (2% methanol/methylene chloride) to give N-hydroxy-2-[[4-methoxybenzenesulfonyl](benzyl)amino]-2-[(2-methyl-5-tetrazolyl)methyl]acetamide, m.p. 72-75 °C.

The starting material is prepared as follows:

D-asparagine (13.2 g, 100.0 mmol) is dissolved in dioxane (75.0 mL) and water (125.0 mL), triethylamine (21.0 mL, 150.0 mmol) is added, and the solution is cooled to 0 °C. To this solution is added 4-methoxybenzenesulfonyl chloride (22.7 g, 110.0 mmol) over 10 minutes. The reaction is warmed to room temperature and stirred for 3 days. The precipitate is then filtered off, the filtrate is acidified to pH = ~4, and extracted well with ethyl acetate. A first crop of pure product precipitates from the ethyl acetate and is collected by filtration. A second crop is obtained by evaporating off the ethyl acetate, and rinsing the solid obtained with water to remove inorganic salts. The two crops are combined to give N-[4-methoxybenzenesulfonyl]-(D)-asparagine.

N-[4-methoxybenzenesulfonyl]-(D)-asparagine (10.1 g, 33.3 mmol) is dissolved in dimethylformamide (167.0 mL). Cesium carbonate (5.43 g, 16.66 mmol) is added, followed by the addition of methyl iodide (2.22 mL, 33.3 mmol), and the reaction is stirred overnight. The reaction is then diluted with saturated ammonium chloride (366.0 mL), and extracted well with ethyl acetate. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated. The crude product is recrystallized from toluene to provide N-[4-methoxybenzenesulfonyl]-(D)-asparagine methyl ester.

To a suspension of N-[4-methoxybenzenesulfonyl]-(D)-asparagine methyl ester (8.54 g, 27.0 mmol) in methylene chloride (47.0 mL) is added pyridine (10.9 mL, 135.0 mmol). Para-toluenesulfonyl chloride (10.3 g, 54.0 mmol) is added, and the reaction mixture is allowed to stand without stirring at room temperature overnight. The next day, saturated sodium bicarbonate is added (125.0 mL), and the mixture is stirred for 1 hour. The mixture is then diluted with water and extracted well with ethyl acetate. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated. The crude product is recrystallized from 20% tetrahydrofuran/methanol to provide methyl 2(R)-[[4-methoxybenzenesulfonyl]-amino]-4-cyano-propionate.

To a suspension of sodium hydride (0.93 g, 23.2 mmol) in dimethylformamide (95.0 mL), is added methyl 2(R)-[[4-methoxybenzenesulfonyl]amino]-4-cyano-propionate (6.92 g, 23.2 mmol) in dimethylformamide (10.0 mL). After stirring at room temperature for 20 minutes, benzyl bromide (3.1 mL, 25.5 mmol) is added, and the reaction is stirred overnight at room temperature. The reaction is then partitioned between ethyl acetate and acidic water (pH = ~5), the organic layer is dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (40% ethyl acetate/hexane) to give methyl 2(R)-[[4-methoxybenzenesulfonyl](benzyl)amino]-4-cyano-propionate.

To a solution of methyl 2(R)-[[4-methoxybenzenesulfonyl](benzyl)amino]-4-cyano-propionate (1.34 g, 3.47 mmol) in dimethylformamide (5.4 mL) is added triethylamine hydrochloride (0.95 g. 6.93 mmol) and sodium azide (0.45 g, 6.93 mmol). The reaction is stirred at 110 °C overnight. The next day, the solvent is evaporated, the residue is acidified with 1N hydrochloric acid (16.0 mL), and extracted well with ethyl acetate. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated to yield methyl 2(R)-[[4-methoxybenzenesulfonyl](benzyl)amino]-2-[(5-tetrazolyl)methyl]acetate.

This crude tetrazole is dissolved in dimethylformamide (17.4 mL). Cesium carbonate (0.56 g, 1.73 mmol) is added, followed by the addition of methyl iodide (0.23 mL, 3.47 mmol), and the reaction is stirred overnight. The reaction is then diluted with brine and extracted well with ethyl acetate. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (40% ethyl acetate/hexane) to give separately the two regioisomers: methyl 2(R)-[[4-methoxybenzenesulfonyl](benzyl)amino]-2-[(1-methyl-5-tetrazolyl)methyl]acetate (0.50 g); and methyl 2(R)-[[4-methoxybenzenesulfonyl](benzyl)amino]-2-[(2-methyl-5-tetrazolyl)methyl]acetate.

Methyl 2(R)-[[4-methoxybenzenesulfonyl](benzyl)amino]-2-[(2-methyl-5 -tetrazolyl)methyl]acetate (1.0 g, 2.27 mmol) is dissolved in tetrahydrofuran (11.3 mL) and water (11.3 mL). To this solution is added lithium hydroxide hydrate (0.095 g, 2.27 mmol), and the reaction is stirred at room temperature for 2 hours. The reaction is then acidified to pH = ~3 using 1N hydrochloric acid, and extracted well with ethyl acetate. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated to provide 2(R)-[[4-methoxybenzenesulfonyl](benzyl)amino]-2-[(2-methyl-5-tetrazolyl)methyl]acetic acid (0.96 g).

2(R)-[[4-methoxybenzenesulfonyl](benzyl)amino]-2-[(2-methyl-5-tetrazolyl)methyl]acetic acid (0.96 g, 2.24 mmol), 1-hydroxybenzotriazole (0.30 g, 2.24 mmol), 4-methylmorpholine (0.86 mL, 7.89 mmol), and O-t-butylhydroxylamine hydrochloride (0.30 g, 2.24 mmol) are dissolved in methylene chloride (75.0 mL). N-[dimethylaminopropyl]-N'-ethylcarbodiimide hydrochloride (0.86 g, 4.48 mmol) is added, and the reaction is stirred overnight. The reaction is then diluted with water and extracted with methylene chloride. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated. The crude product is purified by silica gel chromatography (50% ethyl acetate/hexane) to give N-(t-butyloxy)-2-[[4-methoxybenzenesulfonyl](benzyl)amino]-2-[(2-methyl-5-tetrazolyl)methyl]acetamide.

(b) Similarly prepared is the other tetrazole regioisomer, N-hydroxy-2-[[4-methoxybenzenesulfonyl]-(benzyl)amino]-2-[(1-methyl-5-tetrazolyl)methyl]acetamide, m.p. 92-96 °C, by completing the synthesis as described above.

(c) Similarly prepared is N-hydroxy-2-[[4-methoxybenzenesulfonyl](benzyl)amino]-2-[(5-tetrazolyl)methyl]-acetamide, m.p. 91-94 °C, by completing the synthesis as described above, except trityl chloride is used to protect the tetrazole ring in place of methyl iodide.

(d) Similarly prepared is N-hydroxy-2-[[4-methoxybenzenesulfonyl](4-phenylbenzyl)amino]-2-[(5-tetrazolyl)methyl]acetamide, m.p. 184 °C, by completing the synthesis as described above, except 4-chloromethylbiphenyl is used in place of benzyl bromide in the alkylation step.

Example 28: Oxalyl chloride (106 mL, 1.22 mol) is added over 1 hour to dimethylformamide (92 mL) in methylene chloride (1250 mL) at 0 °C. To this is added a solution of 2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methylbutanoic acid hydrochloride (248 g, 0.6 mol) in dimethylformamide (450 mL) over 1 hour, maintaining the temperature at 0 °C. This solution is stirred an additional 2 hours at room temperature, and then added dropwise to a mixture of hydroxylamine (460 g of a 50% aqueous solution, 6.82 mol) in tetrahydrofuran (2400 mL). The reaction is stirred an additional 3 hours at 5 °C, and then at room temperature overnight. The reaction mixture is filtered, the organic layer is collected, and the solvent is evaporated. The crude product is re-dissolved in methylene chloride (2 L), washed with water (2 X 1 L), saturated sodium bicarbonate (4 X 1 L), brine (1 L), dried (Na_2SO_4), and the solvent is evaporated. The product is dissolved in ethyl acetate (700 mL) and diluted with ether (1400 mL) to induce precipitation. The pure product is collected by filtration to provide N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)-amino]-3-methylbutanamide. After conversion to the hydrochloride salt, a white solid is obtained, m.p. 169-170 °C (dec).

The starting material is prepared as follows:

To a solution of D-valine (2000 g, 17.09 mol) in water (16.9 L) and acetone (9.5 L), cooled to 5 °C, is added triethylamine (4769 mL, 34.22 mol), and the reaction is stirred for 30 minutes. Then a solution of 4-methoxybenzenesulfonyl chloride (3524 g, 18.48 mol) in acetone (7.4 L) is added over 30 minutes, and the reaction is stirred at room temperature overnight. Most of the acetone is evaporated off, and the pH is adjusted to pH = 8.25 with 6N sodium hydroxide. The crude product is washed with toluene (2 X 10 L), and then the pH is re-adjusted to pH = 2.2 with 6N hydrochloric acid. The mixture is then extracted with methylene chloride (3 X 12 L), the combined organic layers are washed with 2N hydrochloric acid, water, dried (Na_2SO_4), and the solvent is evaporated to provide N-[4-methoxybenzenesulfonyl]-(D)-valine.

To a solution of N-[4-methoxybenzenesulfonyl]-(D)-valine (8369 g, 29.13 mol) in methanol (30 L) at 5 °C is added thionyl chloride (2176 mL, 29.7 mol) over 2.5 hours. After stirring for 3 hours at 5 °C, the reaction is stirred for 36 hours at room temperature. Most of the solvent is evaporated, and the crude product is dissolved in toluene (80 L). The toluene layer is then washed with water (20 L), saturated sodium bicarbonate (20 L), water again (20 L), 2N hydrochloric acid (20 L), brine (20 L), dried (Na_2SO_4), and the solvent is evaporated. The solid obtained is dissolved in ethyl acetate (8 L) and heptane (16 L) is added to induce crystallization. The precipitated product is collected by filtration to provide methyl 2(R)-[[4-methoxybenzenesulfonyl]amino]-3-methylbutanoate.

To a solution of methyl 2(R)-[[4-methoxybenzenesulfonyl]amino]-3-methylbutanoate (1662 g, 5.52 mol) in dimethylformamide (10.9 L) is added 3-picolyl chloride hydrochloride (947.3 g, 5.77 mol) followed by powdered potassium carbonate (2409.9 g, 17.36 mol). The reaction mixture is stirred at room temperature for 2 days. At that time, additional quantities of 3-picolyl chloride hydrochloride (95 g) and powdered potassium carbonate (241 g) are added, and the reaction is stirred for 3 more days. The solids are then

filtered away, the crude product is poured into water (22 L), and the pH is adjusted to pH = 8 with 6N sodium hydroxide. This solution is extracted well with toluene (4 X 10 L), the combined organic layers are washed with water (2 X 12 L), and then with 6N hydrochloric acid (3 X 1600 mL). This aqueous layer is then re-adjusted to pH = 8 with 6N sodium hydroxide, extracted with toluene (4 X 10 L), dried (Na_2SO_4), and the solvent is evaporated. The oil obtained is re-dissolved in ethyl acetate (12 L), cooled to 5 °C, and to this is added methanolic HCl (834 mL). After stirring for 2 hours, the precipitated product is collected by filtration to give methyl 2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methylbutanoate hydrochloride.

Methyl 2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methylbutanoate hydrochloride (7164 g, 16.7 mol) is added to a solution of water (27 L) and concentrated hydrochloric acid (9 L), and heated to 120 °C for 3 days. After cooling down to room temperature, charcoal (350 g) is added, stirring is continued for 45 minutes, the reaction is filtered, and the solvent is evaporated. The crude solid is re-dissolved in methanol (7.1 L) and ethyl acetate (73 L), and cooled to 3 °C for 2 hours. The precipitated product is collected by filtration to give 2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methylbutanoic acid hydrochloride.

Example 29: N-Benzyloxy-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methylbutanamide (see example 29a) is reacted with hydrogen in the presence of 10% palladium on charcoal catalyst at room temperature and atmospheric pressure to yield N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)-amino]-3-methylbutanamide. After conversion to the hydrochloride salt, a white solid is obtained, m.p. 169-170 °C (dec).

(a) The N-(benzyloxy) substituted prodrug derivative of the above compound is prepared as follows: 2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methylbutanoic acid hydrochloride is reacted with O-benzylhydroxylamine hydrochloride under conditions described for reaction with O-t-butylhydroxylamine hydrochloride to yield N-(benzyloxy)-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methyl-butanamide, m.p. 74.5-76 °C.

(b) The corresponding N-(4-methoxybenzyloxy) substituted prodrug derivative, N-(4-methoxybenzyloxy)-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methyl-butanamide, is prepared as follows: 2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methylbutanoic acid hydrochloride (2.41 g, 5.82 mmol), 1-hydroxybenzotriazole (0.786 g, 5.82 mmol), 4-methyl-morpholine (1.9 mL, 17.46 mmol), and O-(4-methoxybenzyl)hydroxylamine (1.78 g, 11.63 mmol) (prepared according to Pol. J. Chem. 55, 1163-1167 (1981)) are dissolved in methylene chloride (55 mL). N-[dimethylaminopropyl]-N'-ethylcarbodiimide hydrochloride (1.45 g, 7.57 mmol) is added, and the reaction is stirred overnight. The reaction is then diluted with water and extracted with methylene chloride. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated. The crude product is purified by silica gel chromatography (ethyl acetate followed by 5% methanol/ethyl acetate) to give N-(4-methoxybenzyloxy)-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methylbutanamide, m.p. 45-53 °C.

Similarly prepared are: (c) the N-(2,4-dimethoxybenzyloxy)-substituted prodrug derivative, N-(2,4-dimethoxybenzyloxy)-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methyl-butanamide, m.p. 45-60 °C;

(d) the N-(2-methoxybenzyloxy)-substituted prodrug derivative, N-(2-methoxybenzyloxy)-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methyl-butanamide m.p. 46-56 °C.

Example 30: N-(t-Butyloxy)-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3(R)-(3-picolyloxy)-butanamide (1.3 g, 2.4 mmol) is dissolved in methylene chloride (50 mL) containing ethanol (0.14 mL, 2.4 mmol) in a round bottom flask, and the reaction is cooled to -10 °C. Hydrochloric acid gas (from a lecture bottle) is bubbled through for 20 minutes. The reaction is sealed, allowed to slowly warm to room temperature, and stirred for two days. The solvent is reduced to 1/3 the volume by evaporation and the residue is triturated with ether. The mixture is filtered, the fiter cake is removed and dried in vacuo to provide N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3(R)-(3-picolyloxy)-butanamide dihydrochloride as a white solid; $[\alpha]_D^{25} = +35.26°$ (c = 5.58, DMSO).

The starting material is prepared as follows:

To a solution of D-threonine (5.0 g, 0.042 mol) in water (50 mL) and dioxane (50 mL) containing triethylamine(8.9 mL, 0.063 mol) at room temperature is added 4-methoxybenzenesulfonyl chloride (9.54 g, 0.046 mol). The reaction mixture is stirred overnight at room temperature. Most of the dioxane is evaporated off, and the pH is adjusted to pH = 2 with 1N HCl. The mixture is then extracted with ethyl acetate. The combined organic extracts are washed with brine, dried (Na_2SO_4), and concentrated in vacuo to provide N-[4-methoxybenzenesulfonyl]-(D)-threonine.

N-[4-methoxybenzenesulfonyl]-(D)-threonine (4.0 g, 13.84 mmol), 1-hydroxybenzotriazole (1.87 g, 13.84 mmol), 4-methylmorpholine (7.9 mL, 69.2 mmol), and O-t-butylhydroxylamine hydrochloride (5.22 g, 41.52 mmol) are dissolved in methylene chloride (100 mL). To this solution is added N-[dimethylaminopropyl]-N'-

ethylcarbodiimide hydrochloride (3.45 g, 17.99 mmol), and the reaction is stirred overnight. The mixture is then diluted with water and extracted with methylene chloride. The combined organic extracts are washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product is purified by silica gel chromatography (ethyl acetate) to give N-(t-butyloxy)-2(R)-[[4-methoxybenzenesulfonyl]-amino]-3(R)-hydroxybutanamide.

To a solution of N-(t-butyloxy)-2(R)-[[4-methoxybenzenesulfonyl]amino]-3(R)-hydroxybutanamide (3.04 g, 8.44 mmol) in dimethylformamide (150 mL) is added 3-picolyl chloride hydrochloride (1.45 g, 8.87 mmol) followed by potassium carbonate (11.65 g, 84.4 mmol). The reaction mixture is stirred at room temperature overnight, then heated to 45 °C for 5 hours. An additional amount of 3-picolyl chloride hydrochloride (692.0 mg, 4.23 mmol) is added at this point. The reaction mixture is stirred at 45 °C for 10 hours. The reaction mixture is diluted with water and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product is purified by silica gel chromatography (ethyl acetate, then 5% methanol/methylene chloride) to give N-(t-butyloxy)-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3(R)-(3-picolyloxy)butanamide.

Example 31: (a) N-(t-Butyloxy)-2(R)-[[4-methoxybenzenesulfonyl](4-picolyl)amino]cyclohexylacetamide (1.9 g, 3.9 mmol) is dissolved in dichloroethane (50 mL) containing ethanol (0.21 ml, 3.9 mmol) in a round bottom flask, and the reaction is cooled to -10 °C. Hydrochloric acid gas (from a lecture bottle) is bubbled through for 30 minutes. The reaction is sealed, allowed to slowly warm to room temperature, and stirred for 4 days. The solvent is reduced to 1/3 volume by evaporation and triturated with ether. The mixture is filtered, filter cake removed, and dried in vacuo to provide N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](4-picolyl)amino]-2-cyclohexylacetamide hydrochloride as a white solid, m.p. 154.5-156 °C.

The starting material is prepared as follows:

To a solution of D-cyclohexylglycine hydrochloride (10.4 g, 53.7 mmol) in 1:1 dioxane/water (200 mL) containing triethylamine (37.0 g, 366.0 mmol) at room temperature is added 4-methoxybenzenesulfonyl chloride (15.0 g, 73.0 mmol), and the reaction mixture is stirred at room temperature overnight. The mixture is then diluted with methylene chloride, washed with 1N aqueous hydrochloric acid and water. The organic layer is washed again with brine, dried (Na_2SO_4), and the solvent is evaporated to provide N-[4-methoxybenzenesulfonyl]-(D)-cyclohexylglycine as a crude product. A solution of this crude product in toluene (200 mL) containing N,N-dimethylformamide di-t-butyl acetal (48.5 mL, 200.0 mmol) is heated to 95 °C for 3 hours. The solvent is then evaporated. The crude product is purified by silica gel chromatography (30% ethyl acetate/hexanes) to provide N-[4-methoxybenzenesulfonyl](D)-cyclohexylglycine t-butyl ester.

To a solution of N-[4-methoxybenzenesulfonyl]-(D)-cyclohexylglycine t-butyl ester (2.0 g, 4.1 mmol) in dimethylformamide (100 mL) is added 4-picolyl chloride hydrochloride (0.74 g, 4.5 mmol) followed by potassium carbonate (5.61 g, 40.7 mmol). The reaction mixture is stirred at room temperature for 4 days. The mixture is then diluted with water and extracted with ethyl acetate. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated. The crude product is purified by silica gel chromatography (ethyl acetate) to give t-butyl 2(R)-[[4-methoxybenzenesulfonyl](4-picolyl)amino]-2-cyclohexylacetate.

t-Butyl 2(R)-[[4-methoxybenzenesulfonyl](4-picolyl)amino]-cyclohexyla cetate (2.0 g, 4.2 mmol) is dissolved in methylene chloride (80 mL) and cooled to -10 °C. Hydrochloric acid gas is bubbled into the solution for 10 minuntes. The reaction mixture is then sealed, warmed to room temperature and stirred overnight. The solvent is then evaporated to provide 2(R)-[[4-methoxybenzenesulfonyl](4-picolyl)amino]-2-cyclohexylacetic acid hydrochloride.

2(R)-[[4-Methoxybenzenesulfonyl](4-picolyl)amino]-cyclohexylacetic acid hydrochloride (1.8g, 4.2 mmol), 1-hydroxybenzotriazole (0.65 g, 4.81 mmol), 4-methyl-morpholine (2.4 mL, 24.04 mmol), and O-t-butylhydroxylamine hydrochloride (1.81 g, 14.4 mmol) are dissolved in methylene chloride (100 mL). N-[dimethylaminopropyl]-N'-ethylcarbodiimide hydrochloride (1.2 g, 6.25 mmol) is added, and the reaction is stirred overnight. The reaction is then diluted with water and extracted with methylene chloride. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated. The crude product is purified by silica gel chromatography (5% methanol/methylene chloride) to give N-(t-butyloxy)-2-(R)-[[4-methoxybenzenesulfonyl](4-picolyl)amino]-2-cyclohexylacetamide.

(b) Similarly prepared is N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](2-(2-pyridyl)ethyl)amino]-2-cyclohexylacetamide, m.p. 131.5-134.0 °C.

The first two steps are carried out as described above. A Mitsunobu step is substituted for the alkylation step as described below.

To a stirring solution of N-[4-methoxybenzenesulfonyl]-(D)-cyclohexylglycine-t-butyl ester (2.0 g, 5.25 mmol) in tetrahydrofuran (50 mL) is added triphenylphosphine (4.13 g, 15.75 mmol) and 2-(2-hydrox-

yethyl)-pyridine (646.0 mg, 5.25 mmol) followed by diethyl azodicarboxylate (2.28 g, 13.1 mmol). The reaction mixture is stirred at room temperature for 48 hours. The mixture is concentrated directly in vacuo. The crude mixture is applied to a column of silica gel (30% ethylacetate/hexane) to provide t-butyl 2(R)-[N-[4-methoxybenzenesulfonyl](2-(2-pyridyl)ethyl)amino]- 2-cyclohexylacetate.

All of the subsequent steps are carried out as described under (a).

(c) Similarly prepared is N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](3-(3-pyridyl)propyl)amino]-2-cyclohexylacetamide, m.p. 136.0-138 °C, by using 3-pyridinepropanol in the Mitsunobu step, and carrying out the subsequent steps as described above.

(d) Similarly prepared is N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](2-methylpyrid-5-ylmethyl)amino]-2-cyclohexylacetamide, m.p. 156.5-157.0 °C, by using 6-methyl-3-pyridinemethanol (prepared as in J. Org. Chem. 53 3513 (1988)) in the Mitsunobu step, and carrying out the subsequent steps as described above.

(e) Similarly prepared is N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](4-tetrahydropyranmethyl)amino]-2-cyclohexylacetamide, m.p. 75.0-87.0 °C, by using 4-(hydroxymethyl)tetrahydropyran (prepared as in Okrytiya. Izobret. 82 (1985)) in the Mitsunoba step, and carrying out the subsequent steps as described above.

Example 32: N-(t-Butyloxy)-2(R)-[(4-methoxybenzenesulfonyl)(benzyl)amino]-2-(4-N-methylpiperidinyl)-acetamide (733.0 mg, 1.46 mmol) is dissolved in methylene chloride (60 mL) containing ethanol (67.0 mg, 146 mmol), and the reaction is cooled to -10 °C. Hydrochloric acid gas (from a lecture bottle) is bubbled through for 15 minutes. The reaction is sealed, allowed to slowly warm to room temperature, and stirred for 6 days. The solvent is reduced to 1/3 volume by evaporation and triturated with ether. The mixture is filtered, filter cake removed, and dried in vacuo to provide N-hydroxy-2(R)-[(4-methoxybenzenesulfonyl)-(benzyl)amino]-2-(4-N-methylpiperidinyl)acetamide hydrochloride as a light tan solid, m.p. >160 °C (dec).

The starting material is prepared as follows:

To a solution of ethyl 4-pyridylacetate (11.17 g, 67.62 mmol) in 2N hydrochloric acid (100 mL) is added platinum (IV) oxide (275 mg). The mixture is shaken in a Parr hydrogenation apparatus for 60 hours under a hydrogen pressure of 50 psi (= 3.45 bar). The reaction mixture is basified to pH 8-9 with saturated aqueous sodium carbonate and then washed with methylene chloride. The aqueous layer is concentrated in vacuo providing sodium 4-piperidyl acetate as a white solid. To a solution of the crude product (5.0 g, 30.3 mmol) in 3:1 water/dioxane (200 mL) at 0 °C is added a solution of di-tert-butyldicarbonate (6.38 g, 29.3 mmol) in dioxane (25 mL) in one portion. The cloudy reaction mixture is warmed to room temperature and stirred overnight. The mixture is then filtered, cooled to 0 °C and acidified with cold 6N hydrochloric acid (pH = 2-3).This solution is extracted with ethyl acetate. The combined organic layers are dried (Na_2SO_4), and the solvent is evaporated to provide N-t-BOC-piperidine-4-acetic acid as a white crystalline solid.

To a solution of N-t-BOC-piperidine-4-acetic acid (4.67 g, 19.22 mmol) in tetrahydrofuran at -78 °C is added triethylamine (2.53 g, 24.99 mmol) followed by pivaloyl chloride (2.55 g, 21.14 mmol). The resulting white slurry is stirred at -78 °C for 15 minutes, warmed to 0 °C for 45 minutes, then recooled to -78 °C. In a separate flask, (R)-(+)-4-benzyl-2-oxazolidinone (4.09 g, 23.1 mmol) is dissolved in tetrahydrofuran (50 mL) and 1M n-butyl lithium in hexanes (14.4 mL, 23.06 mmol) is added dropwise at -78 °C. The solution is added via cannula to the aforementioned white slurry at -78 °C. The reaction mixture is stirred at -78 °C for 15 minutes, then warmed to room temperature over 2.5 hours. The mixture is quenched with saturated aqueous sodium carbonate and the tetrahydrofuran is evaporated in vacuo. The remaining aqueous layer is diluted with water and extracted with ethyl acetate. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated under vacuum. The product is purified by silica gel chromatography (75% to 50% hexane/ethyl acetate) to give 3-[2-(N-t-BOC-4-piperidinyl)-1-oxoethyl]-4(R)-(benzyl)-2-oxazolidinone.

To a solution of 3-[2-(N-t-BOC-4-piperidinyl)-1-oxoethyl]-4(R)-(benzyl)-2-oxazolidinone (7.54 g, 18.76 mmol) in tetrahydrofuran (175 mL) at -78 °C is added a 0.5 M solution of potassium bis (trimethylsilylamide in toluene (37.5 mL, 18.76 mmol) dropwise. After stirring for 20 minutes at -78 °C, a pre-cooled solution of trisylazide (7.25 g, 23.4 mmol) in tetrahydrofuran (55 mL) is added via cannula at -78 °C. The mixture is stirred for 15 minutes at -78 °C, then acetic acid 3.38 g, 56.28 mmol) is added followed by immediate warming to room temperature through immersion in a water bath. The reaction mixture is stirred for 1.5 hours at room temperature. The tetrahydrofuran is removed under vacuum and the resulting residue is partitioned between saturated aqueous sodium carbonate and ethyl acetate. The aqueous layer is removed and extracted with ethyl acetate. The combined organic extracts are washed with brine, dried (Na_2S0_4), and concentrated in vacuo. The product is purified by silica gel chromatography (30% to 50% ethyl acetate/hexanes) to give 3-[2-(R)-azido-2-(N-t-BOC-4-piperidinyl)-1-oxoethyl]-4(R)-(benzyl)-2-oxazolidinone.

To a solution of 3-[2-(R)-azido-2-(N-BOC-4-piperidinyl)-1-oxoethyl]-4(R)-(benzyl)-2-oxazolidinone (5.84 g, 13.17 mmol) in 3:1 tetrahydrofuran/water/200 mL) at 0 °C is added 30% aqueous hydrogen peroxide (5.12 mL, 52.67 mmol) followed by lithium hydroxide monohydrate (1.11 g, 26.34 mmol). The reaction mixture is stirred at 0 °C for 1 hour. The mixture is quenched by addition of sodium sulfite (7.1 g) at 0 °C. The tetrahydrofuran is removed in vacuo and the remaining aqueous layer is further diluted with water. This aqueous layer is then washed with methylene chloride and acidified with 1N hydrochloric acid. The resulting acidic aqueous layer is extracted with ethyl acetate. The combined organic extracts are dried (Na_2SO_4) and concentrated in vacuo to provide crude 2-(R)-azido-2-(N-t-BOC-4-piperidinyl)acetic acid.

To a pre-stirred solution of tin (II) chloride (3.14 g, 16.55 mmol) in methanol (100 mL) at 0 °C is added 2-(R)-azido-2-(N-t-BOC-4-piperidinyl)acetic acid (2.35 g, 8.27 mmol) in methanol (25 mL) dropwise. The reaction mixture is stirred at 0 °C for 10 minutes then warmed to room temperature overnight. The methanol is removed in vacuo to provide crude R-(N-t-BOC-4-piperidinyl) glycine, which is used directly in the next reaction without purification. The crude product from the above reaction is dissolved in 2:1 dioxane/water (120 mL) and triethylamine (7.53 g, 74.43 mmol) and cooled to 0 °C. To this mixture is added 4-methoxybenzenesulfonyl chloride (2.22 g, 10.75 mmol) and then the reaction mixture is warmed to room temperature overnight. The dioxane is removed in vacuo and the residue is partitioned between dilute aqueous sodium bicarbonate and ethyl acetate. The basic aqueous layer is removed, acidifed with 1N hydrochloric acid, and extracted with ethyl acetate. The resulting emulsion is passed through a celite pad washing with ethyl acetate. The organic filtrate is dried (Na_2SO_4) and concentrated in vacuo to provide 2(R)-[(4-methoxybenzenesulfonyl)amino]-2-(N-t-BOC-4-piperidinyl) acetic acid as crude product.

A solution of crude 2(R)-[(4-methoxybenzenesulfonyl)amino]-2-(N-t-BOC-4-piperidinyl)-acetic acid (2.88 g) in dimethylformamide (60 mL) containing N,N-dicyclohexylamine (1.22 g, 6.73 mmol) and benzyl bromide (1.15 g, 6.73 mmol) is stirred at room temperature for 3.5 hours. To this same reaction mixture is again added benzyl bromide (1.26 g, 7.4 mmol) followed by potassium carbonate (6.5 g, 47.11 mmol). The reaction mixture is stirred over the weekend at room temperature. The mixture is diluted with water and extracted with ethylacetate. The combined organic extracts are washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product is purified by silica gel chromatography (15% to 25% ethyl acetate/hexanes) to provide benzyl 2(R)-[(4-methoxybenzenesulfonyl)(benzyl)-amino]-2-(N-t-BOC-4-piperidinyl)acetate.

A solution of benzyl 2(R)-[(4-methoxybenzenesulfonyl)(benzyl)amino]-2-(N-t-BOC-4-piperidinyl) acetate (2.0 g, 3.3 mmol) in dichloromethane (50 mL) is cooled to 0 °C and hydrochloric acid gas (from a lecture bottle) is bubbled through for 10 minutes. The reaction mixture is warmed to room temperature over 30 minutes. The solvent is removed in vacuo to yield benzyl 2(R)-[(4-methoxybenzenesulfonyl)(benzyl)amino]-2-(N-t-BOC-4-piperidinyl) acetate hydrochloride as a white foam.

To a solution of benzyl 2(R)-[(4-methoxybenzene sulfonyl)(benzyl)amino]-2-(N-t-BOC-4-piperidinyl) acetate hydrochloride salt (1.28 g, 2.35 mmol) heated to reflux is added sodium formate (480.0 mg, 7.06 mmol) and formaldehyde (0.57 mL, 7.06 mmol). The reaction mixture is refluxed for 10 minutes, then two additional aliquots of formaldehyde (0.57 mL, 7.06 mmol) are added at 10 minute intervals. The reaction mixture is refluxed for an additional 3 hours. The formic acid is removed in vacuo and the residue is partioned between saturated aqueous sodium bicarbonate and ethyl acetate. The basic aqueous layer is further extracted with ethyl acetate. The combined organic extracts are washed with brine, dried (Na_2SO_4) and concentrated in vacuo to provide benzyl 2(R)-[(4-methoxybenzenesulfonyl)benzyl)amino]-2-(4-N-methyl-piperidinyl) acetate as a crude product. A solution of this crude product (1.23 g) in 3N HCl (40 mL) is refluxed at 120 °C for 2 days. The mixture is concentrated in vacuo to provide acid as a crude product. To a solution of this crude product (1.08 g) in methylene chloride (75 mL) is added 1-hydroxybenzotriazole (0.312 g, 2.31 mmol), 4-methylmorpholine (1.64 g, 16.17 mmol), O-t-butylhydroxylamine hydrochloride (870.0 mg, 6.93 mmol), followed by N-[dimethylaminopropyl]-N'-ethylcarbodiimide hydrochloride (576.0 mg, 3.0 mmol). The reaction mixture is stirred at room temperature overnight. The reaction is then diluted with water and extracted with methylene chloride. The combined organic extracts are washed with brine, dried (Na_2SO_4), and the solvent is evaporated. The crude product is purified by silica gel chromatography (3% to 7% methanol/methylene chloride containing 0.5% ammonium hydroxide) to give N-(t-butyloxy)-2(R)-[(4-methoxybenzenesulfonyl)(benzyl)amino]-2-(4-N-methylpiperidinyl)acetamide.

Example 33: Preparation of 3000 capsules each containing 25 mg of the active ingredient, for example, N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)amino]-3-methylbutanamide hydrochloride:

Active ingredient	75.00 g
Lactose	750.00 g
Avicel PH 102 (microcrystalline cellulose)	300.00 g
Polyplasdone XL (polyvinylpyrrolidone)	30.00 g
Purified water	q.s.
Magnesium stearate	9.00 g

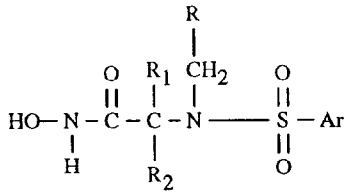
The active ingredient is passed through a No. 30 hand screen.

The active ingredient, lactose, Avicel PH 102 and Polyplasdone XL are blended for 15 minutes in a mixer. The blend is granulated with sufficient water (about 500 mL), dried in an oven at 35 °C overnight, and passed through a No. 20 screen.

Magnesium stearate is passed through a No. 20 screen, added to the granulation mixture, and the mixture is blended for 5 minutes in a mixer. The blend is encapsulated in No. 0 hard gelatin capsules each containing an amount of the blend equivalent to 25 mg of the active ingredient.

Claims

1. A compound of the formula I



(I)

(a) wherein

Ar is carbocyclic or heterocyclic aryl;

R is hydrogen, lower alkyl, carbocyclic aryl-lower alkyl, carbocyclic aryl, heterocyclic aryl, biaryl, biaryl-lower alkyl, heterocyclic aryl-lower alkyl, mono- or poly-halo-lower alkyl, C_3-C_7-cycloalkyl, C_3-C_7-cycloalkyl-lower alkyl, (oxa or thia)-C_3-C_6-cycloalkyl, [(oxa or thia)-C_3-C_6-cycloalkyl]-lower alkyl, hydroxy-lower alkyl, acyloxy-lower alkyl, lower alkoxy-lower alkyl, lower alkyl-(thio, sulfinyl or sulfonyl)-lower alkyl, (amino, mono- or di-lower alkylamino)-lower alkyl, acylamino-lower alkyl, (N-lower alkyl-piperazino or N-carbocyclic or heterocyclic aryl-lower alkylpiperazino)-lower alkyl, or (morpholino, thiomorpholino, piperidino, pyrrolidino, piperidyl or N-lower alkylpiperidyl)-lower alkyl;

R_1 is hydrogen, lower alkyl, carbocyclic aryl-lower alkyl, carbocyclic aryl, heterocyclic aryl, biaryl, biaryl-lower alkyl, heterocyclic aryl-lower alkyl, mono- or poly-halo-lower alkyl, C_3-C_7-cycloalkyl, C_3-C_7-cycloalkyl-lower alkyl, hydroxy-lower alkyl, acyloxy-lower alkyl, lower alkoxy-lower alkyl, (carbocyclic or heterocyclic aryl)-lower alkoxy-lower alkyl, lower alkyl-(thio, sulfinyl or sulfonyl)-lower alkyl, (amino, mono- or di-lower alkylamino)-lower alkyl, (N-lower alkyl-piperazino or N-carbocyclic or heterocyclic aryl-lower alkylpiperazino)-lower alkyl, (morpholino, thiomorpholino, piperidino, pyrrolidino, piperidyl or N-lower alkylpiperidyl)-lower alkyl, acylamino-lower alkyl, piperidyl or N-lower alkylpiperidyl;

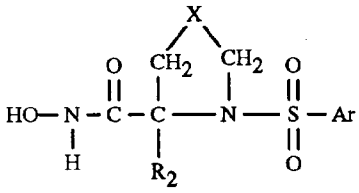
R_2 is hydrogen or lower alkyl;

(b) or wherein R and R_1 together with the chain to which they are attached form a 1,2,3,4-tetrahydroisoquinoline, piperidine, oxazolidine, thiazolidine or pyrrolidine ring, each unsubstituted or substituted by lower alkyl; and Ar and R_2 have meaning as defined under (a);

(c) or wherein R_1 and R_2 together with the carbon atom to which they are attached form a ring system selected from C_3-C_7-cycloalkane which is unsubstituted or substituted by lower alkyl; oxacyclohexane, thia-cyclohexane, indane, tetralin, piperidine or piperidine substituted on nitrogen by acyl, lower alkyl, carbocyclic or heterocyclic aryl-lower alkyl, (carboxy, esterified or amidated carboxy)-lower alkyl or by lower alkylsulfonyl; and Ar and R have meaning as defined under (a);

a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

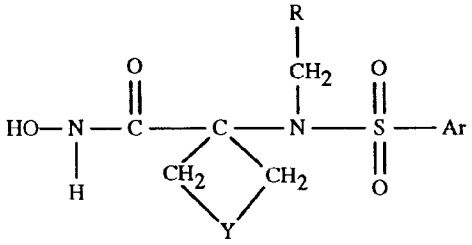
2. A compound according to claim 1 of the formula Ia



(Ia)

wherein X represents methylene or 1,2-ethylene each unsubstituted or substituted by lower alkyl, or X represents oxygen, sulfur, or 1,2-phenylene; Ar and R_2 have meaning as defined in claim 1; or a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

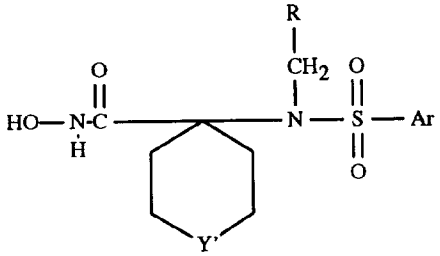
3. A compound according to claim 1 of the formula Ib



(Ib)

wherein Y is a direct bond, C_1-C_4-straight chain alkylene optionally substituted by lower alkyl, CH_2OCH_2, CH_2SCH_2, 1,2-phenylene, CH_2-1,2-phenylene or $CH_2N(R_6)$-CH_2 in which R_6 represents hydrogen, lower alkanoyl, di-lower alkylamino-lower alkanoyl, aroyl, carbocyclic aryl-lower alkanoyl, lower alkyl, carbocyclic or heterocylic aryl-lower alkyl, (carboxy, esterified or amidated carboxy)-lower alkyl or lower alkylsulfonyl; Ar and R have meaning as defined in claim 1; or a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

4. A compound according to claim 3 of the formula Ic



(Ic)

in which Y' represents oxygen, sulfur, a direct bond, methylene or methylene substituted by lower alkyl, or NR_6; R_6 represents hydrogen, lower alkanoyl, di-lower alkylamino-lower alkanoyl, carbocyclic aryl-lower alkanoyl, lower alkyl, carbocyclic or heterocylic aryl-lower alkyl, (carboxy, esterified or amidated carboxy)-lower alkyl or lower alkylsulfonyl; Ar and R have meaning as defined in claim 1; or a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

5. A compound of formula I according to claim 1 wherein Ar is phenyl which is unsubstituted or mono-, di- or tri-substituted by C_1-C_{10}-alkoxy, hydroxy; phenyl-lower alkoxy wherein phenyl is unsubstituted or substituted by lower alkyl, lower alkoxy, halogen or trifluoromethyl; heterocyclic aryl-lower alkoxy wherein heterocyclic aryl is selected from pyridyl, tetrazolyl, triazolyl, thiazolyl, thienyl, imidazolyl and quinolinyl, each unsubstituted or mono- or disubstituted by lower alkyl or halogen; C_3-C_7-cycloalkyl-

lower alkoxy, (lower alkyl, phenyl-lower alkyl or C_3-C_7-cycloalkyl-lower alkyl)-thio, lower alkyloxy-lower alkoxy, halogen, lower alkyl, cyano, nitro, trifluoromethyl, lower alkyl-(sulfinyl or sulfonyl), amino, mono- or di-lower alkylamino or, on adjacent carbon atoms, by C_1-C_2-alkylenedioxy or oxy-C_2-C_3-alkylene; or Ar is thienyl, isoxazolyl or thiazolyl each of which is unsubstituted or mono- or di-substituted by lower alkyl;

R is hydrogen, lower alkyl, phenyl-lower alkyl wherein phenyl is unsubstituted or substituted by lower alkyl, lower alkoxy, halogen or trifluoromethyl; phenyl which is unsubstituted or mono-, di- or tri-substituted by lower alkoxy, hydroxy, halogen, lower alkyl, cyano, nitro, trifluoromethyl, lower alkyl-(thio, sulfinyl or sulfonyl), amino, mono-or di-lower alkylamino or, on adjacent carbon atoms, by C_1-C_2-alkylenedioxy or oxy-C_2-C_3-alkylene; or a heterocyclic aryl radical selected from pyridyl, tetrazolyl, triazolyl, thiazolyl, thienyl, imidazolyl and quinolinyl, each unsubstituted or mono- or disubstituted by lower alkyl or halogen; biphenylyl which is unsubstituted or substituted by lower alkyl, lower alkoxy, halogen, trifluormethyl or cyano; biphenylyl-lower alkyl wherein biphenylyl is unsubstituted or substituted by lower alkyl, lower alkoxy, halogen, trifluormethyl or cyano; (pyridyl, thienyl, quinolinyl or thiazolyl)-lower alkyl, trifluormethyl, C_3-C_7-cycloalkyl, C_3-C_7-cycloalkyl-lower alkyl, (oxa or thia)-C_3-C_6-cycloalkyl, [(oxa or thia)-C_3-C_6-cycloalkyl]-lower alkyl, hydroxy-lower alkyl, lower alkanoyloxy-lower alkyl, lower alkoxy-lower alkyl, lower alkyl-(thio, sulfinyl or sulfonyl)-lower alkyl, (amino, mono- or di-lower alkylamino)-lower alkyl, lower alkanoylamino-lower alkyl, (N-lower alkyl-piperazino or N-phenyl-lower alkylpiperazino)-lower alkyl or (morpholino, thiomorpholino, piperidino, pyrrolidino, piperidyl or N-lower alkylpiperidyl)-lower alkyl;

R_1 is hydrogen, lower alkyl; phenyl-lower alkyl wherein phenyl is unsubstituted or substituted by lower alkyl, lower alkoxy, halogen, trifluoromethyl or, on adjacent carbon atoms, by C_1-C_2-alkylenedioxy or oxy-C_2-C_3-alkylene; phenyl which is unsubstituted or substituted by lower alkyl, lower alkoxy, halogen or trifluoromethyl; pyridyl, thienyl, biphenylyl, biphenylyl-lower alkyl; heterocyclic aryl-lower alkyl wherein heterocyclic aryl is selected from thiazolyl, pyrazolyl, pyridyl, imidazolyl and tetrazolyl each unsubstituted or substituted by lower alkyl; trifluoromethyl, C_3-C_7-cycloalkyl, C_3-C_7-cycloalkyl-lower alkyl, hydroxy-lower alkyl, lower alkanoyloxy-lower alkyl, lower alkoxy-lower alkyl, (phenyl or pyridyl)-lower alkoxy-lower alkyl, lower alkyl-(thio, sulfinyl or sulfonyl)-lower alkyl, (amino, mono- or di-lower alkylamino)-lower alkyl, (N-lower alkyl-piperazino or N-phenyl-lower alkylpiperazino)-lower alkyl, (morpholino, thiomorpholino, piperidino, pyrrolidino, piperidyl or N-lower alkylpiperidyl)-lower alkyl, lower alkanoylamino-lower alkyl; R_3-CONH-lower alkyl wherein R_3 represents (di-lower alkylamino, N-lower alkylpiperazino, morpholino, thiomorpholino, piperidino, pyrrolidino or N-alkylpiperidyl)-lower alkyl; piperidyl or N-lower alkylpiperidyl;

R_2 is hydrogen or lower alkyl;

(b) or wherein R and R_1 together with the chain to which they are attached form a 1,2,3,4-tetrahydroisoquinoline, piperidine, oxazolidine, thiazolidine or pyrrolidine ring, each unsubstituted or mono- or di-substituted by lower alkyl; and Ar and R_2 have meaning as defined under (a);

(c) or wherein R_1 and R_2 together with the carbon atom to which they are attached form a ring system selected from C_3-C_7-cycloalkane which is unsubstituted or substituted by lower alkyl; oxa-cyclohexane, thia-cyclohexane, indane, tetralin and piperidine which is unsubstituted or substituted on nitrogen by lower alkanoyl, di-lower alkylamino-lower alkanoyl, lower alkoxycarbonyl, (morpholino, thiomorpholino or piperidino)-carbonyl, lower alkyl, (phenyl or pyridyl)-lower alkyl, (carboxy, lower alkoxycarbonyl, benzyloxycarbonyl, aminocarbonyl or mono- or di-lower alkylaminocarbonyl)-lower alkyl or by lower alkylsulfonyl; and Ar and R have meaning as defined under (a);

a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

6. A compound of formula I according to claim 1 wherein Ar is phenyl which is unsubstituted or mono-, di- or tri-substituted by C_1-C_7-alkoxy, hydroxy, phenyl-lower alkoxy, C_3-C_7-cycloalkyl-lower alkoxy, lower alkyloxy-lower alkoxy, halogen, lower alkyl, cyano, nitro, trifluoromethyl, lower alkyl-(sulfinyl or sulfonyl), amino, mono- or di-lower alkylamino or, on adjacent carbon atoms, by C_1-C_2-alkylenedioxy or oxy-C_2-C_3-alkylene; or Ar is thienyl, isoxazolyl or thiazolyl each of which is unsubstituted or mono- or di-substituted by lower alkyl;

R is hydrogen, lower alkyl, phenyl-lower alkyl; phenyl which is unsubstituted or mono-, di- or tri-substituted by lower alkoxy, hydroxy, halogen, lower alkyl, trifluoromethyl, or, on adjacent carbon atoms, by C_1-C_2-alkylenedioxy or oxy-C_2-C_3-alkylene; or a heterocyclic aryl radical selected from pyridyl, thiazolyl and quinolinyl, each unsubstituted or mono- or disubstituted by lower alkyl; biphenylyl; biphenylyl-lower alkyl; (pyridyl or thienyl)-lower alkyl, trifluormethyl, C_3-C_7-cycloalkyl, C_3-C_7-cycloalkyl-

lower alkyl, (oxa or thia)-C_3-C_6-cycloalkyl, [(oxa or thia)-C_3-C_6-cycloalkyl]-lower alkyl, hydroxy-lower alkyl, (N-lower alkyl-piperazino or N-phenyl-lower alkylpiperazino)-lower alkyl or (morpholino, thiomorpholino, piperidino, pyrrolidino, piperidyl or N-lower alkylpiperidyl)-lower alkyl;

R_1 is hydrogen, lower alkyl; phenyl-lower alkyl wherein phenyl is unsubstituted or substituted by lower alkyl, lower alkoxy, halogen, trifluoromethyl or, on adjacent carbon atoms, by C_1-C_2-alkylenedioxy; biphenylyl-lower alkyl; heterocyclic aryl-lower alkyl wherein heterocyclic aryl is selected from thiazolyl, pyrazolyl, pyridyl, imidazolyl and tetrazolyl each unsubstituted or substituted by lower alkyl; C_3-C_7-cycloalkyl, C_3-C_7-cycloalkyl-lower alkyl, hydroxy-lower alkyl, (phenyl or pyridyl)-lower alkoxy-lower alkyl, lower alkyl-(thio, sulfinyl or sulfonyl)-lower alkyl, (amino, mono- or di-lower alkylamino)-lower alkyl, (N-lower alkyl-piperazino or N-phenyl-lower alkylpiperazino)-lower alkyl, (morpholino, thiomorpholino, piperidino, pyrrolidino, piperidyl or N-lower alkylpiperidyl)-lower alkyl, lower alkanoylamino-lower alkyl; R_3-CONH-lower alkyl wherein R_3 represents (di-lower alkylamino, N-lower alkylpiperazino, morpholino, thiomorpholino, piperidino, pyrrolidino or N-alkylpiperidyl)-lower alkyl; piperidyl or N-lower alkyl-piperidyl;

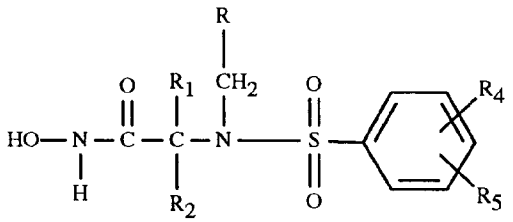
R_2 is hydrogen or lower alkyl;

(b) or wherein R and R_1 together with the chain to which they are attached form a thiazolidine or pyrrolidine ring, each unsubstituted or mono- or di-substituted by lower alkyl; and Ar and R_2 have meaning as defined under (a);

(c) or wherein R_1 and R_2 together with the carbon atom to which they are attached form a ring system selected from C_3-C_7-cycloalkane which is unsubstituted or substituted by lower alkyl; oxa-cyclohexane, thia-cyclohexane and piperidine which is unsubstituted or substituted on nitrogen by lower alkanoyl, di-lower alkylamino-lower alkanoyl, lower alkoxycarbonyl, (morpholino, thiomorpholino or piperidino)-carbonyl, lower alkyl, (phenyl or pyridyl)-lower alkyl, (carboxy, lower alkoxycarbonyl, aminocarbonyl or mono-or di-lower alkylaminocarbonyl)-lower alkyl or by lower alkylsulfonyl; and Ar and R have meaning as defined under (a);

a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

7. A compound according to claim 1 of the formula II



(II)

wherein

R is hydrogen, lower alkyl, carbocyclic aryl-lower alkyl, carbocyclic aryl, heterocyclic aryl, biaryl, biaryl-lower alkyl, heterocyclic aryl-lower alkyl, mono- or poly-halo-lower alkyl, C_3-C_7-cycloalkyl, C_3-C_7-cycloalkyl-lower alkyl, (oxa or thia)-C_3-C_6-cycloalkyl, [(oxa or thia)-C_3-C_6-cycloalkyl]-lower alkyl, hydroxy-lower alkyl, acyloxy-lower alkyl, lower alkoxy-lower alkyl, lower alkyl-(thio, sulfinyl or sulfonyl)-lower alkyl, (amino, mono- or di-lower alkylamino)-lower alkyl, acylamino-lower alkyl, (N-lower alkyl-piperazino or N-carbocyclic or heterocyclic aryl-lower alkylpiperazino)-lower alkyl, or (morpholino, thiomorpholino, piperidino, pyrrolidino or N-lower alkylpiperidyl)-lower alkyl;

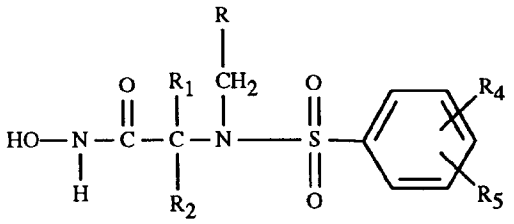
R_1 is hydrogen, lower alkyl, carbocyclic aryl-lower alkyl, carbocyclic aryl, heterocyclic aryl, biaryl, biaryl-lower alkyl, heterocyclic aryl-lower alkyl, mono- or poly-halo-lower alkyl, C_5-C_7-cycloalkyl, C_5-C_7-cycloalkyl-lower alkyl, hydroxy-lower alkyl, acyloxy-lower alkyl, lower alkoxy-lower alkyl, lower alkyl-(thio, sulfinyl or sulfonyl)-lower alkyl, (amino, mono- or di-lower alkylamino)-lower alkyl, (N-lower alkyl-piperazino or N-carbocyclic or heterocyclic aryl-lower alkylpiperazino)-lower alkyl, (morpholino, thiomorpholino, piperidino, pyrrolidino, piperidyl or N-lower alkylpiperidyl)-lower alkyl, piperidyl, N-lower alkyl-piperidyl, or acylamino-lower alkyl represented by R_3-CONH-lower alkyl;

R_2 is hydrogen;

R_3 in R_3-CONH-lower alkyl is lower alkyl, carbocyclic or heterocyclic aryl, di-lower alkylamino, N-lower alkylpiperazino, morpholino, thiomorpholino, piperidino, pyrrolidino, N-alkylpiperidyl, or (di-lower al-

kylamino, N-lower alkylpiperazino, morpholino, thiomorpholino, piperidino, pyrrolidino, pyridyl or N-lower alkylpiperidyl)-lower alkyl;
R_4 is hydrogen, lower alkoxy, hydroxy, carbocyclic or heterocyclic aryl-lower alkoxy, lower alkylthio or carbocyclic or heterocyclic aryl-lower alkylthio, lower alkyloxy-lower alkoxy, halogen, trifluoromethyl, lower alkyl, nitro or cyano;
R_5 is hydrogen, lower alkyl or halogen;
or R_4 and R_5 together on adjacent carbon atoms represent methylenedioxy, ethylenedioxy, oxyethylene or oxypropylene;
or a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

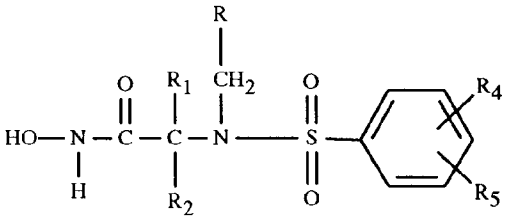
8. A compound according to claim 1 of formula II



(II)

wherein
R and R_1 together with the chain to which they are attached form a 1,2,3,4-tetrahydro-isoquinoline, piperidine, thiazolidine or pyrrolidine ring;
R_2 is hydrogen;
R_4 is hydrogen, lower alkoxy, hydroxy, carbocyclic or heterocyclic aryl-lower alkoxy, lower alkylthio or carbocyclic or heterocyclic aryl-lower alkylthio, lower alkyloxy-lower alkoxy, halogen, trifluoromethyl, lower alkyl, nitro or cyano;
R_5 is hydrogen, lower alkyl or halogen;
or R_4 and R_5 together on adjacent carbon atoms represent methylenedioxy, ethylenedioxy, oxyethylene or oxypropylene;
or a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

9. A compound according to claim 1 of formula II

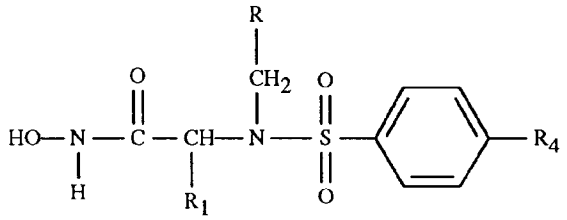


(II)

wherein
R is hydrogen, lower alkyl, carbocyclic aryl-lower alkyl, carbocyclic aryl, heterocyclic aryl, biaryl, biaryl-lower alkyl, heterocyclic aryl-lower alkyl, mono- or poly-halo-lower alkyl, C_3-C_7-cycloalkyl, C_3-C_7-cycloalkyl-lower alkyl, (oxa or thia)-C_3-C_6-cycloalkyl, [(oxa or thia)-C_3-C_6-cycloalkyl]-lower alkyl, hydroxy-lower alkyl, acyloxy-lower alkyl, lower alkoxy-lower alkyl, lower alkyl-(thio, sulfinyl or sulfonyl)-lower alkyl, (amino, mono- or di-lower alkylamino)-lower alkyl, acylamino-lower alkyl, (N-lower alkyl-piperazino or N-carbocyclic or heterocyclic aryl-lower alkylpiperazino)-lower alkyl, or (morpholino, thiomorpholino, piperidino, pyrrolidino or N-lower alkylpiperidyl)-lower alkyl;
R_1 and R_2 together with the carbon atom to which they are attached form a ring system selected from cyclohexane, cyclopentane, oxacyclohexane, thiacyclohexane, indane, tetralin, piperidine or piperidine

substituted on nitrogen by acyl, lower alkyl, carbocyclic or heterocyclic aryl-lower alkyl or by lower alkylsulfonyl;
R_4 is hydrogen, lower alkoxy, hydroxy, carbocyclic or heterocyclic aryl-lower alkoxy, lower alkylthio or carbocyclic or heterocyclic aryl-lower alkylthio, lower alkyloxy-lower alkoxy, halogen, trifluoromethyl, lower alkyl, nitro or cyano;
R_5 is hydrogen, lower alkyl or halogen;
or R_4 and R_5 together on adjacent carbon atoms represent methylenedioxy, ethylenedioxy, oxyethylene or oxypropylene;
or a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

10. A compound according to claim 1 of formula III



(III)

wherein R represents lower alkyl, trifluoromethyl, C_5-C_7-cycloalkyl, (oxa or thia)-C_4-C_5-cycloalkyl, biaryl, carbocyclic monocyclic aryl or heterocyclic monocyclic aryl; R_1 represents hydrogen, lower alkyl, C_5-C_7-cycloalkyl, monocyclic carbocyclic aryl, carbocyclic aryl-lower alkyl, heterocyclic aryl-lower alkyl, lower alkoxy-lower alkyl, lower alkyl-(thio, sulfinyl or sulfonyl)-lower alkyl, di-lower alkylamino-lower alkyl, (N-lower alkylpiperazino, morpholino, thiomorpholino, piperidino or pyrrolidino)-lower alkyl or R_3-CONH-lower alkyl; R_3 represents lower alkyl, carbocyclic aryl, heterocyclic aryl, di-lower alkylamino, N-lower alkylpiperazino, morpholino, thiomorpholino, piperidino, pyrrolidino, N-alkylpiperidyl, or (di-lower alkylamino, N-lower alkylpiperazino, morpholino, thiomorpholino, piperidino, pyrrolidino or N-alkyl-piperidyl)-lower alkyl; R_4 represents lower alkoxy or carbocyclic or heterocyclic aryl-lower alkoxy; or a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

11. A compound of formula III according to claim 10 wherein R represents heterocyclic monocyclic aryl selected from tetrazolyl, triazolyl, thiazolyl, imidazolyl and pyridyl, each unsubstituted or substituted by lower alkyl; or R represents phenyl or phenyl substituted by lower alkyl, lower alkoxy, halogen or trifluoromethyl; R_1 represents lower alkyl, cyclohexyl, or R_3-CONH-lower alkyl wherein R_3 represents (di-lower alkylamino, N-lower alkylpiperazino, morpholino, thiomorpholino, piperidino, pyrrolidino or N-alkylpiperidyl)-lower alkyl; and R_4 represents lower alkoxy or phenyl-lower alkoxy; or a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

12. A compound of formula III according to claim 10 wherein R represents 2-, 3- or 4-pyridyl or phenyl; R_1 represents C_1-C_4-alkyl, cyclohexyl or R_3-CONH-C_1-C_4-alkyl wherein R_3 represents di-C_1-C_4-alkylamino-C_1-C_4-lower alkyl; and R_4 represents lower alkoxy; or a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

13. A compound of formula III according to claim 10 wherein R represents 3-pyridyl or 4-pyridyl; R_1 represents isopropyl or cyclohexyl; and R_4 represents lower alkoxy; or a pharmaceutically acceptable prodrug derivative thereof; or a pharmaceutically acceptable salt thereof.

14. A compound according to any one of claims 1-13 wherein the asymmetric carbon to which is attached R_1 is assigned the (R)-configuration.

15. A compound according to claim 1 which is N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)-amino]-3-methylbutanamide, a pharmaceutically acceptable prodrug derivative thereof or a pharmaceutically acceptable salt thereof.

16. A compound according to claim 1 which is N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)-amino]-3-methylbutanamide or a pharmaceutically acceptable salt thereof.

17. A compound according to claim 1 which is N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](3-picolyl)-amino]-2-cyclohexylacetamide or a pharmaceutically acceptable salt thereof.

18. A compound according to claim 1 which is N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](benzyl)amino]-4-methylpentanamide or a pharmaceutically acceptable salt thereof.

19. A compound according to claim 1 which is N-hydroxy-2(R)-[[4-methoxybenzenesulfonyl](benzyl)amino]-6-[(N,N-dimethylglycyl)amino] hexanamide hydrochloride or a pharmaceutically acceptable salt thereof.

20. A pharmaceutical composition comprising a compound according to any one of claims 1 to 19 and a pharmaceutically acceptable carrier.

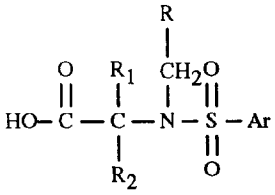
21. A compound according to any one of claims 1 to 19 for use in a method for the therapeutic treatment of the animal or human body.

22. A compound according to any one of claims 1 to 19 for use in the treatment of stromelysin and collagenase dependent conditions.

23. The use of a compound according to any one of claims 1 to 19 for the manufacture of a pharmaceutical composition.

24. The use of a compound according to any one of claims 1 to 19 for the manufacture of a pharmaceutical composition for the treatment of stromelysin and collagenase dependent conditions.

25. A process for the preparation of a compound of formula I according to claim 1, which comprises condensing a carboxylic acid of formula IV,



(IV)

or a reactive functional derivative thereof, wherein R, R_1, R_2 and Ar having meaning as defined in claim 1, with hydroxylamine of formula V,

NH_2-OH (V)

optionally in protected form, or a salt thereof;
and, if necessary, temporarily protecting any interfering reactive group(s), and then liberating the resulting compound of the invention; and, if required or desired, converting a resulting compound of the invention into another compound of the invention, and/or, if desired, converting a resulting free compound into a salt or a resulting salt into a free compound or into another salt; and/or separating a mixture of isomers or racemates obtained into the single isomers or racemates; and/or, if desired, resolving a racemate into the optical antipodes.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 93 81 0896

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
A	CHEMICAL ABSTRACTS, vol. 59, no. 4, 19 August 1963, Columbus, Ohio, US; abstract no. 3824b, 'N-Arylglycine chemotherapeutics' column 3824 ; * abstract * compound Acetohydroxamic acid, 2-[N-(p-methoxyphenyl)benzenesulfonamido]- & YAKUGAKU ZASSHI vol. 83 , 1963 pages 130 - 134 KAORU KONDO ET AL ---	1-25	C07D213/42 C07C311/29 C07D317/62 C07D317/58 C07D405/14 C07D277/28 C07D215/12 C07D277/06 C07D207/48 C07D277/30 A61K31/44 A61K31/18
A	US-A-4 885 027 (P.B. POMIDOR) 5 December 1989 *see formula I, especially definitions of R and R1* ---	1-25	
A	TRENDS BIOTECHNOL. vol. 10, no. 6 , 1992 , UK pages 200 - 207 A.J.P.DOCHERTY ET AL 'The matrix metalloproteinases and their natural inhibitors: prospects for treating degenerative tissue disease' ---	1-25	TECHNICAL FIELDS SEARCHED (Int.Cl.5) C07D C07C A61K
A	WO-A-90 05719 (BRITISH BIO-TECHNOLOGY LIMITED) 31 May 1990 *see formula I and whole document* ---	1-25	
A	EP-A-0 236 872 (F.HOFFMANN-LA ROCHE & CO) 16 September 1987 * the whole document * -----	1-25	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	17 March 1994	Scruton-Evans, I

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
O : non-written disclosure
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
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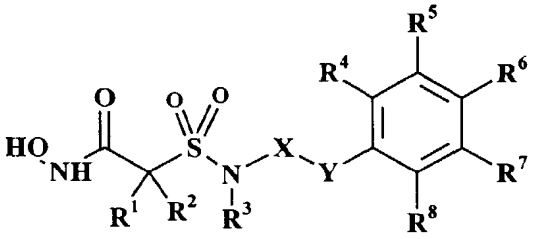
(54) Metalloprotease inhibitors

(57) Compounds of formula (I):



(I)

where the substituents are as defined herein, and salts thereof, are matrix metalloprotease inhibitors.

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Description

[0001] This invention relates to a series of substituted α-aminosulphonyl-acetohydroxamic acids which are inhibitors of zinc-dependent metalloprotease enzymes. In particular, the compounds are inhibitors of certain members of the matrix metalloprotease (MMP) family.

[0002] Matrix metalloproteases (MMPs) constitute a family of structurally similar zinc-containing metalloproteases, which are involved in the remodelling and degradation of extracellular matrix proteins, both as part of normal physiological processes and in pathological conditions. Since they have high destructive potential, MMPs are usually under close regulation and failure to maintain MMP regulation has been implicated as a component of a number of diseases and conditions including pathological conditions, such as atherosclerotic plaque rupture, heart failure, restenosis, periodontal disease, tissue ulceration, wound repair, cancer metastasis, tumour angiogenesis, age-related macular degeneration, fibrotic disease, rheumatoid arthritis, osteoarthritis and inflammatory diseases dependent on migratory inflammatory cells.

[0003] Another important function of certain MMPs is to activate various enzymes, including other MMPs, by cleaving the pro-domains from their protease domains. Thus some MMPs act to regulate the activities of other MMPs, so that over-production of one MMP may lead to excessive proteolysis of extracellular matrix by another. Moreover, MMPs have different substrate preferences (shown in the following Table for selected family members) and different functions within normal and pathological conditions. For recent reviews of MMPs, see Current Pharmaceutical Design, 1996, 2, 624 and Exp. Opin. Ther. Patents, 1996, 6, 1305.

TABLE

Enzyme	Other Names	Preferred Substrates
MMP-1	collagenase-1; interstitial collagenase	collagens I, II, III, VII, X; gelatins
MMP-2	gelatinase A; 72kDa gelatinase	gelatins; collagens IV, V, VII, X; elastin; fibronectin; activates pro-MMP-13
MMP-3	stromelysin-1	proteoglycans; laminin; fibronectin; gelatins
MMP-8	collagenase-2; neutrophil collagenase	collagens I, II, III
MMP-9	gelatinase B; 92kDa gelatinase	gelatins; collagens IV, V; elastin
MMP-13	collagenase-3	collagens I, II, III; gelatins
MMP-14	MT-MMP-1	activates pro-MMP-2 & 13; gelatins

[0004] Excessive production of MMP-3 is thought to be responsible for pathological tissue breakdown which underlies a number of diseases and conditions. For example, MMP-3 has been found in the synovium and cartilage of osteoarthritis and rheumatoid arthritis patients, thus implicating MMP-3 in the joint damage caused by these diseases: see Biochemistry, 1989, 28, 8691 and Biochem. J., 1989, 258, 115. MMP-13 is also thought to play an important role in the pathology of osteoarthritis and rheumatoid arthritis: see Lab. Invest., 1997, 76, 717 and Arthritis Rheum., 1997, 40, 1391. The compounds of the present invention inhibit both MMP-3 and MMP-13 and thus may be of utility in treating these diseases.

[0005] The over-expression of MMP-3 has also been implicated in the tissue damage and chronicity of chronic wounds, such as venous ulcers, diabetic ulcers and pressure sores: see Brit. J. Dermatology, 1996, 135, 52.

[0006] Furthermore, , the production of MMP-3 may also cause tissue damage in conditions where there is ulceration of the colon (as in ulcerative colitis and Crohn's disease: see J. Immunol., 1997 158, 1582 and J. Clin. Pathol., 1994, 47, 113) or of the duodenum (see Am. J. Pathol., 1996, 148, 519).

[0007] Moreover, MMP-3 is also thought to be involved in skin diseases such as dystrophic epidermolysis bullosa (see Arch. Dermatol. Res., 1995, 287, 428) and dermatitis herpetiformis (see J. Invest. Dermatology, , 1995, 105, 184).

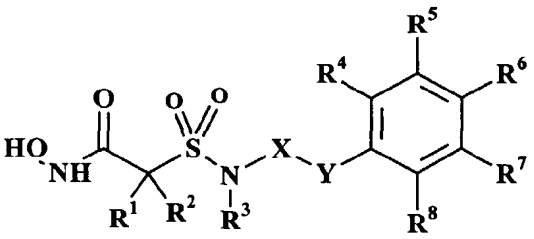
[0008] Rupture of atherosclerotic plaques by MMP-3 has also been described (see e.g. Circulation, 1997, 96, 396). Thus, MMP-3 inhibitors may find utility in the treatment of conditions caused by or complicated by embolic phenomena such as cardiac or cerebral infarctions.

[0009] Studies of human cancers have shown that MMP-2 is activated on the invasive tumour cell surface (see J. Biol.Chem., 1993, 268, 14033) and BB-94, a non-selective peptidic hydroxamate MMP inhibitor, has been reported to decrease the tumour burden and prolong the survival of mice carrying human ovarian carcinoma xenografts (see Cancer Res., 1993, 53, 2087). Certain compounds of the present invention inhibit MMP-2 and therefore may be useful in the treatment of cancer metastasis and tumour angiogenesis.

[0010] Various series of MMP inhibitors have appeared in the literature which have a carbonyl moiety (CO) and a sulphone moiety (SO_2) with a two atom "spacer" interposed between them. For example, α-arylsulphonamido-substituted acetohydroxamic acids are disclosed in EP-A-0606046, WO-A-9627583 and WO-A-9719068, whilst EP-A-0780386 discloses certain related sulphone-substituted hydroxamic acids.

[0011] The compounds of the present invention represent a new class of compounds, and are inhibitors of some of the members of the MMP family. In particular, they are potent inhibitors of MMP-3 and MMP-13, with certain compounds exhibiting varying degrees of selectivity over other MMPs, such as MMP-1, MMP-2 and MMP-9. Certain of the compounds are potent MMP-2 inhibitors.

[0012] Thus, according to one aspect of the present invention, there is provided a compound of formula (I):



(I)

and a pharmaceutically- and/or veterinarily-acceptable salt thereof, and a solvate of such compound and salt, wherein

R^1 and R^2 are each independently H,
C_{2-6} alkenyl, aryl(C_{1-6} alkyl), heteroaryl(C_{1-6} alkyl), aryloxy(C_{1-6} alkyl), heteroaryloxy(C_{1-6} alkyl),
C_{1-6} alkyl optionally substituted by NH_2, C_{2-6} acylamino, OH, or by CO_2H,
or R^1 and R^2 can be taken together with the carbon atom to which they are attached, to form a 4- to 8-membered saturated carbocyclic or heterocyclic ring, which heterocyclic ring has 1 or 2 hetero-groups selected from O, $S(O)_n$ or NR^9 in the ring,
R^3 is H, C_{1-6} alkyl or (C_{1-6} alkoxy)C_{1-6} alkyl,
R^4, R^5, R^7 and R^8 are each independently H, C_{1-6} alkyl, C_{1-6} alkoxy, CN or halogen,
R^6 is H, aryl, heteroaryl, aryloxy or heteroaryloxy, C_{1-6} alkyl, C_{1-6} alkoxy, CN or halogen,
R^9 is H or C_{1-6} alkyl,
n is 0,1 or 2,
X is C_{1-6} alkylene or C_{2-6} alkenylene,
Y is a direct link, CH=CH or O,

wherein "aryl" is phenyl optionally fused with another ring selected from furan, dioxolan, and pyran,
which group is optionally mono- or disubstituted by substituents independently seleceted from halogen, CN, C_{1-6} alkyl optionally substituted by OH or NH_2, C_{1-6} alkoxy, perfluoro(C_{1-6} alkyl) and perfluoro(C_{1-6} alkoxy),
and wherein "heteroaryl" is a 5- or 6-membered aromatic heterocycle with one or two heteroatoms in the ring, which heteroatoms are independently selected from O, N and S, which heteroaryl is optionally mono- or disubstituted by substituents independently selected from halogen, CN, C_{1-6} alkyl optionally substituted by OH or NH_2, C_{1-6} alkoxy, perfluoro(C_{1-6} alkyl) and perfluoro(C_{1-6} alkoxy).

[0013] In the above definition, unless otherwise indicated, alkyl, alkenyl, alkylene and alkenylene groups having three or more carbon atoms may be straight chain or branched chain.

[0014] The compounds of formula (I) may contain one or more chiral centres and therefore can exist as stereoisomers, i.e. as enantiomers or diastereoisomers, as well as mixtures thereof. The invention includes both the individual stereoisomers of the compounds of formula (I) and any mixture thereof. Separation of diastereoisomers may be achieved by conventional techniques, e.g. by fractional crystallisation or chromatography (including HPLC) of a diastereoisomeric mixture of a compound of formula (I) or a suitable salt or derivative thereof. An individual enantiomer of a compound of formula (I) may be prepared from a corresponding optically pure intermediate or by resolution, either by HPLC of the racemate using a suitable chiral support or, where appropriate, by fractional crystallisation of the diastereoisomeric salts formed by reaction of the racemate with a suitable optically active base or acid, as appropriate to the

specific compound to be resolved. Furthermore, compound of formula (I) which contain alkenyl groups can exist as *cis*- or *trans*- geometric isomers. Again, the invention includes both the separated individual geometric isomers as well as mixtures thereof.

[0015] Also included in the invention are radiolabelled derivatives of compounds of formula (I) which are suitable for biological studies.

[0016] The pharmaceutically acceptable salts of the compounds of the formula (I) include the acid addition and the base salts thereof.

[0017] Suitable acid addition salts are formed from acids which form non-toxic salts and examples are the hydrochloride, hydrobromide, hydroiodide, sulphate, hydrogen sulphate, nitrate, phosphate, hydrogen phosphate, acetate, maleate, fumarate, lactate, tartrate, citrate, gluconate, succinate, benzoate, methanesulphonate, benzenesulphonate and p-toluenesulphonate salts.

[0018] Suitable base salts are formed from bases which form non-toxic salts and examples are the aluminum, calcium, lithium, magnesium, potassium, sodium, zinc and diethanolamine salts.

[0019] For a review on suitable salts see Berge et al, J. Pharm. Sci., 66, 1-19 (1977).

Preferably R^1 is H.

Preferably R^2 is H.

Preferably R^3 is H or C_{1-6} alkyl.
More preferably R^3 is H or CH_3.

Preferably R^4 is H.

Preferably R^5 is H or C_{1-6} alkyl.
More preferably $R^5$is H or CH_3.

Preferably R^6 is H, $aryl^1$ or $aryl^1$oxy wherein "$aryl^1$" is phenyl optionally mono- or disubstituted by substituents selected from halogen and CN.
More preferably R^6 is H, $aryl^2$ or $aryl^2$oxy wherein "$aryl^2$" is phenyl optionally 4-substituted by substituents selected from Cl and CN.
Most preferably R^6 is H, phenyl, phenoxy, 4-cyanophenyl or 4-chlorophenyl.

Preferably R^7 is H.

Preferably R^8 is H.

Preferably X is CH_2, $(CH_2)_2$, $(CH_2)_3$, or is $CH_2CH{=}CH$ wherein the terminal methinyl carbon of this group is linked to the Y moiety.

[0020] A preferred group of compounds, salts and solvates is that in which at least two of the groups R^4, R^5, R^7 and R^8 are all H.

[0021] Another preferred group of compounds, salts and solvates is that in which R^4, R^7 and R^8 are all H and R^5 is CH_3.

[0022] Yet another preferred group of compounds, salts and solvates is that in which R^1,R^2, R^4, R^7 and R^8 are all H,
R^3 is H or CH_3,
$R^5$is H or CH_3,
R^6 is H, phenyl, phenoxy, 4-cyanophenyl or 4-chlorophenyl,
X is CH_2, $(CH_2)_2$, $(CH_2)_3$, or $CH_2CH{=}CH$,
and the salts and solvates thereof.

[0023] The most preferred compounds, , salts and solvates are those of the Examples and the salts and solvates thereof.

[0024] The invention further provides synthetic methods for the production of compounds, salts and solvates of the invention, which are described below and in the Examples. The skilled man will appreciate that the compounds, salts and solvates of the invention could be made by methods other than those herein described, by adaptation of the methods herein described and/or adaptation of methods known in the art, for example the art described herein.

[0025] In the Methods below, unless otherwise specified, the substituents are as defined above with reference to the compounds of formula (I).

[0026] Where desired or necessary, the compound of formula (I) can be converted into a pharmaceutically or veterinarily acceptable salt thereof, conveniently by mixing together solutions of a compound of formula (I) and the desired acid or base, as appropriate. The salt may be precipitated from solution and collected by filtration, or may be collected by other means such as by evaporation of the solvent. In some cases, the salt may be the direct product of a reaction to make a compound or salt of the invention in a solvent, in which case no further transformation step would be necessary.

[0027] Where desired or necessary, solvates of the compounds and salts of the invention may be made by standard methods well known in the art. In some cases, the solvate may be the direct product of a reaction to make a compound or salt of the invention, in which case no further transformation step would be necessary.

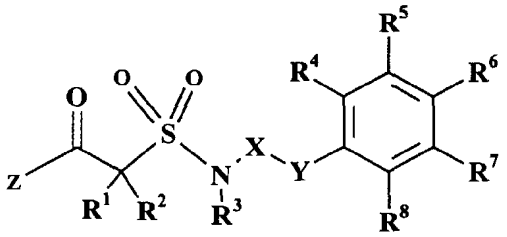
[0028] It is to be understood that the synthetic transformation methods mentioned herein may be carried out in various different sequences in order that the desired compounds can be efficiently assembled. The skilled chemist will exercise his judgement and skill as to the most efficient sequence of reactions for synthesis of a given target compound.

[0029] It will be apparent to those skilled in the art that sensitive functional groups may need to be protected and deprotected during synthesis of a compound of the invention. This may be achieved by conventional methods, for example as described in "Protective Groups in Organic Synthesis" by TW Greene and PGM Wuts, John Wiley & Sons Inc (1991).

[0030] The following processes are illustrative of the general synthetic procedures which may be adopted in order to obtain the compounds of the invention.

[0031] Unless otherwise stated, the substituents of the intermediates described below are as defined above for formula (I).

[0032] A compound of formula (I) may be prepared directly from an acid derivative of formula (II):



(II)

where Z is chloro, bromo, iodo, C_{1-3} alkyloxy or HO.

[0033] When prepared directly from the ester of formula (II), where Z is C_{1-3} alkyloxy, the reaction may be carried out by treatment of the ester with hydroxylamine, preferably up to a 3-fold excess of hydroxylamine, in a suitable solvent at from about room temperature to about 85°C. The hydroxylamine is conveniently generated in situ from a suitable salt such as its hydrochloride salt by conducting the reaction in the presence of a suitable base such as an alkali metal carbonate or bicarbonate, e.g. potassium carbonate. Preferably the solvent is a mixture of methanol and tetrahydrofuran and the reaction is temperature is from about 65 to 70°C.

[0034] Alternatively, the ester (II, where Z is C_{1-3} alkyloxy) may be converted by conventional hydrolysis to the corresponding carboxylic acid (II, Z is HO) which is then transformed to the required hydroxamic acid of formula (I).

[0035] Preferably the hydrolysis of the ester is effected under basic conditions using up to about a 6-fold excess of an alkali metal hydroxide in aqueous solution, optionally in the presence of a co-solvent, at from about room temperature to about 85°C. Typically the co-solvent is a mixture of methanol and tetrahydrofuran or a mixture of methanol and 1,4-dioxan and the reaction temperature is from about 40 to about 70°C.

[0036] The subsequent coupling step may be achieved using conventional amide-bond forming techniques, e.g. via the acyl halide derivative (II, Z is Cl, I or Br) and hydroxylamine hydrochloride in the presence of an excess of a tertiary amine such as triethylamine or pyridine to act as acid-scavenger, optionally in the presence of a catalyst such as 4-dimethylaminopyridine, in a suitable solvent such as dichloromethane, at from about 0°C to about room temperature. For convenience, pyridine may also be used as the solvent.

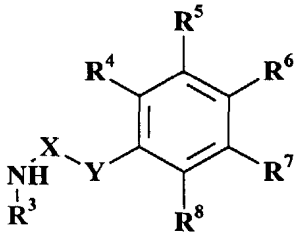
[0037] In particular, any one of a host of amino acid coupling variations may be used. For example, the acid of formula (II) wherein Z is HO may be activated using a carbodiimide such as 1 ,3-dicyclohexylcarbodiimide or 1 -ethyl-3-(3-dimethylaminoprop-1-yl)carbodiimide optionally in the presence of 1 -hydroxybenzotriazole and/or a catalyst such as 4-dimethylaminopyridine, or by using a halotrisaminophosphonium salt such as bromotris(pyrrolidino)-phosphonium hex-

afluorophosphate. Either type of coupling is conducted in a suitable solvent such as dichloromethane or dimethylformamide, optionally in the presence of a tertiary amine such as N-methylmorpholine or N-ethyldiisopropylamine (for example when either the hydroxylamine or the activating reagent is presented in the form of an acid addition salt), at from about 0°C to about room temperature. Typically, from 1.1 to 2.0 molecular equivalents of the activating reagent and from 1.0 to 4.0 molecular equivalents of any tertiary amine present are employed.

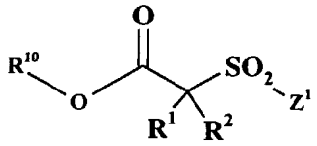
[0038] A preferred reagent for mediating the coupling reaction is O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU).

[0039] Preferably a solution of the acid (II, Z is HO) and N-ethyldiisopropylamine in a suitable solvent such as anhydrous dimethylformamide or anhydrous 1-methylpyrrolidin-2-one, under nitrogen, is treated with up to a 1.5-fold excess of HATU at about room temperature followed, after about 15 to 30 minutes, with up to about a 3-fold excess of hydroxylamine hydrochloride and up to about a 4-fold excess of N-ethyldiisopropylamine, optionally in the same solvent, at the same temperature.

[0040] An ester of formula (II, Z is C_{1-3} alkyloxy) may be prepared from an amine of formula (III) by sulphonylation with a compound of formula (IV), wherein R^{10} is C_{1-3} alkyloxy and Z^1 is a leaving group such as Br, I or Cl.



(III)



(IV)

Preferably, Z^1 is chloro.

[0041] The reaction may be effected in the presence of an appropriate base in a suitable solvent at from about 0°C to about room temperature. For example, when both R^1 and R^2 are hydrogen, an appropriate base is 1,8-diazabicyclo[5.4.0]undec-7-ene and a suitable solvent is dichloromethane.

[0042] Certain esters of formula (II, Z is C_{1-3} alkyloxy) wherein at least one of R^1 and R^2 is other than hydrogen may be conveniently obtained from the α-carbanion of an ester of formula (II) wherein at least one of R^1 and R^2 is hydrogen by conventional C-alkylation procedures using an alkylating agent of formula (VA) or (VB):

(VA) (VB)

wherein R is as previously defined for R^1 or R^2 but is not hydrogen, Z^2 and Z^3 may be the same or different and are suitable leaving groups such as chloro, bromo, iodo, C_1-C_4 alkanesulphonyloxy, trifluoromethanesulphonyloxy or arylsulphonyloxy (e.g. benzenesulphonyloxy or p-toluenesulphonyloxy), and q is 3, 4, 5, 6 or 7.

[0043] Preferably, Z^2 and Z^3 are selected from bromo, iodo and p-toluenesulphonyloxy.

[0044] The carbanion may be generate using an appropriate base in a suitable solvent. Typical base-solvent combinations may be selected from lithium, sodium or potassium hydride, lithium, sodium or potassium bis(trimethylsilyl)amide, lithium diisopropylamide and butyllithium, together with toluene, ether, 1,2-dimethoxyethane, tetrahydrofuran, 1,4-dioxan, dimethylformamide, N,N-dimethylacetamide, 1-methylpyrrolidin-2-one and any mixture thereof.

[0045] Preferably the base is sodium hydride and the solvent is dimethylformamide, optionally with tetrahydrofuran as co-solvent, or 1-methylpyrrolidin-2-one. For monoalkylation up to about a 10% excess of base is employed whilst, for dialkylation, from about 2 to about 3 molar equivalents are generally appropriate.

[0046] Typically, the carbanion is generated at about room temperature, under nitrogen, and subsequently treated with the required alkylating agent at the same temperature. Clearly, when dialkylation is required and R^1 and R^2 are different, the substituents may be introduced in tandem in a "one-pot reaction" or in separate steps.

[0047] An amine of formula (III) may be obtained by standard chemical procedures. Other amines of formula (III),

when neither commercially available nor subsequently described, can be obtained either by analogy with the processes described in the Preparations section below or by conventional synthetic procedures, in accordance with standard textbooks on organic chemistry or literature precedent, from readily accessible starting materials using appropriate reagents and reaction conditions.

[0048] Moreover, persons skilled in the art will be aware of variations of, and alternatives to, those processes described hereinafter in the Examples and Preparations sections which allow the compounds defined by formula (I) to be obtained.

[0049] The biological activities of the compounds of the present invention were determined by the following test methods, which are based on the ability of the compounds to inhibit the cleavage of various fluorogenic peptides by MMPs 1, 2, 3, 9, 13 and 14.

[0050] The assays for MMPs 2, 3, 9 and 14 are based upon the original protocol described in fed.Euro.Biochem.Soc., 1992, 296, 263, with the minor modifications described below.

Inhibition of MMP-1

Enzyme Preparation

[0051] Catalytic domain MMP-1 was prepared in Pfizer Central Research laboratories. A stock solution of MMP-1(1μM) was activiated by the addition of aminophenylmercuric acetate (APMA), at a final concentration of 1mM, for 20 minutes at 37°C. MMP-1 was then diluted in Tris-HCl assay buffer (50mM Tris, 200mM NaCl, 5mM $CaCl_2$, 20μM $ZnSO_4$ and 0.05% Brij 35, pH 7.5) to a concentration of 10nM. The final concentration of enzyme used in the assay was 1nM.

Substrate

[0052] The fluorogenic substrate used in this assay was Dnp-Pro-β-cyclohexyl-Ala-Gly-Cys(Me)-His-Ala-Lys-(N-Me-Ala)-NH_2 as originally described in Anal. Biochem., 1993, 212, 58. The final substrate concentration used in the assay was 10μM.

Determination of Enzyme Inhibition

[0053] The test compound was dissolved in dimethyl sulphoxide and diluted with assay buffer so that no more than 1% dimethyl sulphoxide was present Test compound and enzyme were added to each well of a 96 well plate and allowed to equilibrate for 15 minutes at 37°C in an orbital shaker prior to the addition of substrate. Plates were then incubated for 1 hour at 37°C prior to determination of fluorescence (substrate cleavage) using a fluorimeter (Fluostar; BMG LabTechnologies, Aylesbury, UK) at an excitation wavelength of 355 nm and emission wavelength of 440 nm. The potency of inhibition was measured from the amount of substrate cleavage obtained using a range of test compound concentrations and, from the resulting dose-response curve, an IC_{50} value (the concentration of inhibitor required to inhibit 50% of the enzyme activity) was calculated.

Inhibition of MMP-2, MMP-3 and MMP-9

Enzyme Preparation

[0054] Catalytic domains MMP-2, MMP-3 and MMP-9 were prepared in Pfizer Central Research laboratories. A stock solution of MMP-2, MMP-3 or MMP-9 (1μM) was activated by the addition of APMA. For MMP-2 and MMP-9, a final concentration of 1mM APMA was added, followed by incubation for 1 hour at 37°C. MMP-3 was activated by the addition of 2mM APMA, followed by incubation for 3 hours at 37°C. The enzymes were then diluted in Tris-HCl assay buffer (100mM Tris, 100mM NaCl, 10mM $CaCl_2$ and 0.16% Brij 35, pH 7.5) to a concentration of 10nM. The final concentration of enzyme used in the assays was 1nM.

Substrate

[0055] The fluorogenic substrate used in this screen was Mca-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH_2 (Bachem Ltd., Essex, UK) as originally described in J.Biol.Chem., 1994, 269 20952. This substrate was selected because it has a balanced hydrolysis rate against MMPs 2, 3 and 9 (k_{cat}/k_m of 54,000, 59,400 and 55,300 $^{s-1}$ M^{-1} respectively). The final substrate concentration used in the assay was 5μM.

Determination of Enzyme Inhibition

[0056] The test compound was dissolved in dimethyl sulphoxide and diluted with assay buffer so that no more than 1% dimethyl sulphoxide was present. Test compound and enzyme were added to each well of a 96 well plate and allowed to equilibrate for 15 minutes at 37°C in an orbital shaker prior to the addition of substrate. Plates were then incubated for 1 hour at 37°C, prior to determination of fluorescence using a fluorimeter (Fluostar; BMG LabTechnologies, Aylesbury, UK) at an excitation wavelength of 328nm and emission wavelength of 393nm. The potency of inhibition was measured from the amount of substrate cleavage obtained using a range of test compound concentrations and, from the resulting dose-response curve, an IC_{50} value (the concentration of inhibitor required to inhibit 50% of the enzyme activity) was calculated.

Inhibition of MMP-13

Enzyme Preparation

[0057] Human recombinant MMP-13 was prepared by PanVera Corporation (Madison, Wisconsin) and characterised at Pfizer Central Research laboratories. A 1.9 mg/ml stock solution was activated with 2mM APMA for 2 hours at 37°C. MMP-13 was then diluted in assay buffer (50mM Tris, 200mM NaCl, 5mM $CaCl_2$, 20μM $ZnCl_2$ and 0.02% Brij 35, pH 7.5) to a concentration of 5.3nM. The final concentration of enzyme used in the assay was 1.3nM.

Substrate

[0058] The fluorogenic substrate used in this screen was Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2. The final substrate concentration used in the assay was 10μM.

Determination of Enzyme Inhibition

[0059] The test compound was dissolved in dimethyl sulphoxide and diluted with assay buffer so that no more than 1% dimethyl sulphoxide was present. Test compound and enzyme were added to each well of a 96 well plate. The addition of substrate to each well initiated the reaction. Fluorescence intensity was determined using a 96 well plate fluorimeter (Cytofluor II; PerSeptive Biosystems, Inc., Framingham, MA) at an excitation wavelength of 360nm and emission wavelength of 460nm. The potency of inhibition was measured from the amount of substrate cleavage obtained using a range of test compound concentrations and, from the resulting dose-response curve, an IC_{50} value (the concentration of inhibitor required to inhibit 50% of the enzyme activity) was calculated.

Inhibition of MMP-14

Enzyme Preparation

[0060] Catalytic domain MMP-14 was prepared in Pfizer Central Research laboratories. A 10μM enzyme stock solution was activated for 20 minutes at 25°C following the addition of 5μg/ml of trypsin (Sigma, Dorset, UK). The trypsin activity was then neutralised by the addition of 50μg/ml of soyabean trypsin inhibitor (Sigma, Dorset, UK), prior to dilution of this enzyme stock solution in Tris-HCl assay buffer (100mM Tris, 100nM NaCl, 10mM $CaCl_2$, 0.16% Brij 35, pH 7.5) to a concentration of 10nM. The final concentration of enzyme used in the assay was 1nM.

Substrate

[0061] The fluorogenic substrate used in this screen was Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (Bachem Ltd., Essex, UK) as described in J.Biol.Chem. 1996, 271, 17119.

Determination of enzyme inhibition

[0062] This was performed as described for MMPs 2, 3 and 9.

[0063] For use in mammals, including humans, the compounds of formula (I) or their salts or solvates of such compounds or salts, can be administered alone, but will generally be administered in admixture with a pharmaceutically or veterinarily acceptable diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice. For example, they can be administered orally, including sublingually, in the form of tablets containing such excipients as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the

form of elixirs, solutions or suspensions containing flavouring or colouring agents. The compound or salt could be incorporated into capsules or tablets for targetting the colon or duodenum via delayed dissolution of said capsules or tablets for a particular time following oral administration. Dissolution could be controlled by susceptibility of the formulation to bacteria found in the dudodenum or colon, so that no substantial dissolution takes places before reaching the target area of the gastrointestinal tract. The compounds or salts can be injected parenterally, for example, intravenously, intramuscularly or subcutaneously. For parenteral administration, they are best used in the form of a sterile aqueous solution or suspension which may contain other substances, for example, enough salt or glucose to make the solution isotonic with blood. They can be administered topically, in the form of sterile creams, gels, suspensions, lotions, ointments, dusting powders, sprays, drug-incorporated dressings or via a skin patch. For example they can be incorporated into a cream consisting of an aqueous or oily emulsion of polyethylene glycols or liquid paraffin, or they can be incorporated into an ointment consisting of a white wax soft paraffin base, or as hydrogel with cellulose or polyacrylate derivatives or other viscosity modifiers, or as a dry powder or liquid spray or aerosol with butane/propane, HFA or CFC propellants, or as a drug-incorporated dressing either as a tulle dressing, with white soft paraffin or polyethylene glycols impregnated gauze dressings or with hydrogel, hydrocolloid, alginate or film dressings. The compound or salt could also be administered intraocularly as an eye drop with appropriate buffers, viscosity modifiers (e.g. cellulose derivatives), preservatives (e.g. benzalkonium chloride (BZK)) and agents to adjust tenicity (e.g. sodium chloride). Such formulation techniques are well-known in the art.

[0064] For veterinary use, a compound of formula (I), or a veterinarily acceptable salt thereof, or a veterinarily acceptable solvate of either entity, is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

[0065] All such formulations may also contain appropriate stabilisers and preservatives.

[0066] Reference to treatment includes prophylaxis as well as alleviation of established conditions, or the symptoms thereof.

[0067] For oral and parenteral administration to animal (inc. human) patients, the daily dosage level of the compounds of formula (I) or their salts will be from 0.001 to 20, preferably from 0.01 to 20, more preferably from 0.1 to 10, and most preferably from 0.5 to 5 mg/kg (in single or divided doses). Thus tablets or capsules of the compounds will contain from 0.1 to 500, preferably from 50 to 200, mg of active compound for administration singly or two or more at a time as appropriate.

[0068] For topical administration to animal (inc. human) patients with chronic wounds, the daily dosage level of the compounds, in suspension or other formulation, could be from 0.00001 to 1mg/ml, preferably from 0.001 to 0.1 mg/ml.

[0069] The physician or veterinary surgeon in any event will determine the actual dosage which will be most suitable for a an individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case; there can of course be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

[0070] Thus the invention provides a pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable solvate of either entity, together with a pharmaceutically acceptable diluent or carrier.

[0071] It further provides a veterinary formulation comprising a compound of formula (I), or a veterinarily acceptable salt thereof or a veterinarily acceptable solvate of either entity, together with a veterinarily acceptable diluent or carrier.

[0072] The invention also provides a compound of formula (I), or a pharmaceutically acceptable salt thereof or a pharmaceutically acceptable solvate of either entity, or a pharmaceutical composition containing any of the foregoing, for use as a human medicament.

[0073] In addition, it provides a compound of formula (I), or a veterinarily acceptable salt thereof, or a veterinarily acceptable solvate of either entity, or a veterinary formulation containing any of the foregoing, for use as a medicament for non-human animal.

[0074] In yet another aspect, the invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate of either entity, for the manufacture of a human medicament for the treatment of a condition mediated by one or more MMPs.

[0075] It also provides the use of a compound of formula (I), or a veterinarily acceptable salt thereof, or a veterinarily acceptable solvate of either entity, for the manufacture of an animal medicament for the treatment of a condition mediated by one or more MMPs.

[0076] Moreover, the invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate of either entity, for the manufacture of a human medicament for the treatment of atherosclerotic plaque rupture, myocardial infarction, heart failure, restenosis, stroke, periodontal disease, tissue ulceration, wound repair, skin diseases, cancer metastasis, tumour angiogenesis, age-related macular degeneration, fibrotic disease, rheumatoid arthritis, osteoarthritis and inflammatory diseases dependent on migratory inflammatory cells.

[0077] It also provides the use of a compound of formula (I), or a veterinarily acceptable salt thereof, or a veterinarily acceptable solvate containing either entity, for the manufacture of an animal medicament for the treatment of atherosclerotic plaque rupture, myocardial infarction, heart failure, restenosis, stroke, periodontal disease, tissue ulceration, wound repair, skin diseases, cancer metastasis, tumour angiogenesis, age-related macular degeneration, fibrotic disease, rheumatoid arthritis, osteoarthritis and inflammatory diseases dependent on migratory inflammatory cells.

[0078] Additionally, the invention provides a method of treating or preventing a medical condition for which a MMP inhibitor is indicated, in an animal such as a mammal (including a human being), which comprises administering to said animal a therapeutically effective amount of a compound of formula (I), or a pharmaceutically or veterinarily acceptable salt thereof, or a pharmaceutically or veterinarily acceptable solvate of either entity, or a pharmaceutical composition or veterinary formulation containing any of the foregoing.

[0079] Still further, the invention provides a method of treating or preventing atherosclerotic plaque rupture, myocardial infarction, heart failure, restenosis, stroke, periodontal disease, tissue ulceration, wound repair, skin diseases, cancer metastasis, tumour angiogenesis, age-related macular degeneration, fibrotic disease, rheumatoid arthritis, osteoarthritis and inflammatory diseases dependent on migratory inflammatory cells, in a animal (including a human being), which comprises administering to said animal a therapeutically effective amount of a compound of formula (I), or a pharmaceutically or veterinarily acceptable salt thereof, or a pharmaceutically or veterinarily acceptable solvate of either entity, or a pharmaceutical composition or veterinary formulation containing any of the foregoing.

[0080] The invention also includes any novel intermediates described herein, for example those of formula (II).

[0081] The syntheses of the compounds of the invention and of the intermediates for use therein are illustrated by the following Examples and Preparations.

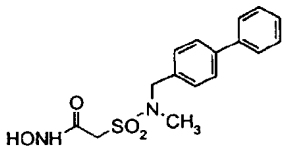
EXAMPLES AND PREPARATIONS

[0082] Room temperature means 20 to 25°C. Flash chromatography refers to column chromatography on silica gel (Kieselgel 60, 230-400 mesh). Melting points are uncorrected. ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded using a Bruker AC300, a Varian Unity Inova-300 or a Varian Unity Inova-400 spectrometer and were in all cases consistent with the proposed structures. Characteristic chemical shifts are given in parts-per-million downfield from tetramethylsilane using conventional abbreviations for designation of major peaks: e.g. s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectra were recorded using a Finnigan Mat. TSQ 7000 or a Fisons Instruments Trio 1000 mass spectrometer. LRMS means low resolution mass spectrum and the calculated and observed ions quoted refer to the isotopic composition of lowest mass._Hexane refers to a mixture of hexanes (hplc grade) b.p. 65-70°C. Ether refers to diethyl ether. Acetic acid refers to glacial acetic acid. 1-Hydroxy-7-aza- 1H-1,2,3-benzotriazole (HOAt), *N*-[(dimethylamino)-1H-1,2,3-triazolo[4,5-*b*]pyridin- 1-ylmethylene]-*N*-methylmethaninium hexafluorophosphate *N*-oxide (HATU) and 7-azabenzotriazol-1-yloxy*tris*(pyrrolidino)phosphonium hexafluorophosphate (PyAOP) were purchased from PerSeptive Biosystems U.K. Ltd.

Example 1

N-Hydroxy 2-({methyl[(biphen-4-yl)methyl]amino}sulfonyl)acetamide

[0083]



(a) Methyl 2-({methyl[(biphen-4-yl)methyl]amino}sulfonyl)acetate

[0084] *N*-Methyl-*N*-[(biphen-4-yl)methyl]amine (Preparation 1, 500 mg, 2.5 mmol) and 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU, 0.38 ml, 2.5 mmol) were dissolved in dichloromethane (5 ml) and cooled to 0°C. Methyl chlorosulfonylacetate (0.44 g, 2.5 mmol) in dichloromethane (5 ml) was added dropwise to the solution, and the stirred mixture was allowed to warm to ambient temperature for 20 hours. The mixture was diluted with dichloromethane and washed with aqueous phosphate buffer (at pH 7), dried ($MgSO_4$), and the solvents were evaporated under reduced pressure. The

residue was purified by flash chromatography on silica gel (dichloromethane as eluent) and the isolated product was crystallised from diisopropyl ether to give the title compound as a colourless solid (388 mg).

m.p. 82-84°C
^{1}H NMR (400 MHz, $CDCl_3$): 2.90 (s, 3H), 3.86 (s, 3H), 4.05 (s, 2H), 4.46 (s, 2H), 7.33-7.40 (m, 1H), 7.40-7.45 (m, 4H), 7.54-7.67 (m, 4H).
LPMS (Thermospray): 334.8 (MH^+).

(b) *N*-Hydroxy-2-({methyl[(biphen-4-yl)methyl]amino}sulfonyl)acetamide

[0085] Potassium carbonate (124 mg, 0.9 mmol) was added to a mixture of methyl 2-({methyl[(biphen-4-yl)methyl]amino}sulfonyl)acetate (100 mg, 0.3 mmol) and hydroxylamine hydrochloride (63 mg, 0.9 mmol) in methanol (3 ml). The mixture was heated to reflux for 18 hours. The mixture was cooled and partitioned between ethyl acetate and 0.1M aqueous hydrochloric acid. The layers were separated, and the organic layer was dried ($MgSO_4$), and the solvents were removed under reduced pressure. The residue was triturated with diisopropyl ether to give the titled compound as a colourless solid (88 mg).

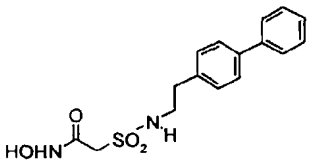
m.p. 176-178°C
^{1}H NMR (300 MHz, DMSO-d_6): 2.75 (s, 3H), 3.98 (s, 2H), 4.33 (s, 2H), 7.33-7.52 (m, 5H), 7.61-7.74 (m, 4H), 9.22 (s, 1H), 10.84 (br s, 1H).
LRMS (Thermospray): 335.7 (MH^+)

Analysis:	Found:	C, 57.32;	H, 5.40;	N, 8.24.
$C_{16}H_{18}N_2O_4S$	Requires:	C, 57.47;	H, 5.43;	N, 8.38.

Example 2

N-Hydroxy 2-({[2-(biphen-4-yl]ethyl]amino} sulfonyl)acetamide

[0086]



(a) Methyl 2-({[2-(biphen-4-yl)ethyl]amino}sulfonyl)acetate

[0087] In a manner similar to Example 1 (a), 2-(biphen-4-yl)ethylamine (Preparation 2) was reacted with methyl chlorosulfonylacetate to give the title compound as a colourless solid.

m.p. 130-131°C
^{1}H NMR (300 MHz, $CDCl_3$): 2.97 (t, 2H), 3.49 (q, 2H), 3.76 (s, 3H), 3.93 (s, 2H), 4.76 (br t, 1H), 7.22-7.40 (m, 3H), 7.40-7.50 (m, 2H), 7.52-7.64 (m, 4H).
LRMS (Thermospray): 351.1 (MNH_4^+)

Analysis:	Found:	C, 61.39;	H, 5.74;	N, 4.19.
$C_{17}H_{19}NO_4S$,	Requires:	C, 61.24;	H, 5.74;	N, 4.20.

(b) *N*-Hydroxy 2-({[2-(biphen-4-yl)ethyl]amino}sulfonyl)acetamide

[0088] In a manner similar to Example 1 (b), methyl 2-({[2-(biphen-4-yl)ethyl]amino}sulfonyl)acetate was reacted with hydroxylamine to give the title compound as a colourless solid.

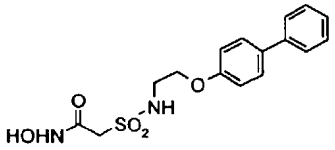
m.p. 202-204°C
^{1}H NMR (300 MHz, DMSO-d_6): 2.81 (t, 2H), 3.16-3.29 (m, 2H), 3.78 (s, 2H), 7.24-7.39 (m, 3H), 7.40-7.50 (m, 2H), 7.54-7.68 (m, 4H), 9.13 (s, 1H), 10.74 (br s, 1H).
LRMS (Thermospray): 336.2 (MH^+)

Analysis:	Found:	C, 57.45;	H, 5.40;	N, 8.35.
$C_{16}H_{18}N_2O_4S$	Requires:	C, 57.47;	H, 5.43;	N, 8.38.

Example 3

N-Hydroxy 2-({[2-(biphen-4-yloxy)ethyl]amino} sulfonyl)acetamide

[0089]



(a) Methyl 2-({[2(biphen-4-yloxy)ethyl]amino}sulfonyl)acetate

[0090] In a manner similar to Example 1 (a), 2-(biphen-4-yloxy)ethylamine (Preparation 3) was reacted with methyl chlorosulfonylacetate to give the title compound as a colourless solid.

m.p. 123-124°C
^{1}H NMR (400 MHz, $CDCl_3$): 3.62 (q, 2H), 3,79 (s, 3H), 4.10 (s, 2H), 4.18 (t, 2H), 5.26 (br t, 1H), 6.98 (d, 2H), 7.31-7.34 (m, 1H), 7.39-7.46 (m, 2H), 7.50-7.60 (m, 4H).

Analysis:	Found:	C, 58.33;	H, 5.44;	N, 3.99.
$C_{17}H_{19}NO_5S$	Requires:	C, 58.43;	H, 5.48;	N, 4.01.

(b) *N*-Hydroxy 2-({[2-(biphen-4-yloxy)ethyl]amino}sulfonyl)acetamide

[0091] In a manner similar to Example 1 (b), methyl 2-({[2-(biphen-4yloxy)ethyl]amino} sulfonyl)acetate was reacted with hydroxylamine to give the title compound as a colourless solid.

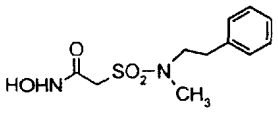
m.p. 222-224°C
^{1}H NMR (400 MHz, DMSO-d_6): 3.39 (d, 2H), 3.86 (s, 2H), 4.07 (t, 2H), 7.07 (d, 2H), 7.29-7.33 (m, 1H), 7.37-7.51 (m, 3H), 7.57-7.65 (m, 4H), 9.13 (s, 1H), 10.73 (s, 1H).
LPMS (Thermospray): 352.0 (MH^+)

Analysis:	Found:	C, 54.69;	H, 5.13;	N, 7.92.
$C_{19}H_{22}N_2O_4S$	Requires:	C, 54.84;	H, 5.18;	N, 8.00.

Example 4

N-Hydroxy 2-[methyl(phenethyl)amino]sulfonylacetamide

[0092]



(a) Methyl 2-[methyl(phenethyl)amino]sulfonylacetate

[0093] In a manner similar to Example 1 (a), *N*-methyl-*N*-phenethylamine was reacted with methyl chlorosulfonylacetate to give the titled compound as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): 2.88-2.96 (m, 5H), 3.48 (t, 2H), 3.77 (s, 3H), 3.81 (s, 2H), 7.18-7.36 (m, 5H).

(b) *N*-Hydroxy 2-[methyl(phenethyl)amino]sulfonylacetamide

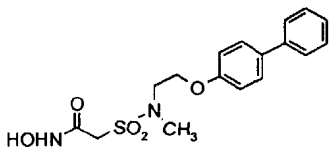
[0094] In a manner similar to Example 1 (b), methyl 2-[methyl(phenethyl)amino]sulfonylacetate was reacted with hydroxylamine to give the title compound as a colourless solid.

m.p. 149-151°C
^{1}H NMR (300 MHz, DMSO-d_6): 2.76-2.86 (m, 5H), 3.28 (s, 2H), 3.80 (s, 2H), 7.15-7.35 (m, 5H), 9.14 (s, 1H), 10.73 (s, 1H).
LRMS (Thermospray): 290.0 (MNH_4^+)
$C_{11}H_{16}N_2O_4S$.

Example 5

N-Hydroxy 2-({methyl-[2-(biphen-4-yloxy)ethyl]amino} sulfonyl)acetamide

[0095]



(a) Methyl 2-({methyl-[2-(biphen-4-yloxy)ethyl]amino}sulfonyl)acetate

[0096] Sodium hydride (23 mg of 60% dispersion in mineral oil, 0.58 mmol) was added to a stirred solution of methyl 2-({[2-(biphen-4-yloxy)ethyl]amino}sulfonyl)acetate (Example 3(a), 185 mg, 0.53 mmol) in anhydrous dimethylforma-

mide (2 ml) at ambient temperature under a nitrogen atmosphere. After 30 minutes methyl *p*-toluenesulfonate (0.99 g, 0.53 mmol) was added, and stirring continued for an additional 3 hours. The mixture was partitioned between ethyl acetate and aqueous phosphate buffer (pH 7). The organic layer was separated and washed with water, dried ($MgSO_4$) and the solvents were removed under reduced pressure. The residue was crystallised from diisopropyl ether to give the titled compound as a colourless solid (170 mg).

m.p. 73-75°C
^{1}H NMR (400 MHz, $CDCl_3$): d = 3.11 (s, 3H), 3.69 (t, 2H), 3,78 (s, 3H), 4.08 (s, 2H), 4.18 (t, 2H), 6.97 (d, 2H), 7.28-7.32 (m, 1H), 7.38-7.46 (m, 2H), 7.47-7.58 (m, 4H).
LRMS (Thermospray): 381.1 (MNH_4^+)

Analysis:	Found:	C, 59.39;	H, 5.88;	N, 3.74.
$C_{18}H_{21}NO_5S$	Requires:	C,59.48;	H, 5.82;	N, 3.86.

(b) *N*-Hydroxy 2-({methyl-[2-(biphen-4 yloxy)ethyl]amino}sulfonyl)acetamide

[0097] In a manner similar to Example 1 (b), methyl 2-({methyl-[2-(biphen-4-yloxy)ethyl] amino} sulfonyl)acetate was reacted with hydroxylamine to give the title compound as a colourless solid.

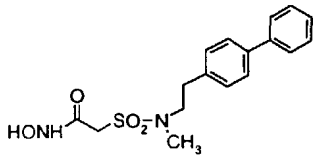
m.p. 153-155°C
^{1}H NMR (400 MHz, DMSO-d_6): d = 2.93 (s, 3H), 3.47-3.58 (m, 2H), 3.90 (s, 2H), 4.10-4.20 (m, 2H), 7.03 (d, 2H), 7.25-7.33 (m, 1H), 7.37-7.46 (m, 2H), 7.54-7.66 (m, 4H), 9.18 (s, 1H), 10.79 (s, 1H).
LRMS (APCI): 368.8 (MH^+)

Analysis:	Found:	C, 55.56;	H, 5.47;	N, 7.24.
$C_{17}H_{20}N_2O_5S$	Requires:	C, 56.03;	H, 5.53;	N, 7.69.

Example 6

N-Hydroxy 2-({methyl-[2-(biphen-4-yl)ethyl]amino) sulfonyl)acetamide

[0098]



(a) Methyl 2-({methyl-[2-(biphen-4-yl)ethyl]amino} sulfonyl)acetate

[0099] In a manner similar to Example 5 (a), methyl 2-({[2-(biphen-4-yl)ethyl]amino}-sulfonyl)acetate (Example 2 (a)) was reacted with sodium hydride and methyl *p*-toluenesulfonate to give the title compound as a colourless solid.

m.p. 72-74°C
^{1}H NMR (400 MHz, $CDCl_3$): 2.87-2.97 (m, 5H), 3.48 (t, 2H), 3.75 (s, 3H), 3.82 (s, 2H), 7.24-7.33 (m, 3H), 7.37-7.44 (m, 2H), 7.47-7.59 (m, 4H).
LRMS (Thermospray): 365.0 (MNH_4^+)
$C_{18}H_{21}NO_4S$

(b) *N*-Hydroxy 2-({methyl-[2-(biphen-4-yl)ethyl]amino}sulfonyl)acetamide

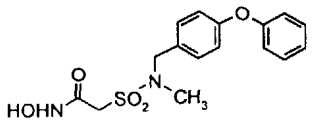
[0100] In a manner similar to Example 1 (b), methyl 2-({methyl-[2-(biphen-4-yl)ethyl] amino}sulfonyl)acetate was reacted with hydroxylamine to give the title compound as a colourless solid.

m.p. 166-168°C
^{1}H NMR (400 MHz, DMSO-d_6): 2.77-2.88 (m, 5H), 3.32 (t, 2H), 3.78 (s, 2H), 7.24-7.33 (m, 3H), 7.37-7.45 (m, 2H), 7.53-7.63 (m, 4H).
LRMS (Thermospray): 365.9 (MNH_4^+)
$C_{17}H_{20}N_2O_4S$.

Example 7

N-Hydroxy 2-({methyl[4-phenoxybenzyl]amino} sulfonyl)acetamide

[0101]



(a) Methyl 2-({methyl[4-phenoxybenzyl]amino}sulfonyl)acetate

[0102] In a manner similar to Example 1 (a), *N*-methyl-*N*-(4-phenoxybenzyl)amine (Preparation 4) was reacted with methyl chlorosulfonylacetate to give the title compound as a colourless solid.

m.p. 63-64°C
^{1}H NMR (300 MHz, $CDCl_3$): 2.84 (s, 3H), 3.81 (s, 3H), 4.00 (s, 2H), 4.35 (s, 2H), 6.95-7.06 (m, 4H), 7.06-7.16 (m, 1H), 7.21-7.40 (m, 4H).
LRMS (Thermospray): 350.6 (MH^+)
$C_{17}H_{19}NO_5S$.

(b) *N*-Hydroxy 2-({methyl[4-phenoxybenzyl]amino}sulfonyl)acetamide

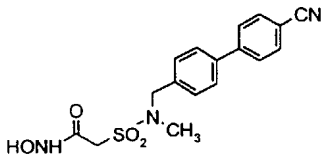
[0103] In a manner similar to Example 1(b), methyl 2-({methyl[4-phenoxybenzyl]amino}-sulfonyl) acetate was reacted with hydroxylamine to give the title compound as a colourless solid.

m.p. 154-157°C
^{1}H NMR (400 MHz, DMSO-d_6): d = 2.72 (s, 3H), 3.95 (s, 2H), 4.26 (s, 2H), 6.94-7.04 (m, 4H), 7.10-7.18 (m, 1H), 7.29-7.43 (m, 4H).
LRMS (Thermospray): 373.5 (MNa^+)

Example 8

N-Hydroxy 2-({methyl[(4'-cyanobiphen-4-yl)methyl]amino} sulfonyl)acetamide

[0104]



(a) Methyl 2-({methyl[(4-bromophenyl)methyl]amino}suflonyl)acetate

[0105] In a manner similar to Example 1(a), *N*-methyl-*N*-(4-bromobenzyl)amine (Preparation 5) was reacted with methyl chlorosulfonylacetate to give the title compound as a pale yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$): 2.83 (s, 3H), 3.82 (s, 3H), 4.03 (s, 2H), 4.33 (s, 2H), 7.25 (d, 2H), 7.50 (d, 2H).
LRMS (Thermospray): 354.3 (MNH_4^+)
$C_{11}H_{14}BrNO_4S$.

(b) Methyl-2-({methyl[(4'-cyanobiphen-4-yl)methyl]amino}sulfonyl)acetate

[0106] To a solution of methyl 2({methyl[(4-bromophenyl)methyl]amino}sulfonyl)acetate (300 mg, 0.9 mmol) in dimethoxyethane (5 ml) was added 4-cyanophenylboronic acid (Preparation 6,150 mg, 1.0 mmol), caesium fluoride (290 mg), tri-*ortho*-tolyl phosphine (28 mg, 0.09 mmol) and bis(benzylideneacetone)palladium(0) (25 mg, 0.04 mmol) and the mixture was heated to reflux for 1 hour under an atmosphere of nitrogen. The mixture was cooled to ambient temperature, diluted with dichloromethane (30 ml) and washed with water. The organic layer was dried (Na_2SO_4), the solvent was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel (hexane/ethyl acetate 2:1 as eluent) to give the titled compound as a pale yellow low melting solid (230mg).

^{1}H NMR (300 MHz, $CDCl_3$): 2.88 (s, 3H), 3.84 (s, 3H), 4.06 (s, 2H), 4.45 (s, 2H), 7.48 (d, 2H), 7.60 (d, 2H), 7.67 (d, 2H), 7.75 (d, 2H).

(c) 2-({methyl[(4'-cyanobiphen-4-yl)methy]lamino}sulfonyl)acetic acid

[0107] To a solution of methyl-2-({methyl[(4'-cyanobiphen-4-yl)methyl]amino}-sulfonyl)acetate (200 mg, 0.56 mmol) in methanol (2 ml) and tetrahydrofuran (5 ml) was added 1M aqueous sodium hydroxide solution (1.2 ml, 1.2 mmol) and the mixture was stirred at ambient temperature for 2 hours. The solution was diluted with water (10 ml), acidified to pH 2 with 2M aqueous hydrochloric acid and extracted with dichloromethane (2 x 30 ml). The combined organic layers were dried Na_2SO_4), and the solvent was evaporated under reduced pressure to give the title compound as a pale yellow solid (130 mg).

m.p. 149-152°C
^{1}H NMR (300 MHz, DMSO-d_6): 2.74 (s, 3H), 4.16 (s, 2H), 4.57 (s, 2H), 7.46 (d, 2H), 7.78 (d, 2H), 7.87 (d, 2H), 7.90 (d, 2H).

(d) *N*-Hydroxy 2-({methyl[(4'-cyanobiphen-4-yl)methyl]amino}sulfonyl)-acetamide

[0108] *O*-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU 263 mg, 0.72 mmol) was added to a solution of 2-({methyl[(4'-cyanobiphenyl-4-yl)methyl]amino}sulfonyl)acetic acid (185 mg, 0.48 mmol) and *N*-ethyl-*N,N*-diisopropylamine (0.08 ml, 0.48 mmol) in anhydrous dimethylformamide (3 ml) at ambient temperature under an atmosphere of nitrogen. After stirring for 20 minutes a solution of hydroxylamine hydrochloride (131 mg, 1.92 mmol) and *N*-ethyl-*N,N*-diisopropylamine (0.33 ml, 1.92 mmol) in anhydrous dimethylformamide (1 ml) was added and the solution was stirred for a further 16 hours. The mixture was partitioned between aqueous phosphate buffer (at pH 7)

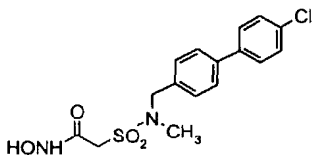
and ethyl acetate. The organic layer was washed with water, dried (MgSO4) and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (dichloromethane/methanol/aqueous ammonia 90:10:1 as eluent) to give the title compound as a colourless solid (14 mg).

m.p. 128-130°C
^{1}H NMR (400 MHz, DMSO-d_6): 2.73 (s, 3H), 3.97 (s, 2H), 4.33 (s, 2H), 7.44 (d, 2H), 7.74 (d, 2H), 7.85 (d, 2H), 7.91 (d, 2H).
LRMS (Thermospray): 361.0 ($M+2H^+$).

Example 9

N-Hydroxy 2-({methyl[(4'-chlorobiphen-4-yl)methyl]amino}sulfonyl)acetamide

[0109]



(a) Methyl-2-({methyl[(4'-chlorobiphen-4-yl)methyl]amino}sulfonyl)acetate

[0110] In a manner similar to Example 8 (b), methyl 2({methyl[(4'-bromophenyl-4-yl)methyl]amino}sulfonyl)acetate (Example 8 (a)) was reacted with 4-chlorophenylboronic acid to give the titled compound as a pale yellow solid.

m.p. 103-106°C
^{1}H NMR (400 MHz, $CDCl_3$): 2.87 (s, 3H), 3.83 (s, 3H), 4.04 (s, 2H), 4.43 (s, 2H), 7.38-7.46 (m, 4H), 7.48-7.59 (m, 4H).
LRMS (Thermospray): 385.2 ($M+H^+$)

(b) *N*-Hydroxy-2-({methyl[(4'-chlorobiphen-4-yl)methyl]amino}-sulfonyl)acetamide

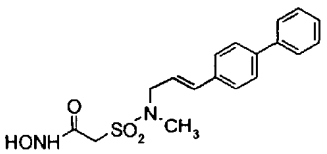
[0111] In a manner similar to Example 1 (b), methyl-2-({methyl[(4'-chlorobiphenyl-4-yl)methyl]amino}sulfonyl)acetate was reacted with hydroxylamine to give the title compound as a colourless solid.

m.p. 158-161°C
^{1}H NMR (400 MHz, DMSO-d_6): 2.72 (s, 3H), 3.95 (s, 2H), 4.32 (s, 2H), 7.40 (d, 2H), 7.49 (d, 2H), 7.66 (d, 2H), 7.69 (d, 2H), 9.22 (s, 1H), 10.83 (s, 1H).
LRMS (Thermospray): 369.8 ($M+H^+$).

Example 10

N-Hydroxy 2-({methyl[3-(biphen-4-yl)-*trans*-prop-2-enyl]amino}sulfonyl) acetamide

[0112]



(a) Methyl 2-({methyl[allyl]amino}sulfonyl)acetate

[0113] In a manner similar to Example 1 (a), *N*-methyl-*N*-allylamine was reacted with methyl chlorosulfonylacetate to give the title compound as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): 2.89 (s, 3H), 3.81 (s, 3H), 3.81 (d, 2H), 3.97 (s, 2H), 5.03-5.15 (m, 2H), 5.74-5.88 (m, 1H).

(b) Methyl- 2-({methyl[3-(biphen-4-yl)-*trans*-prop-2-enyl]amino}sulfonyl)acetate

[0114] To a solution of methyl 2-({methyl[allyl]amino}sulfonyl)acetate (300 mg, 1.4 mmol) and 4-bromobiphenyl (370 mg, 1.54 mmol) in acetonitrile (4 ml) was added triethylamine (0.3 ml, 2.1 mmol), palladium(II) acetate (17 mg, 0.07 mmol) and tri-*ortho*-tolyl phosphine (52 mg, 0.14 mmol) and the solution was heated to reflux under an atmosphere of nitrogen for 3 hours. The mixture was cooled to ambient temperature, the solvent was evaporated and the residue was purified by flash chromatography on silica gel (dichloromethane as eluent) to give the title compound as a pale yellow solid (300 mg).

m.p. 104-107°C
^{1}H NMR (300 MHz, $CDCl_3$): 2.97 (s, 3H), 3.86 (s, 3H), 4.00-4.13 (m, 4H), 6.24 (dt, 1H), 6.66 (d, 1H), 7.33-7.40 (m, 1H), 7.41-7.54 (m, 4N), 7.58-7.71 (m, 4H).
LRMS (Thermospray): 377.2 (MNH_4^+).

(c) *N*-Hydroxy-2-({methyl[3-(biphen-4-yl)-*trans*-prop-2-enyl]amino}sulfonyl)-acetamide

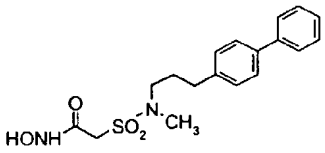
[0115] In a manner similar to Example 1 (b), methyl 2-({methyl[3-(1,1'-biphenyl-4-yl)*trans*-prop-2-enyl]amino} sulfonyl)acetate was reacted with hydroxylamine to give the title compound as a colourless solid.

m.p. 153-155°C
^{1}H NMR (300 MHz, DMSO-d_6): 2.82 (s, 3H), 3.88-3.97 (m, 4H), 6.34 (dt, 1H), 6.66 (d, 1H), 7.36 (d, 1H), 7.43 (d, 1H), 7.46 (d, 1H), 7.56 (d, 2H), 7.64 (d, 2H), 7.67 (d, 2H), 9.20 (s, 1H), 10.81 (s, 1H).
LRMS (Thermospray): 362.2 ($M+2H^+$).

Example 11

N-Hydroxy 2-({methyl[3-(biphen-4-yl)-prop-1-yl]amino}sulfonyl)acetamide

[0116]



(a) Methyl- 2-({methyl[3-(biphen-4-yl)-propyl]amino}sulfonyl)acetate

[0117] To a solution of methyl- 2-({methyl[3-(biphen-4-yl)-*trans*-prop-2-enyl]amino}sulfonyl)acetate (Example 10 (b), 200 mg, 0.56 mmol) and ammonium formate (175 mg, 2.8 mmol) in methanol (5 ml) was added 20% palladium hydroxide on carbon (50 mg) and the mixture was heated to reflux for 4 hours. The mixture was cooled to ambient temperature, filtered through arbocel and the filtrate was concentrated under reduced pressure to give the title compound as a pale yellow solid (193 mg).

m.p. 66-70°C

^{1}H NMR (300 MHz, $CDCl_3$): 1.89-2.04 (m, 2H), 2.72 (t, 2H), 2.95 (s, 3H), 3.30 (t, 2H), 3.80 (s, 3H), 3.97 (s, 2H), 7.23-7.38 (m, 3H), 7.40-7.47 (m, 2H), 7.54 (d, 2H), 7.59 (d, 2H).
LRMS (Thermospray): 379.2 (MNH_4^+).

(b) *N*-Hydroxy 2-({methyl[3-(biphen-4-yl)-propyl]amino}sulfonyl)acetamide

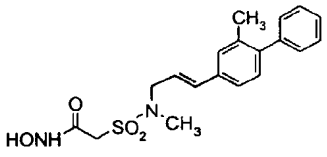
[0118] In a manner similar to Example 1 (b), methyl-2-({methyl[3-(biphen-4-yl)-propyl]amino}sulfonyl)acetate was reacted with hydroxylamine to give the title compound as a colourless solid.

m.p. 137-140°C
^{1}H NMR (300 MHz, DMSO-d_6): 1.75-1.93 (m, 2H), 2.61 (t, 2H), 2.82 (s, 3H), 3.18 (t, 2H), 3.83 (s, 2H), 7.25-7.36 (m, 3H), 7.40-7.50 (m, 2H), 7.57 (d, 2H), 7.64 (d, 2H), 9.05-9.28 (br s, 1H).
LRMS (Thermospray): 380.2 (MNH_4^+).

Example 12

N-Hydroxy 2-({methyl-[3-(2-methylbiphen-4-yl)-*trans*-prop-2-enyl]amino}-sulfonyl)acetamide

[0117]



(a) Methyl 2-({methyl-[3-(2-methylbiphen-4-yl)-*trans*-prop-2-enyl]amino}sulfonyl)acetate

[0120] In a manner similar to Example 10 (b), methyl 2-({methyl[allyl]amino}sulfonyl)acetate (Example 10 (a)) was reacted with 4-bromo-2-methylbiphenyl (Preparation 7) to give the title compound as a pale yellow low melting solid.

^{1}H NMR (400 MHz, $CDCl_3$): 2.29 (s, 3H), 2.97 (s, 3H), 3.93 (s, 3H), 4.00-4.07 (m, 4H), 6.23 (dt, 1H), 6.62 (d, 1H), 7.18-7.47 (m, 8H).
LPMS (Thermospray): 391.9 (MNH_4^+).

(b) *N*-Hydroxy 2-({methyl-[3-(2-methylbiphen-4-yl)-*trans*-prop-2-enyl]amino}-sulfonyl)acetamide

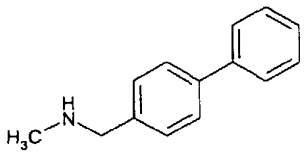
[0121] In a manner similar to Example 1 (b), methyl 2-({methyl-[3-(2-methylbiphen-4-yl)-*trans*-prop-2-enyl]amino}sulfonyl)acetate was reacted with hydroxylamine to give the titled compound as a colourless solid.

m.p. 146-149°C
^{1}H NMR (400 MHz, DMSO-d_6): 2.23 (s, 3H), 2.81 (s, 3H), 3.82-4.02 (m, 4H), 6.33 (dt, 1H), 6.62 (d, 1H), 7.17 (d, 1H), 7.25-7.49 (m, 7H), 9.21 (s, 1H), 10.82 (s, 1H).
LPMS (Thermospray): 376.1 ($M+2H^+$)

Preparation 1

N-Methyl-*N*-[(biphen-4-yl)methyl]amine

[0122]



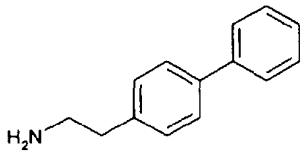
[0123] To a solution of biphenyl-4-carboxaldehyde (4.6 g, 25 mmol) in ethanol (50 ml) was added methylamine (3.0 ml of 33% solution in ethanol, 25 mmol) and acetic acid (1.4 ml, 25 mmol), and the mixture was stirred under an atmosphere of nitrogen. After 20 minutes sodium tri(acetoxy)borohydride (10.5 g, 50 mmol) was added and stirring was continued for 16 hours. The mixture was diluted with 2M aqueous hydrochloric acid (200 ml) and washed with ethyl acetate (3 x 100 ml). The aqueous layer was basified to pH 12 with concentrated aqueous ammonia solution and extracted with dichloromethane (4 x 100 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to give the title compound as a pale yellow oil (2.5 g).

^{1}H NMR (300 MHz, $CDCl_3$):1.38 (br s, 1H), 2.50 (s, 3H), 3.80 (s, 2H), 7.30-7.48 (m, 5H), 7.52-7.64 (m, 4H).

Preparation 2

2-(Biphen-4-yl)ethylamine

[0124]

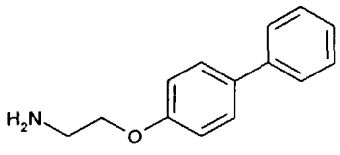


[0125] This was prepared according to the method described by W. W. Zacac Jr, J. F. Siuda, M. J. Nolan and T. M. Santususso, in *J. Org. Chem.* 1971, *36*, 3539.

Preparation 3

2-(Biphen-4-yloxy)ethylamine

[0126]



(a) 2-([Biphen-4-yloxy]ethyl)isoindoline-1,3-dione

[0127] Potassium phthalimide (1.2 g, 6.5 mmol) was added to a solution of 4-(2-chloroethoxy)-1,1'-biphenyl (1.0 g, 5.4 mmol) in anhydrous dimethylformamide (3 ml) and anhydrous dimethylsulfoxide (3 ml) and the mixture was heated to 70°C under an atmosphere of nitrogen for 5 hours. The mixture was cooled to ambient temperature and partitioned between water and dichloromethane. The organic layer was washed with water, dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to give the title compound as a colourless solid (1.51 g).

^{1}H NMR (300 MHz, $CDCl_3$): 4.13 (t, 2H), 4.26 (t, 2H), 6.96 (d, 2H), 7.23-7.34 (m, 1H), 7.34-7.44 (m, 2H), 7.44-7.58 (m, 4H), 7.67-7.80 (m, 2H), 7.83-7.93 (m, 2H).
LPMS (Thermospray): 343.3 (M^+).

(b) 2-(Biphen-4-yloxy)ethylamine

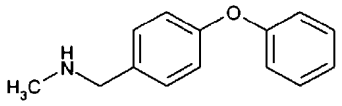
[0128] To a solution of 2-([biphen-4-yloxy]ethyl)isoindoline-1,3-dione (1.5 g, 4.4 mmol) in dichloromethane (30 ml) was added methylamine (33% solution in ethanol, 50 ml) and the solution was heated to reflux under an atmosphere of nitrogen for 2 hours. The mixture was cooled to ambient temperature, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (dichloromethane/methanol/aqueous ammonium solution 95:5:0 to 94:5:1 as eluent) to give the title compound as a colourless solid (505 mg).

^{1}H NMR (400 MHz, $CDCl_3$): 1.40 (s, 2H), 3.05-3.19 (m, 2H), 3.98-4.12 (m, 2H), 6.98 (d, 2H), 7.22-7.66 (m, 7H).
LRMS (Thermospray): 214.0 (MH^+).

Preparation 4

N-Methyl-*N*(4-phenoxybenzyl)amine

[0127]



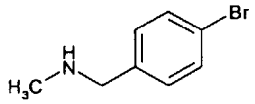
[0130] To a solution of 4-phenoxybenzaldehyde (4.4 ml, 25 mmol) in ethanol (50 ml) was added methylamine (3.0 ml of 33% solution in ethanol, 25 mmol) and acetic acid (1.4 ml, 25 mmol), and the mixture was stirred under an atmosphere of nitrogen. After 20 minutes sodium tri(acetoxy)borohydride (10.5 g, 50 mmol) was added and stirring was continued for 16 hours. The mixture was diluted with 2M aqueous hydrochloric acid (200 ml) and washed with diethyl ether (2 x 100 ml). The aqueous layer was basified to pH 12 with concentrated aqueous ammonia solution and extracted with dichloromethane (4 x 100ml). The combined organic layers were dried (Na_2SO_4), the solvent was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel (dichloromethane/methanol/aqueous ammonia solution 95:5:0 to 94:5:1) to give the titled compound as a colourless oil (3.3 g).

^{1}H NMR (300 MHz, $CDCl_3$): 2.33 (s, 1H), 2.47 (s, 3H), 3.73 (s, 2H), 6.93-7.02 (m, 4H), 7.02-7.13 (m, 1H), 7.23-7.37 (m, 4H).

Preparation 5

N-Methyl-*N*-(4-bromobenzyl)amine

[0131]

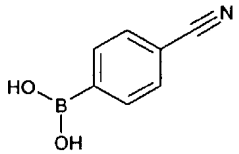


[0132] This was prepared according to the method of G. M. Singer et al, described in *J. Med. Chem.* 1986, *29,* 40.

Preparation 6

4-Cyano-phenylboronic acid

[0133]

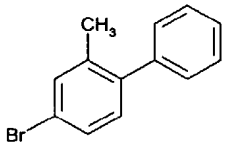


[0134] This was prepared according to the method of G. J. Pernia et al, described in *J. Am. Chem. Soc.* 1996, *118,* 10220.

Preparation 7

4-Bromo-2-methylbiphenyl

[0135]



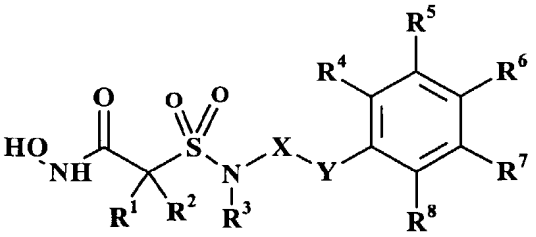
[0136] This was prepared according to the method of M. Gomberg et al, described in *J. Am Chem. Soc.* 1926, *48,* 1372.

Biological Data

[0137] The substances of Examples 1-12 had MMP-3 IC_{50} values of 1.5μM or less. The substances of Examples 1-12 had MMP-2 IC_{50} values of 6.3μM or less. Certain of the substances of the Examples had MMP-13 IC_{50} values of 0.05μM or less.

Claims

1. A compound of formula (I):



(I)

and a pharmaceutically- and/or veterinarily-acceptable salt thereof, and a solvate of such compound and salt, wherein

R^1 and R^2 are each independently H,
C_{2-6} alkenyl, aryl(C_{1-6} alkyl), heteroaryl(C_{1-6} alkyl), aryloxy(C_{1-6} alkyl), heteroaryloxy-(C_{1-6} alkyl),
C_{1-6} alkyl optionally substituted by NH_2, C_{2-6} acylamino, OH, or by CO_2H
or R^1 and R^2 can be taken together with the carbon atom to which they are attached, to form a 4- to 8-membered saturated carbocyclic or heterocyclic ring, which heterocyclic ring has 1 or 2 hetero-groups selected from O, $S(O)_n$ or NR^9 in the ring,
R^3 is H, C_{1-6} alkyl or (C_{1-6} alkoxy)C_{1-6} alkyl,
R^4, R^5, R^7 and R^8 are each independently H, C_{1-6} alkyl, C_{1-6} alkoxy, CN or halogen,
R^6 is H, aryl, heteroaryl, aryloxy or heteroaryloxy, C_{1-6},alkyl, C_{1-6} alkoxy, CN or halogen,
R^9 is H or C_{1-6} alkyl,
n is 0,1 or 2,
X is C_{1-6} alkylene or C_{2-6} alkenylene,
Y is a direct link, CH=CH or O,

wherein "aryl" is phenyl optionally fused with another ring selected from furan, dioxolan, and pyran,
which group is optionally mono- or disubstituted by substituents independently seleceted from halogen, CN, C_{1-6} alkyl optionally substituted by OH or NH_2, C_{1-6} alkoxy, perfluoro(C_{1-6} alkyl) and perfluoro(C_{1-6} alkoxy),
and wherein "heteroaryl" is a 5- or 6-membered aromatic heterocycle with one or two heteroatoms in the ring, which heteroatoms are independently selected from O, N and S, which heteroaryl is optionally mono- or disubstituted by substituents independently selected from halogen, CN, C_{1-6} alkyl optionally substituted by OH or NH_2, C_{1-6} alkoxy, perfluoro(C_{1-6} alkyl) and perfluoro(C_{1-6} alkoxy).

2. A substance according to claim 1 wherein R^1 is H.

3. A substance according to any preceding claim wherein R^2 is H.

4. A substance according to any preceding claim wherein R^3 is H or C_{1-6} alkyl.

5. A substance according to any preceding claim wherein R^4 is H.

6. A substance according to any preceding claim wherein R^5 is H or C_{1-6} alkyl.

7. A substance according to any preceding claim wherein R^6 is H, aryl[1] or aryl[1]oxy wherein "aryl" is phenyl optionally mono- or disubstituted by substituents selected from halogen and CN.

8. A substance according to any preceding claim wherein R^7 is H.

9. A substance according to any preceding claim wherein R^8 is H.

10. A substance according to any preceding claim wherein X is CH_2, $(CN_2)_2$, $(CN_2)_3$, or is $CH_2CH=CH$ wherein the terminal methinyl carbon of this group is linked to the Y moiety.

11. A substance according to any preceding claim wherein R^3 is H or CH_3.

12. A substance according to any preceding claim wherein $R^5$is H or CH_3.

13. A substance according to any preceding claim wherein R^6 is H, $aryl^2$ or $aryl^2oxy$ wherein "$aryl^2$" is phenyl optionally 4-substituted by substituents selected from Cl and CN.

14. A substance according to any preceding claim wherein R^6 is H, phenyl, phenoxy, 4-cyanophenyl or 4-chlorophenyl.

15. A substance according to any preceding claim wherein at least two of the groups R^4, R^5, R^7 and R^8 are all H.

16. A substance according to any preceding claim wherein R^4, R^7 and R^8 are all H and R^5 is CH_3.

17. A substance according to any preceding claim wherein R^1, R^2, R^4, R^7 and R^8 are all H,

R^3 is H or CH_3,
$R^5$is H or CH_3,
R^6 is H, phenyl, phenoxy, 4-cyanophenyl or 4-chlorophenyl,
X is CH_2, $(CH_2)_2$, $(CH_2)_3$, or is $CH_2CH=CH$ wherein the terminal methine carbon of this group is linked to the Y moiety,
and the salts and solvates thereof.

18. A substance according to claim 1 as described herein in the Examples and the salts and solvates thereof.

19. A pharmaceutical composition comprising a substance according to any one of claims 1 to 18, together with a pharmaceutically acceptable diluent or carrier.

20. A veterinary composition comprising a substance according to any one of claims 1 to 18, together with a veterinarally acceptable diluent or carrier.

21. A substance according to any one of claims 1 to 18 for use as a medicament.

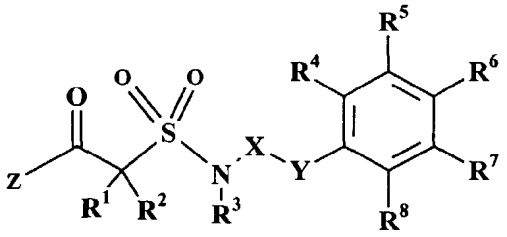
22. The use of a substance according to any one of claims 1 to 18 in the manufacture of a medicament for the treatment of a condition mediated by one or more MMPs.

23. The use of a substance according to any one of claims 1 to 18 in the manufacture of a medicament for the treatment of atherosclerotic plaque rupture, myocardial infarction, heart failure, restenosis, stroke, periodontal disease, tissue ulceration, wounds, skin diseases, cancer metastasis, tumour angiogenesis, age-related macular degeneration, fibrotic disease, rheumatoid arthritis, osteoarthritis and inflammatory diseases dependent on migratory inflammatory cells.

24. A method of treating a condition mediated by one or more MMPs, in an animal such as a mammal (including a human being), which comprises administering to said animal an effective amount of a substance according to any one of claims 1 to 18.

25. A method of treating atherosclerotic plaque rupture, myocardial infarction, heart failure, restenosis, stroke, periodontal disease, tissue ulceration, wounds, skin diseases, cancer metastasis, tumour angiogenesis, age-related macular degeneration, fibrotic disease, rheumatoid arthritis, osteoarthritis and inflammatory diseases dependent on migratory inflammatory cells, in an animal such as a mammal (including a human being), which comprises administering to said animal an effective amount of a substance according to any one of claims 1 to 18.

26. A compound of formula (II):



(II)

where Z is chloro, bromo, iodo, C_{1-3} alkyloxy or HO, and X, Y, R^1, R^2, R^3, R^4, R^5, R^6, R^7 and R^8 are as defined in claim 1, or a salt thereof.

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31/16		31/16	４Ｃ０８４
31/19		31/19	４Ｃ０８６
31/197		31/197	４Ｃ２０６
31/4015		31/4015	

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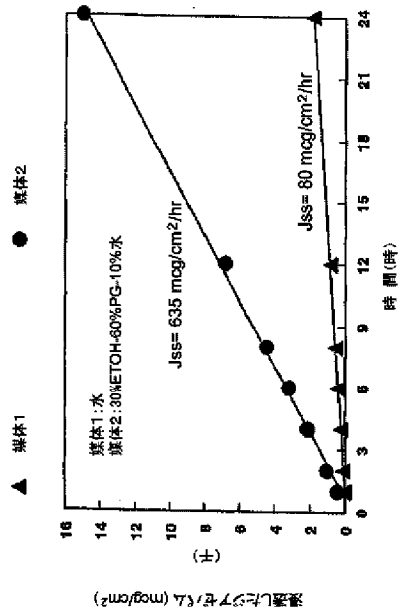
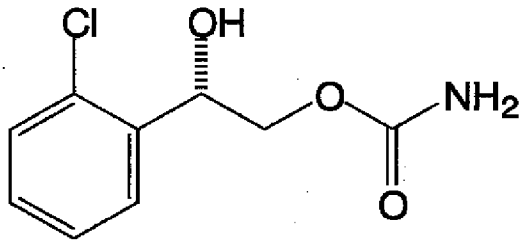
(54)【発明の名称】　経鼻抗痙攣組成物及び調節方法

(57)【要約】
媒体によって調節して、ヒト及び動物の粘膜に抗痙攣剤を投与する新規な方法を開示する。媒体系は、胆汁塩又はレシチンのような生物学的活性剤と共に、脂肪族アルコール（１０－８０％）、又はグリコール（１０－８０％）、及びそれらの組み合わせを含む、水性の薬学的担体である。薬学的組成物は、１回又は複数回投与することにより、粘膜を介した薬剤の浸透及び吸収の速度及び量を調節及び促進するための手段を提供する。薬学的組成物の経鼻投与は、静脈内投与と同じ位素早く、抗痙攣剤の高い血漿濃度をもたらす。そのような組成物は、発作重積状態及び他の発熱によって引き起こされる発作の救急及び／又は緊急治療における、患者の迅速かつ適時の薬物治療に特に適している。




Cl
OH
O
NH2
O

ウサギにおける本発明の調製物のＩＶ及びＩＮ投与後のジアゼパムの生物学的利用能及び薬学動力学パラメーター

経路/製剤	投薬(mg/kg)	C_{max} (ng/ml)	T_{max}(分)	$AUC_{(0-120分)}$ (ng×分/ml)	F(%)
IV 処方 1[a]	1回 (1mg/kg×1)	398.8 (63.0)[d]	2.0	17582 (407)[d]	100.0 (n=3)
IN 処方 2[b]	1回 (1mg/kg×1)	273.6 (62.2)[d]	5.0	10383 (692)[d]	59.1 (n=3)
IN 処方 3[c]	1回 (1mg/kg×1)	273.7 (26.4)[d]	2.0	13300 (972)[d]	75.7 (n=4)
IN 処方 3[c]	2回 (1mg/kg×2)[f]	327.1 (29.7)[d] 556.9 (130.5)[d]	2.0 10.0	26787 (4859)[d]	76.2[e] (n=3)
IN 処方 4[g]	1回 (1mg/kg×1)	73.3 (11.9)[d]	30.0	7497 (1445)[d]	42.6 (n=3)

[a]ＩＶ処方１：０．５％ジアゼパム注射、ＵＳＰ、エルキンスーシン社(Elkins-Sinn,Inc.)（ＰＧ／ＥＴＯＨ／ベンジルアルコール／ベンゾエートナトリウム塩／安息香酸／注射用の水）

[b]ＩＮ処方２：２％のジアゼパム溶液を含む６０％ＰＧ、３０％ＥＴＯＨ、及び１０％水

[c]ＩＮ処方３：２％のジアゼパム溶液を含む１％ＳＧＣ、６０％ＰＧ、３０％ＥＴＯＨ、及び１０％水

[d]標準偏差

[e]以下の式を用いて決定された標準化したデータ：

$$F = \{AUC_{IN,1\ mg\ x\ 2} / 2 \times AUC_{IV,\ 1\ mg\ x\ 1)} \times 100\}$$

[f]適用時間：$t_{ゼロ}$：最初の経鼻投薬

$t_{5分}$：２回目の経鼻投薬

[g]ＩＮ処方４：２％のジアゼパム溶液を含むクレモフォア(Cremophor)ＥＬ

ウサギにおける本発明の調製物のＩＶ及びＩＮ投与後のジアゼパムの薬学動力学パラメーターへの媒体のＥＴＯＨ／ＰＧ容量比の効果

経路/製剤	投薬(mg/kg)	C_{max} (ng/ml)	T_{max}(分)	$AUC_{(0-120分)}$ (ng×分/ml)	F(%)
IV 処方１[a]	１回 (1mg/kg×1)	398.8 (63.0)[e]	2.0	17582 (407)[e]	100.0 (n=3)
IN 処方Ａ[b]	１回 (1mg/kg×1)	313.2 (17.3)[e]	2.0	13592 (692)[e]	77.3 (n=3)
IN 処方Ｂ[c]	１回 (1mg/kg×1)	273.7 (26.4)[e]	2.0	13300 (972)[e]	75.7 (n=4)
IN 処方Ｃ[d]	１回 (1mg/kg×１)	246.3 (32.2)[e]	2.0	12860 (827)[e]	73.1 (n=3)

[a]ＩＶ処方１：０．５％ジアゼパム注射、ＵＳＰ、エルキンスーシン社(Elkins-Sinn,Inc.)（ＰＧ／ＥＴＯＨ／ベンジルアルコール／ベンゾエートナトリウム塩／安息香酸／注射用の水）

[b]ＩＮ処方Ａ：２％のジアゼパム溶液を含む１％ＳＧＣ、３０％ＰＧ、６０％ＥＴＯＨ、及び１０％水

[c]ＩＮ処方Ｂ：２％のジアゼパム溶液を含む１％ＳＧＣ、６０％ＰＧ、３０％ＥＴＯＨ、及び１０％水

[d]ＩＮ処方Ｃ：２％のジアゼパム溶液を含む１％ＳＧＣ、７０％ＰＧ、２０％ＥＴＯＨ、及び１０％水

[e]標準偏差

ウサギへの調製物のＩＶ及びＩＮ投与後のクロナゼパムの生物学的利用能及び薬学動力学パラメーター

経路/製剤	投薬(mg/kg)	C_{max} (ng/ml)	T_{max}(分)	$AUC_{(0-120\ 分)}$ (ng×分/ml)	F(%)
IV 処方[a]	１回 (0.2mg/kg ×1)	104.8	2.0	7437.7	100.0 (n=2)
IN 処方[b]	１回 (0.2mg/kg ×1)	32.9 (5.9)[c]	2.0	3356.4 (544.8)[c]	45.1 (n=3)
IN 処方[b]	２回[f] (0.2mg/kg ×2)	49.5 (5.3)[c]	10.0	4896.8 (836.6)[c]	32.9[d] (n=4)
IN 処方[b]	３回[f] (0.2mg/kg ×3)	80.2 (21.3)[c]	15.0	7766.1 (2077.9)[c]	34.8[e] (n=3)

[a]ＩＶ処方：０．１５％クロナゼパム溶液を含む４０％ＰＧ、３０％ＥＴＯＨ、及び３０％水

[b]ＩＮ処方：０．４２％のクロナゼパム溶液を含む１％ＳＧＣ、６０％ＰＧ、３０％ＥＴＯＨ、及び１０％水

[c]標準偏差

[f]以下の式を用いて算出された標準化したデータ：

$$F = \{AUC_{IN,0.2\ mg\ x\ 2} / 2 \times AUC_{IV,\ 0.2\ mg\ x\ 1}\} \times 100\}$$

[l]以下の式を用いて算出された標準化したデータ：

$$F = \{AUC_{IN,0.2\ mg\ x\ 3} / 3 \times AUC_{IV,\ 0.2\ mg\ x\ 1}\} \times 100\}$$

[f]適用時間：　$t_{ゼロ}$：最初の経鼻投薬
$t_{5分}$：２回目の経鼻投薬
$t_{10分}$：３回目の経鼻投薬

２つの投与強度での、１回のＩＶおよびＩＮ投与後の（Ｓ）－２－カルバモイロキシル－１－ｏ－クロロフェニルエタノールの薬学動力学パラメーター

経路/製剤	投薬(mg/kg)	最大濃度 (ng/ml)	T_{max}(分)	$AUC_{(0-240\ 分)}$ (ng×分/ml)	F(%)
IV 処方[a]	5.0	6267.7 (408.0)[d]	2.0	473176 (56105)[d]	100.0 (n=4)
IN 処方１[b]	5.0	2404.9 (130.0)[d]	30.0	373991 (5077)[d]	79.1 (n=3)
IV 処方[a]	2.5	4179.9	2.0	221291	100.0 (n=2)
IN 処方２[c]	2.5	1407.2	5.0	160269	72.4 (n=2)

[a]ＩＶ処方：０．１５％（Ｓ）－２－カルバモイロキシル－１－ｏ－クロロフェニルエタノール溶液を含む４０％ＰＥＧ４００及び６０％水

[b]ＩＮ処方１：　１０％の（Ｓ）－２－カルバモイロキシル－１－ｏ－クロロフェニルエタノール溶液を含む１％ＳＧＣ、６０％ＰＧ、３０％ＥＴＯＨ、及び１０％水

[b]ＩＮ処方２：　５％の（Ｓ）－２－カルバモイロキシル－１－ｏ－クロロフェニルエタノール溶液を含む１％ＳＧＣ、６０％ＰＧ、３０％ＥＴＯＨ、及び１０％水

１回及び３回の投与計画における調製物のＩＶ及びＩＮ投与後の（Ｓ）－２－カルバモイロキシル－１－ｏ－クロロフェニルエタノールの生物学的利用能及び薬学動力学パラメーター

経路/製剤	投薬(mg/kg)	最大濃度 (ng/ml)	T_{max}(分)	$AUC_{(0-120\ 分)}$ (ng×分/ml)	F(%)
IV 処方[a]	１回 (5mg/kg×1)	6267.7 (408.0)[c]	2.0	473176 (56105)[c]	100.0 (n=4)
IN 処方[b]	１回 (5mg/kg×1)	2404.9. (130.0)[c]	30.0	373991 (5077)[c]	79.1 (n=3)
IN 処方[b]	２回[e] (5mg/kg×2)	4332.3 (979.3)[c]	30.0	700475 (114195)[c]	74.1[d] (n=3)

[a]ＩＶ処方：１．５％（Ｓ）－２－カルバモイロキシル－１－ｏ－クロロフェニルエタノール溶液を含む４０％ＰＥＧ及び６０％水

[b]ＩＮ処方：１０％の（Ｓ）－２－カルバモイロキシル－１－ｏ－クロロフェニルエタノール溶液を含む１％ＳＧＣ、６０％ＰＧ、３０％ＥＴＯＨ、及び１０％水

[c]標準偏差

[d]以下の式を用いて決定された標準化したデータ：

$$F = \{AUC_{IN,5\ mg\ x\ 2} / 2 \times AUC_{IV,\ 5\ mg\ x\ 1)} \times 100\}$$

[e]適用時間：$t_{ゼロ}$：最初の経鼻投薬

$t_{5分}$：２回目の経鼻投薬

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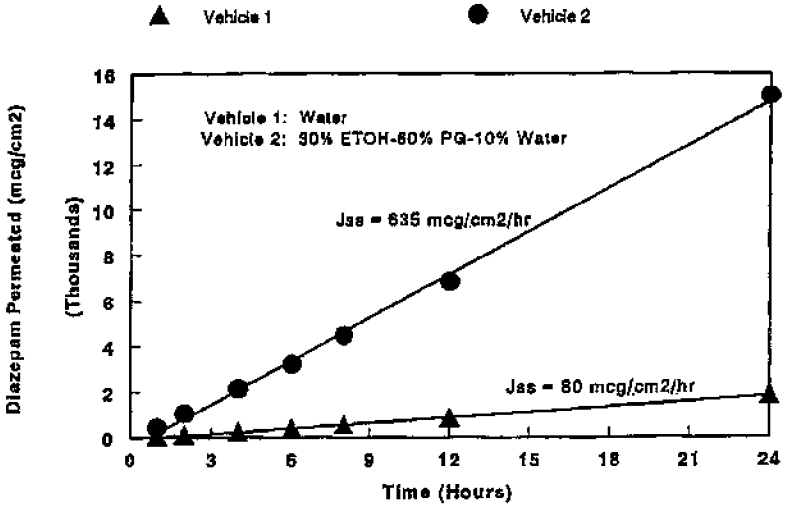

(57) Abstract: A novel method of vehicle modulated administration of an anticonvulsive agent to the mucous membranes of humans and animals is disclosed. The vehicle system is an aqueous pharmaceutical carrier comprising an aliphatic alcohol (10-80 %) or a glycol (10-80 %), and their combinations with a biological surfactant such as a bile salt or a lecithin. The pharmaceutical composition provides a means to control and promote the rate and extent of transmucosal permeation and absorption of the medicaments via a single and multiple administration. Nasal administration of the pharmaceutical preparation produces a high plasma concentration of the anticonvulsant nearly as fast as intravenous administration. Such compositions are particularly suitable for a prompt and timely medication of patients in the acute and/or emergency treatment of status epilepticus and other fever-induced seizures.

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TRANSNASAL ANTICONVULSIVE COMPOSITIONS AND MODULATED PROCESS

FIELD OF THE INVENTION

The present invention is directed to pharmaceutical compositions for transmucosal delivery of biologically active agents. More particularly, this invention relates to a novel method for controlling and promoting the rate and extent of transmucosal permeation and absorption of an anticonvulsive agent by coadministration of the medicament with a pharmaceutically acceptable co-solvent system comprising an aliphatic alcohol, a glycol, and water, and their combinations with a biological surfactant such as a bile salt or a lecithin. Even more particularly, this invention relates to the pharmaceutical compositions to provide a patient-acceptable transnasal anticonvulsive delivery system, which may be useful for the emergency management of status epilepticus and fever seizures in a prompt and convenient manner of administration.

BACKGROUND OF THE INVENTION

Status epilepticus is a neurological emergency in which mortality ranges from 3 – 35%. The major goal of treatment is rapid management of pathological seizure activity; the longer that the episode of status epilepticus is untreated, the more difficult it is to control and the greater the risk of permanent brain damage. Thus, critical to the management of the patient is a clear plan, involving prompt treatment with effective drugs in adequate doses having a proper pharmaceutical formulation as well as attention to hypoventilation and hypotension.

Currently several drug regimens have been proven to be applicable in treating status epilepticus. Diazepam and lorazepam are the most widely used benzodiazepines for this purpose. Intravenous administration of anticonvulsants is the most rapid way to suppress epileptic convulsions. However, other routes of administration may be highly desirable when intravenous administration is inconvenient and delaying, for instance, because of technical difficulties such as requirements for sterile equipment and skilled

personnel, and because of the possible development of thrombophlebitis. In addition, intravenous medication is often associated with hypotension, cardiac dysrhythmia or central nervous system depression. In this regard Moolenaar [Moolenaar et al., *Int. J. Pharm.*, 5: 127-137 (1986)] attempted to administer diazepam in humans via several other routes such as intramuscular injection, oral tablet and rectal solution. Only the rectal administration was found to provide a fairly rapid absorption and thus, it might be looked upon as an alternative route to IV injection. However, the rectal route is a very inconvenient way of drug administration particularly in emergency treatment. In U.S. Patent No. 4,863,720 of Burghardt, a sublingual sprayable pharmaceutical preparation is disclosed, in which the active drug can be a benzodiazepine, optimally comprising polyethylene glycol (PEG) and requiring ethanol, di- and/or triglyceride of fatty acids and a pharmaceutically acceptable propellant gas.

More recently, it appears that the mucosal membrane of the nose offers a practical route of administration for therapeutic effect of many medicinal substances. Intranasal administration has the advantages that drugs may be administered readily and simply to achieve a systemic or localized effect, as required. However, the major problem associated with intranasal drug administration is the fact that most drug molecules diffuse poorly and slowly through the nasal mucosal membrane and thus the desired levels of the therapeutic agent cannot be achieved by means of simple transnasal administration. An additional constraint concerning nasal administration is that a small administration volume is needed; it is not generally possible to administer more than approximately 150 µl per nostril; above this, the formulation will be drained out into the pharynx and swallowed. Therefore, a great need exists for solvent vehicles, in which the solubility of the drug is high and which, on the other hand, are non-irritating to the nasal mucosa. The intranasal absorption of drugs can be increased by coadministering a chemical adjuvant or permeation enhancers. For example, Lau and Slattery [Lau et al., *Int. J. Pharm.*, 54: 171-174 (1989)] attempted to administer a benzodiazepine such as diazepam and lorazepam by dissolving these medicaments in a variety of solvents; triacetin, dimethylsulfoxide, PEG 400, Cremophor EL, Lipal-9-LA, isopropyl adipate and Azone. While many of the solvents dissolved diazepam and lorazepam in the desired concentrations, they were too

irritable to be used when administered to the nose. Cremophor EL was found to be the least irritating for nasal mucosal tissue, but the nasal absorption in the use of this vehicle in humans was rather slow ($T_{max} \cong 1.4$ hours) and the peak concentration was low relative to that observed after IV administration. In U.S. Patent No. 4,950,664 Rugby described the nasal administration of a benzodiazepine hypnotic in a pharmaceutically acceptable nasal carrier. The carrier may be an aqueous saline solution, an alcohol, a glycol, a glycol ether or mixtures thereof. The results of pharmacokinetic studies in dogs showed that the time to maximum plasma concentration for triazolam was achieved at 18 minutes after the nasal administration, while an effective treatment within 5 minutes is considered to be an attractive goal. Bechgaard and Hjortkjer [Bechgaard et al., *J. Pharm. Pharmacol.*, 49: 747-750 (1997)] described the use of pure organic solvents such as glycofurol and tetraethyleneglycol, and their combinations as carriers for nasal delivery of diazepam. The absolute bioavailability, measured during the first 30 minutes after the nasal administration, was 49-62% for the most promising carrier systems examined. In PCT WO 95/31217, Dumex described the use of a pharmaceutical emulsion preparation based on tocopherol and its derivatives for intranasal administration of biologically active compounds including benzodiazepines.

SUMMARY OF INVENTION

The present invention is a novel method of vehicle modulated administration of an anticonvulsive agent to the mucous membranes of humans and animals. The vehicle system is an aqueous pharmaceutical carrier comprising an aliphatic alcohol or a glycol and their combinations with a biological surfactant such as a bile salt or a lecithin.

An objective of the present invention is to provide a pharmaceutically acceptable carrier system which is capable of enhancing the transmucosal permeation and absorption of an anticonvulsive agent. The ingredients used in the pharmaceutical composition are preferably those of GRAS materials (generally recognized as safe), so there are no major toxicity issues of concern. Another objective of the present invention is to provide a method of controlling the transmucosal delivery of an anticonvulsant at an appropriately

adjusted rate so as to achieve an optimum therapeutic effect, while avoiding or reducing adverse side effects. Such compositions are particularly suitable for intranasal administration of the medicaments in the acute treatment of status epilepticus and fever seizures.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph showing the effect of a vehicle on the *in vitro* transnasal permeation of diazepam preparations of the invention.

Fig. 2 is a graph showing the effect of drug concentration level on the *in vitro* transnasal permeation of diazepam from a vehicle of the invention.

Fig. 3 is a graph showing the influence of sodium glycocholate (SGC) on the *in vitro* transnasal permeation of diazepam from a vehicle of the invention.

Fig. 4 is a graph showing the mean plasma concentration-time profiles of diazepam after intravenous (IV) administration and intranasal administration of a preparation in accordance with the invention (a single dose application).

Fig. 5 is a graph showing the mean plasma concentration-time profiles of diazepam after intravenous and intranasal administration of a preparation in accordance with the invention (a multiple dose application).

Fig. 6 is graph showing the mean plasma concentration-time profiles of diazepam after intranasal administration of a preparation as a function of propylene glycol/ethanol volume ratio in the preparation according to the invention.

Fig. 7 is a graph showing the mean plasma concentration profiles of clonazepam after intravenous administration and intranasal administration of a preparation in accordance with the invention (a single and multiple dose application).

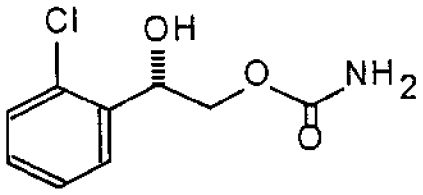
Fig. 8 is a graph showing the mean plasma concentration-time profiles of (S)-2-carbamoyloxyl-1-o-chlorophenylethanol after intravenous administration and intranasal administration of a preparation according to the invention as a function of dose strength.

Fig. 9 is a graph showing the mean plasma concentration-time profiles of (S)-2-carbamoyloxyl-1-o-chlorophenylethanol after intravenous administration and intranasal

administration of a preparation according to the invention (a single and multiple dose application).

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, a certain aqueous co-solvent system comprising one aliphatic alcohol, one glycol and a biological surfactant provides a rate-controlled and enhanced transnasal delivery of an anticonvulsive agent. The alcohol of the present invention is selected from C_1 to C_5 aliphatic alcohols; a glycol is selected from propylene glycol (PG), polyethylene glycol (PEG) 200, PEG 300 and PEG 400, and PEG 600; and a biological surfactant is selected from bile salts such as sodium cholate, sodium deoxycholate, sodium taurocholate, sodium glycocholate, and sodium ursodeoxycholate or a lecithin such as lysophosphatidylcholines, phosphatidylcholines, phosphatidylserines, phosphatidylinositols, phosphatidylethanolamines, and phosphatidylglycerols. The above-described compositions can be used for medicinal preparations comprising anticonvulsive agents applicable to the mucosal membranes of humans and animals. More specifically, these compositions are ones which comprise a benzodiazepine such as diazepam, clonazepam, and lorazepam, and a monocarbamate based new anticonvulsive compound, (S)-2-carbamoyloxyl-1-o-chlorophenylethanol represented by the following formula:



adapted for intranasal administration in a solution, suspension, gel or other useful nasal formulation. These nasal compositions may be employed for any of the known therapeutic purposes for which such anticonvulsants are known including phenytoins

(phenytoin, mephenytoin and ethotoin), barbiturates (phenobarbital, mephobarbital, and primidone), iminostilbenes (carbamazepine), succinimides (ethosuximide), valproic acid, oxazolidinediones (trimethadione) and other antiseizure agents (gabapentin, lamotrigine, acetazolamide, felbamate, and γ-vinyl GABA). The utilization of an intranasal formulation of the anticonvulsant greatly facilitates administration. As compared with parenteral administration, for example, a simple sprayer, dropper or nebulizer will suffice for prompt and convenient delivery of the medicaments, in particular, for the emergency treatment of acute convulsive attack phenomena of epilepsy. From a clinical point of view, intranasal administration often provides an improved duration of anticonvulsive effect. By the present invention, the therapeutic effect, in terms of onset, intensity, and duration, can be more efficiently and accurately controlled by varying the proportion of aliphatic alcohol and glycol in the vehicle and by a single-dose and/or multiple-dose administration of the preparation of the invention. Although this invention has been described with respect to an anticonvulsant as a model compound, it is understood that this invention is also applicable to the other biologically active agents that are applicable to the mucosal membranes of humans and animals.

The invention is further illustrated by the following examples, which are illustrative of a specific mode of practicing the invention and are not intended as limiting the scope of the appended claims.

Example 1

In Vitro Nasal Membrane Permeation Studies

The nasal mucous membrane used in these *in vitro* experiments was obtained from New Zealand White rabbits (2.5 – 3.0 kg). Rabbits were sacrificed by IV injection of phenobarbital. The nasal septum was carefully removed from a bone block using surgical scissors and a bone-cutting saw. Two pieces of nasal mucous membranes were then carefully stripped from the nasal septum without touching the center of the membrane surface and rinsed with normal saline solution. The mucosal membrane was mounted between two half-cells of a glass diffusion cell apparatus. The exposed area of

the nasal membrane was approximately 0.64 cm^2. A test solution or suspension (3.5 ml) was introduced into the mucosal side of the membrane in the donor compartment while 3.5 ml of 10% ethanol, 40% propylene glycol, and 50% pH 7.4 isotonic phosphate buffer solution was added to the receptor compartment. The entire diffusion system was maintained at 37°C throughout the experiment. At predetermined time intervals, 100 µl of the receptor solution was withdrawn for the assay and refilled with the same volume of fresh receptor medium to keep the volume constant. The steady-state flux value was determined from the slope of the straight line attained from the plot of the cumulative amount of drug permeated as a function of time. Each experiment was carried out in at least duplicate. This method was used in Examples 2 – 6.

A high pressure liquid chromatographic system equipped with a multi-solvent delivery system (Model 600E, Waters Associates, Milford, Mass.), an auto-injector (Model 717 Plus, Waters Ass.), a photodiode array detector (Model 996, Waters Ass.), a reverse phase Symmetric C_{18} column (150 mm x 3.9 mm ID, 5 µm), and a Millenium 2010 software computer system was used in this study. The mobile phases and UV wavelengths utilized for the analysis of diazepam, clonazepam, and (S)-2-carbamoyloxyl-1-o-chlorophenylethanol were 70% methanol, 30% water at 254 nm; 60% methanol, 40% water at 252 nm; and 25% acetonitrile, and 75% water at 262 nm, respectively.

Example 2

This example shows the effect of a bile salt and a lecithin dissolved in an aqueous medium at a 1% w/v level on the *in vitro* permeation of a model drug diazepam through the freshly excised nasal membrane. In these studies, a series of bile salts such as sodium cholate, sodium deoxycholate, sodium taurocholate, and sodium glycocholate, and a lecithin such as lysophosphatidylcholine were examined. The permeation rates were measured using the method described under the *in vitro* membrane permeation test method. The average steady-state transnasal flux data obtained in this manner are presented in Table I.

Table I

Effect of Bile Salts and Lecithin on the *In Vitro* Permeation of Diazepam across the Rabbit Nasal Mucosal Membrane at 37°C

Vehicle	Mean Transnasal Flux ($\mu g/cm^2/hr$) (n = 2)
Water	79.5
1% Sodium Cholate/H_2O	66.3
1% Sodium Deoxycholate/H_2O	74.9
1% Sodium Taurocholate/H_2O	87.0
1% Sodium Glycocholate/H_2O	96.4
1% Lysophosphotidylcholine/H_2O	125.5

As seen from Table I, a bile salt such as sodium glycocholate and a lecithin such as lysophosphotidylcholine produce a significant enhancing effect on the diazepam permeation through the nasal membrane.

Example 3

This example exhibits the influence of a vehicle on the *in vitro* membrane permeation of diazepam across the rabbit nasal mucous membrane at 37°C. In this experiment, a 1% diazepam suspension and solution were prepared using water and a co-solvent vehicle consisting of 30% ethanol (ETOH), 60% propylene glycol (PG), and 10% water (WT), respectively. The permeation rates were determined utilizing the method described in Example 1. The transnasal permeation profiles of diazepam obtained in this manner are presented in Fig. 1.

As seen from Fig. 1, a co-solvent vehicle comprising ethanol, propylene glycol, and water provides an approximately 8 times increase in the transnasal permeation rate of diazepam when compared with that obtained with an aqueous suspension.

Example 4

This example shows the influence of the drug concentration in the donor compartment on the permeation of diazepam through the nasal mucous membrane, *in vitro*. In this study, 0.5 – 2% diazepam formulations were prepared using a co-solvent mixture comprising 30% ethanol, 60% propylene glycol, and 10% water. The *in vitro* membrane permeation rates were measured using the test method described in Example 1. The *in vitro* transnasal flux data obtained with diazepam formulations over 0.5 – 2% level are shown in Fig. 2.

As seen from Fig 2, the steady-state transnasal flux of diazepam increases linearly with increasing the drug concentration in the donor compartment over the 0.5 – 2.0% concentration level.

Example 5

This example shows the effect of the incorporation of a bile salt into a nasal formulation according to the invention on the *in vitro* transnasal membrane permeation of diazepam. In this experiment, the inclusion of sodium glycocholate to a vehicle consisting of 30% ethanol, 60% propylene glycol, and 10% water at a 1% level was examined. Sample drug solutions (10 mg/ml) were prepared with the vehicle with and without the bile salt. The membrane permeation rates were measured in the use of the test method described in Example 1. The *in vitro* permeation profiles obtained in this manner are presented in Fig. 3.

As seen from Fig. 3, the inclusion of a 1% level of sodium glycocholate enhances the transnasal permeation rate of diazepam significantly. An approximately 50% increase in the steady-state flux is noticed when the bile salt is incorporated into the vehicle.

Example 6

This example shows the comparative transnasal permeabilities of three model drugs such as diazepam, clonazepam, and (S)-2-carbamoyloxyl-1-o-chlorophenylethanol. In this experiment, a co-solvent vehicle consisting of 30% ethanol, 60% propylene glycol, and 10% water was used. The *in vitro* permeation experiments were performed using the test method described in Example 1. The comparative transnasal permeability coefficient and steady-state flux data obtained with the medicaments at an initial drug concentration of 5mg/ml are presented in Table II.

Table II

Comparative Transnasal Permeability of Model Drug Substances across the Rabbit Nasal Mucous Membrane *In Vitro*

Drug Compound	Permeability Coefficient (cm/hr)	Transnasal Flux ($\mu g/cm^2/hr$)
Diazepam	4.92×10^{-2}	246.0
Clonazepam	6.95×10^{-2}	347.7
(S)-2-carbamoyloxyl-1-o-chlorophenylethanol	9.77×10^{-2}	487.6

As seen from Table II, the monocarbamate based anticonvulsant, (S)-2-carbamoyloxyl-1-o-chlorophenylethanol appears to have approximately two times greater transnasal permeability as compared with that of diazepam.

Example 7

Bioavailability and Pharmacokinetics of Diazepam Preparations

The bioavailability and pharmacokinetic characteristics of the preparations of the invention containing diazepam were tested after intranasal application to New Zealand White rabbits (n = 3-4). For comparison, a diazepam injection (Formula 1 on Table III) was examined *in vivo* after intravenous administration of the same dose. IV Formula 1

(10 mg/2 ml) was obtained from Elkins-Sinn, Inc., which was prepared with propylene glycol (0.4 ml), alcohol (0.1 ml), benzyl alcohol (0.015 ml), sodium benzoate/benzoic acid (50 mg), and a sufficient quantity of water for injection to make 1 ml. For intranasal application, two formulations were prepared using a vehicle system of the invention consisting of 30% ethanol, 60% propylene glycol, and 10% water with (Formula 3 on Table III) and without (Formula 2 on Table III) 1% sodium glycocholate, respectively. Another nasal formulation (Formula 4 on Table III), prepared with a non-ionic surfactant vehicle of polyoxyethylated castor oil (Cremophor EL), was also tested after intranasal application for comparison since this formulation was tested in humans by Lau and Slattery (1989). All of the nasal formulations were prepared just prior to the experiments by dissolving 20-mg diazepam (Sigma Chemical) in 1 ml of the vehicles described above.

Just prior to the experiment, rabbits (n=3-4) were weighed and restrained in rabbit restrainers while they were facing up. Each rabbit received 100 µl of the Formula 2 or 3 into each nostril by means of a Pfeiffer spray device within 5 seconds. Rabbits (n=3) having IV administration received 1mg/kg of Formula 1 as an ear-vein infusing during 20 seconds. For the repeated dosing studies, the same volume of Formula 3 (100µl) was sprayed into each nostril 5 minutes after the first dosing. Blood samples (1 ml) were collected at 0, 2, 5, 10, 20, 30, 45, 60, and 120 minutes after the IV and IN administration. From the blood samples, plasma was separated by centrifugation and stored at – 20°C until analysis. For analysis, plasma samples (0.5 ml) were accurately transferred into a 1.5 ml polypropylene centrifuge tube. To the plasma sample, 0.5 ml of 0.01% v/v perchloric acid in an acetonitrile containing internal standard (clonazepam 1 µg/ml) was added. The mixture was vortexed for 30 seconds and centrifuged at 4000 rpm for 10 minutes. The plasma concentration of diazepam was assayed by HPLC. The analysis was performed with the Waters HPLC as described in Example 1. The column used in this study was a 3.9 mm x 150 mm x 5 µm Symmetric C_{18} column. The mobile phase was 50% methanol: 10% acetonitrile: 40% pH 3.5 phosphate buffer by volume. The flow rate of the mobile phase was 1 ml/min and the UV detection was made at 228.5

nm. The detection limit for diazepam was 70 nmol/l. The areas (AUC) under the drug plasma concentration-time curves, from 0 min to 120 minutes, were calculated by means of the linear trapezoidal method. The bioavailability and pharmacokinetic data obtained in this manner are listed in Table III. The comparative pharmacokinetic profiles obtained after a single IV administration (Formula 1) and single and double IN applications of the preparations of the invention (Formulas 3 and 4) are depicted in Figs. 4 and 5, respectively.

Table III

Bioavailability and Pharmacokinetic Parameter of Diazepam after IV and IN Administration of the Preparation of the Invention in Rabbits

Route/ Formulation	Dosing (mg/kg)	C_{max} (ng/ml)	T_{max} (min)	A U C $_{(0-120\ min)}$ (ng x min/ml)	F (%)
IV Formula 1[a]	Single (1 mg/kg x 1)	398.8 (63.0)[d]	2.0	17582 (407)[d]	100.0 (n=3)
IN Formula 2[b]	Single (1 mg/kg x 1)	273.6 (62.2)[d]	5.0	10383 (692)[d]	59.1 (n=3)
IN Formula 3[c]	Single (1 mg/kg x 1)	273.7 (26.4)[d]	2.0	13300 (972)[d]	75.7 (n=4)
IN Formula 3[c]	Double[f] (1 mg/kg x 2)	327.1 (29.7)[d] 556.9 (130.5)[d]	2.0 10.0	26787 (4859)[d]	76.2[e] (n=3)
IN Formula 4[g]	Single (1 mg/kg x 1)	73.3 (11.9)[d]	30.0	7497 (1445)[d]	42.6 (n=3)

[a] IV Formula 1: 0.5% Diazepam Injection, USP, Elkins-Sinn, Inc., (PG / ETOH /Benzyl Alcohol / Sodium Benzoate/Benzoic Acid/Water for Injection)

[b] IN Formula 2: 2% Diazepam Solution in 60% PG, 30% ETOH, and 10% Water

[c] IN Formula 3: 2% Diazepam Solution in 1% SGC, 60% PG, 30% ETOH, and 10% Water

[d] Standard deviation
[e] Normalized data determined using the following equation:
$F = \{AUC_{IN,\ 1\ mg\ x\ 2} / 2 \times AUC_{IV,\ 1\ mg\ x\ 1}\} \times 100\}$
[f] Application time: t_{zero}: First dosing for nasal administration
$t_{5\ minutes}$: Second dosing for nasal administration
[g] IN Formula 4: 2% Diazepam Solution in Cremophor EL

As seen from Fig. 4 and Table III, IN Formula 3 prepared with 1% SGC, 30% ethanol, 60% PG, and 10% water increases the transnasal absorption markedly when compared with the Cremophor EL Formula 4. The C_{max} and $AUC_{0\text{-}120\ minutes}$ for the IN Formula 3 are approximately 69% and 76% with reference to the IV administration, respectively. On the other hand, the C_{max} and $AUC_{0\text{-}120\ minutes}$ for the Cremophor EL Formula 4 are about 19% and 42.6% of the IV injection. These comparative results appear to be consistent with the human pharmacokinetic data reported by Lau and Slattery (1989). According to the reported data, the Cremophor EL formulation yielded the T_{max} of 1.4 hours after intranasal administration in humans and the C_{max} was only about 27% relative to the IV injection . Surprisingly enough, as seen from Fig. 5 and Table III, a repeated intranasal application 5 minutes after the first dosing produces a marked increase in the transnasal absorption of diazepam. The C_{max} and AUC values were exactly doubled after the second application relative to those obtained with the first administration. In addition, the plasma diazepam level attained after the second dosing exceeds that of the single IV administration within 7 minutes. These findings clearly demonstrate that a repeated dosing regimen (within a short period of time) can be effectively utilized for the acute management of epileptic seizures when a single intranasal dosing is incapable of producing the desired therapeutic effect.

Example 8

Control of Peak Plasma Level Pharmacokinetics

Two mg of diazepam in a 100 µl vehicle was prepared and applied to rabbits (n=3) in a manner analogous to that described in Example 7. The following vehicles were tested: 60% ETOH, 30% PG, and 10% water (WT) with 1% SGC, 30% ETOH, 60% PG,

and 10% water (WT) with 1% SGC, and 20% ETOH, 70% PG and 10% water (WT) with 1% SGC. Blood samples were collected from the ear vein at the following time intervals: 0, 2, 5, 10, 20, 30, 45, 60, and 120 minutes. The diazepam concentration in plasma was determined by HPLC. The pharmacokinetic profiles obtained after IV and IN administration of the preparations are presented in Table IV and Fig. 6.

Table IV

Effect of ETOH/PG Volume Ratio of the Vehicle on the Pharmacokinetic Parameter of Diazepam after IV and IN Administration of the Preparation of the Invention in Rabbits

Route/ Formulation	Dosing (mg/kg)	C_{max} (ng/ml)	T_{max} (min)	A U C $_{(0-120\ min)}$ (ng x min/ml)	F (%)
IV Formula 1[a]	Single (1 mg/kg x 1)	398.8 (63.0)[e]	2.0	17582 (407)[e]	100.0 (n=3)
IN Formula A[b]	Single (1 mg/kg x 1)	313.2 (17.3)[e]	2.0	13592 (692)[e]	77.3 (n=3)
IN Formula B[c]	Single (1 mg/kg x 1)	273.7 (26.4)[e]	2.0	13300 (972)[e]	75.7 (n=4)
IN Formula C[d]	Single (1 mg/kg x 1)	246.3 (32.2)[e]	2.0	12860 (827)[e]	73.1 (n=3)

[a] IV Formula 1: 0.5% Diazepam Injection, USP, Elkins-Sinn, Inc., (PG / ETOH /Benzyl Alcohol / Sodium Benzoate/Benzoic Acid/Water for Injection)

[b] IN Formula A: 2% Diazepam Solution in 1% SGC, 30% PG, 60% ETOH, and 10% Water

[c] IN Formula B: 2% Diazepam Solution in 1% SGC, 60% PG, 30% ETOH, and 10% Water

[d] IN Formula C: 2% Diazepam Solution in 1% SGC, 70% PG, 20% ETOH, and 10% Water

[e] Standard deviation

As seen from Table IV and Fig. 6, the peak plasma concentration of the drug, observed within 2 minutes after the IN administration, can be controlled depending on the ETOH/PG volume ratio in the vehicles examined. The C_{max} increases gradually with increasing the ETOH/PG volume ratio from 0.3 to 2. In addition, the peak plasma concentration for the IN vehicle consisting of 60% ETOH, 30% PG and 10% water (WT) with 1% SGC at 2 minutes is approximately 79% of an IV injection of the same dose.

In addition, modulating the ETOH/PG volume ratio in the vehicles can also control the plasma level-time profile in the elimination phase.

Example 9

Pharmacological Response of Diazepam Preparations

The pharmacological response was examined in New Zealand White rabbits by evaluating the muscle relaxation effect of diazepam after IV administration and IN administration of the preparations of the invention at a dosing level of 1 mg/kg. The vehicle of nasal formulation consisted of 30% ethanol, 60% propylene glycol, and 10% water containing 1% SGC. The sample formulation was prepared by dissolving 20 mg diazepam in 1 mL of the vehicle by ultrasonification. The IV formulation was the same as that used in Example 7. The pharmacological response was measured in rabbits after application of 100 μL of nasal formulation into each nostril while the rabbit was in a lying position after being firmly tipped with a finger on the hip. The mean response times that the rabbits remained in a lying position with its hind legs stretched to one side after IV and IN administration are listed in Table V.

Table V

Mean Pharmacological Response Times after IV and IN Administration of Diazepam Preparations

Route/Formulation	Response Time (Min.)	N
IV Injection	1.1 ± 0.2	3

IN Formula 3	1.5 ± 0.5	3

As seen from Table V, the nasal formulation of the invention provides a very fast response. The time to pharmacological response was 1.5 minutes.

Example 10

Bioavailability and Pharmacokinetics of Clonazepam Preparations

An intranasal formulation was prepared by dissolving 8.36 mg clonazepam in 2 ml of a vehicle of the invention consisting of 30% ETOH, 60% PG, and 10% water containing 1% SGC. A formulation for IV injection was prepared by dissolving 3-mg of clonazepam in 2 mL of a 40% PG, 30% ETOH, and 30% water solution and filtering the solution through a sterile filter under aseptic conditions. The formulations were administered to rabbits (n=3) at a dose of 0.2 mg/kg in a manner analogous to those described in Example 7. A repeated dosing regimen (double and triple applications) at 5 minutes time intervals was also tested. Blood samples were obtained from the ear vein at the following time intervals: 0, 2, 5, 10, 20, 30, 45, 60, and 120 minutes. From the blood samples, plasma was separated by centrifugation and stored at – 20°C until analysis. For analysis, plasma samples (0.5 ml) were accurately transferred into a 15-ml test tube. To the plasma sample, 10µl of an internal standard solution (diazepam - 5 µg/ml) and 50µl NaOH (0.5M) were added. To the above mixture, 5 ml of diethyl ether was added and this mixture was vortexed for 60 seconds and centrifuged at 4000 rpm for 10 minutes. The upper ethereal solution was transferred to a 5 ml test tube and evaporated in a vacuum evaporator at 40°C for 30 minutes. The residue was reconstituted with 100 µl of the mobile phase for HPLC analysis consisting of 20% methanol, 30% acetonitrile, and a 50% pH 3.5 KH_2PO_4/H_3PO_4 buffer solution. The clonazepam concentration in the plasma was determined by HPLC using a flow rate of 1 ml/minute and the UV detection at 254 nm. The detection limit for clonazepam was 16 nmol/l. The bioavailability and pharmacokinetic data obtained after IV and IN administration in a single or multiple

dosing schedule are listed in Table VI and the mean plasma concentration-time profiles are shown in Fig. 7.

Table VI

Bioavailability and Pharmacokinetic Parameters for Clonazepam after IV and IN Administration of the Preparations to Rabbits

Route/ Formulation	Dosing (mg/kg)	C_{max} (ng/ml)	T_{max} (min)	A U C $_{(0-120\ min)}$ (ng x min/ml)	F (%)
IV Formula[a]	Single (0.2mg/kg x 1)	104.8	2.0	7437.7	100.0 (n=2)
IN Formula[b]	Single (0.2mg/kg x 1)	32.9 (5.9)[c]	2.0	3356.4 (544.8)[c]	45.1 (n=3)
IN Formula[b]	Double[f] (0.2mg/kg x 2)	49.5 (5.3)[c]	10.0	4896.8 (836.6)[c]	32.9[d] (n=3)
IN Formula[b]	Triple[f] (0.2mg/kg x 3)	80.2 (21.3)[c]	15.0	7766.1 (2077.9)c	34.8[e] (n=3)

[a] IV Formula: 0.15% Clonazepam Solution in 40% PG, 30% ETOH and 30% Water
[b] IN Formula: 0.42% Clonazepam Solution in 1% SGC, 60% PG, 30% ETOH, and 10% Water
[c] Standard deviation
[d] Normalized data calculated using the following equation:
$F = \{AUC_{IN,\ 0.2\ mg\ x\ 2}/2 \times AUC_{IV,\ 0.2\ mg\ x\ 1}\} \times 100$
[e] Nomalized data calculated using the following equation:
$F = \{AUC_{IN,\ 0.2mg\ x\ 3}/3 \times AUC_{IV,\ 0.2\ mg\ x\ 1}\} \times 100$
[f] Application times: t_{zero}: First dosing for nasal administration
$t_{5\ minutes}$: Second dosing for nasal administration
$t_{10\ minutes}$: Third dosing for nasal administration

As seen from Table VI and Fig. 7, the initial peak plasma concentration is attained within 2 minutes after the first intranasal application of the preparation. The peak plasma level was about 32% of the IV injection. However, after the third application at 5

minutes intervals, the peak plasma concentration observed at 15 minutes was nearly identical to that of the single IV injection of clonazepam.

Example 11

Pharmacological Response of Clonazepam Preparations

The pharmacological response of clonazepam preparations was examined in New Zealand White rabbits after application of 100 μL of the 4.18 mg clonazepam/mL vehicle into each nostril in a manner analogous to that described in Example 9. The vehicle consisted of 30% ETOH, 60% PG, and 10% water containing 1% SGC. Clonazepam was dissolved in the vehicle by ultrasonification. The IV formulation used in the study was the same as described in Example 10. The mean response times measured after the IV and IN administration are presented in Table VII.

Table VII

Mean Pharmacological Response Times after IV and IN Administration of Clonazepam Preparations

Route/Formulation	Response Time (Minutes)	N
IV Injection	1.7 ± 0.5	3
IN Formulation	1.4 ± 0.7	3

As shown in Table VII, the intranasal application of the clonazepam formulation of the invention provides a faster response time (1.4 minutes) when compared with that of IV injection (1.7 minutes).

Example 12

Bioavailability and Pharmacokinetics of (S)-2-carbamoyloxyl-1-o-chlorophenylethanol Preparations

An intranasal formulation was prepared by dissolving 50 mg or 100 mg of a monocarbamate based new anticonvulsive agent (S)-2-carbamoyloxyl-1-o-chlorophenylethanol in 1 mL of a vehicle of the invention consisting of 30% ETOH, 60% PG, and 10% water containing 1% SGC. A formulation for IV injection was prepared by dissolving 15 mg (S)-2-carbamoyloxyl-1-o-chlorophenylethanol in 1 mL of 40% PEG 400 and 60% water and filtering through a sterile membrane filter under aseptic conditions. The formulations were administered to rabbits (n = 2-4) at the two dosing levels of 2.5 mg/kg and 5 mg/kg in a manner analogous to that described in Example 7. A repeated dosing regimen at 5 minute intervals was also studied in the nasal application of the preparation of the invention. Blood samples were obtained from the ear vein at the following time intervals: 0, 2, 5, 10, 20, 30, 45, 60, 120, 180 and 240 minutes. From the blood samples, plasma was separated by centrifugation and stored at – 20°C until analysis. For analysis, plasma samples (0.5 ml) were accurately transferred into a 15-ml test tube. To the plasma sample, 50µl of an internal standard solution (2-(2,6-dichlorophenyl)-2-carbamoyloxyethyl)oxocarboxamide - 10 µg/ml) and 5 ml of methyl-butyl ether were added. The mixture was vortexed for 60 seconds and centrifuged at 3500 rpm for 10 minutes. The upper ethereal solution was transferred to a 5 ml test tube and evaporated in a vacuum evaporator at 40°C for 30 minutes. The residue was reconstituted with 200 µl of deionized water. The (S)-2-carbamoyloxyl-1-o-chlorophenylethanol concentration in the plasma was determined by HPLC in the use of a mobile phase consisting of 20% acetonitrile and 80% water with a flow rate of 1 ml/minute and UV detection at 210 nm. The detection limit for (S)-2-carbamoyloxyl-1-o-chlorophenylethanol was 23 nmol/l. The pharmacokinetic parameters determined after IV and IN administration of (S)-2-carbamoyloxyl-1-o-chlorophenylethanol at two dose strengths are presented in Table VIII. The bioavailability and pharmacokinetic parameters obtained after IV administration and IN administration of the preparations of the invention in a single and double dosing regimen are listed in Table IX. The mean plasma concentration-time profiles obtained after IV and IN administration of (S)-2-

carbamoyloxyl-1-o-chlorophenylethanol preparations in single and double dosing schedules are presented in Figs. 8 and 9.

Table VIII

Pharmacokinetic Parameters of (S)-2-carbamoyloxyl-1-o-chlorophenylethanol after a Single IV and IN Administration at Two Dosing Strengths

Route/ Formulation	Dose (mg/kg)	Maximum Conc.(ng/ml)	T_{max} (min)	AUC $_{(0-240\ min)}$ (ng x min/ml)	F (%)
IV Formula[a]	5.0	6267.7 (408.0)[d]	2.0	473176 (56105)[d]	100.0 (n=4)
IN Formula 1[b]	5.0	2404.9. (130.0)[d]	30.0	373991 (5077)[d]	79.1 (n=3)
IV Formula[a]	2.5	4179.9	2.0	221291	100.0 (n=2)
IN Formula 2[c]	2.5	1407.2	5.0	160269	72.4 (n=2)

[a] IV Formula : 1.5 % (S)-2-carbamoyloxyl-1-o-chlorophenylethanol solution in 40% PEG 400 and 60% Water

[b] IN Formula 1: 10% (S)-2-carbamoyloxyl-1-o-chlorophenylethanol solution in 1% SGC, 60% PG, 30% ETOH and 10% Water

[c] IN Formula 2: 5% (S)-2-carbamoyloxyl-1-o-chlorophenylethanol solution in 1% SGC, 60% PG, 30% ETOH, and 10% Water

[d] Standard deviation

Table IX

Bioavailability and Pharmacokinetic Parameters of (S)-2-carbamoyloxyl-1-o-chlorophenylethanol after IV and IN Administration of the Preparations in Single and Double Dosing Regimen

Route/ Formulation	Dose (mg/kg)	Maximum Conc.(ng/ml)	T_{max} (min)	A U C $_{(0-240\ min)}$ (ng x min/ml)	F (%)
IV Formula[a]	Single (5 mg/kg x 1)	6267.7 (408.0)[c]	2.0	473176 (56105)[c]	100.0 (n=4)
IN Formula[b]	Single (5 mg/kg x 1)	2404.9. (130.0)[c]	30.0	373991 (5077)[c]	79.1 (n=3)
IN Formula [b]	Double[e] (5 mg/kg x 2)	4332.3 (979.3)[c]	30.0	700475 (114195)[c]	74.0[d] (n=3)

[a] IV Formula: 1.5% (S)-2-carbamoyloxyl-1-o-chlorophenylethanol solution in 40% PEG 400, and 60% Water

[b] IN Formula : 10% (S)-2-carbamoyloxyl-1-o-chlorophenylethanol solution in 1% SGC 60% PG, 30% ETOH, and 10% Water

[c] Standard deviation

[d] Normalized data determined using the following equation:

$F = \{AUC_{IN,\ 5mg\ x\ 2}/2 \times AUC_{IV,\ 5\ mg\ x\ 1)} \times 100\}$

[e] Application times: t_{zero}: First dosing for nasal administration

$t_{5\ minutes}$: Second dosing for nasal administration

As seen from Table XIII, after the intranasal application the initial peak concentrations observed within 5 - 30 minutes increased proportionally with increasing the dose strength. The bioavailability of the nasal preparations is found to be 73-79% of the IV injection. The pharmacokinetic results presented in Table IX and Fig. 9 clearly demonstrate that the second application of the intranasal formulation 5 minutes after the first dosing produces a nearly identical bioavailability to that obtained after the first dosing. The C_{max} and $AUC_{0-240\ minutes}$ are doubled after the second intranasal application. In addition, the plasma concentration of (S)-2-carbamoyloxyl-1-o-chlorophenylethanol

achieved after the second dosing exceeded the plasma level obtained with a single IV injection at 30 minutes.

Example 13

Stability Studies

In an effort to optimize the stability of the medicaments in the pharmaceutical compositions according to the present invention, an accelerated stability study was performed at a storage temperature of 37°C over a 10 - 14 weeks time period. Sample drug solutions (0.1 mg/ml) were prepared using a vehicle of the invention consisting of 30% ETOH, 60% PG, and 10% water. The drug solutions were stored in an oven set at 37°C. At appropriate time intervals, a 100 µl sample was withdrawn and analyzed by means of HPLC. The chemical stability data determined in terms of the percent drug recovery are presented in Table X.

Table X

Chemical Stability of the Preparations of the Invention at 37°C.

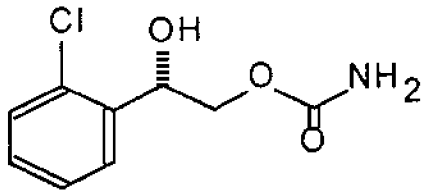
Drug Formulation	Storage Time (Weeks)	% Recovery
Diazepam Formulation	0	100.0
	4	100.3
	10	102.4
	14	102.6
Clonazepam Formulation	0	100.0
	4	101.7
	11	100.9
(S)-2-carbamoyloxyl-1-o-chlorophenylethanol Formulation	0	100.0
	3	100.2
	4	98.2
	9	98.0
	12	97.6

WHAT IS CLAIMED IS:

1. A method for administering a therapeutically effective amount of an anticonvulsive agent to the mucosal membranes of a mammal in a rate-controlled manner of absorption by means of a pharmaceutical composition comprising a therapeutically effective amount of the medicament dissolved or dispersed in a water-containing vehicle containing 10-80% by volume of an aliphatic alcohol, 10-80% by volume of a glycol and 0.1-5% by weight of a bile salt or a lecithin.

2. The composition of Claim 1, wherein the anticonvulsive agent is selected from the group consisting of diazepam, clonazepam, lorazepam , phenytoin, mephenytoin, ethotoin, phenobarbital, mephobarbital, primidone, carbamazepine, ethosuximide, valproic acid, trimethadione, gabapentin, lamotrigine, felbamate, γ-vinyl GABA, and acetazolamide.

3 The composition of Claim 1, wherein the anticonvulsive agent comprises a mono-carbamate anticonvulsive agent (S)-2-carbamoyloxyl-1-o-chlorophenylethanol by the following formula:



4. The composition of Claim 1, wherein the alcohol comprises an aliphatic alcohol containing 1 to 5 carbons.

5. The composition of Claim 1, wherein the glycol is selected from the group consisting of propylene glycol, polyethylene glycol 200, polyethylene glycol 300, polyethylene glycol 400, and polyethylene glycol 600.

6. The composition of Claim 1, wherein the bile salt is selected from the group consisting of sodium cholate, sodium deoxycholate, sodium glycocholate, sodium taurocholate, and sodium ursodeoxycholate.

7. The composition of Claim 1, wherein the lecithin is selected from the group consisting of lysophosphatidylcholines, phosphatidylcholines, phosphatidylserines, phosphatidylinositols, phosphatidylethanolamines, and phosphatidylglycerols.

8. A method for providing absorption in a rate-controlled manner in a mammal through the nasal administration of a pharmaceutical composition comprising a therapeutically effective amount of an anticonvulsant agent by modulating the aliphatic alcohol/glycol volume ratio in an intranasal vehicle system.

9. The method of Claim 8, wherein the anticonvulsant agent is dissolved or dispersed in the intranasal vehicle system to produce a rapid onset and a high plasma concentration level of medicament by increasing the aliphatic alcohol/glycol volume ratio in the vehicle from 0.1 to 8.0.

10. The method of Claim 8, wherein the anticonvulsant agent is dissolved or dispersed in the intranasal vehicle system to produce a rapid onset and a prolonged plasma concentration level of medicament by reducing the aliphatic alcohol/glycol volume ratio in the vehicle from 8.0 to 0.1.

11. The method of Claim 8, wherein the pharmaceutical composition comprising the anticonvulsive agent and the intranasal vehicle system is intranasally administered to a mammal in an amount effective for the treatment of epilepsy or other fever-induced seizures using single or multiple dosing regimens.

Fig. 1

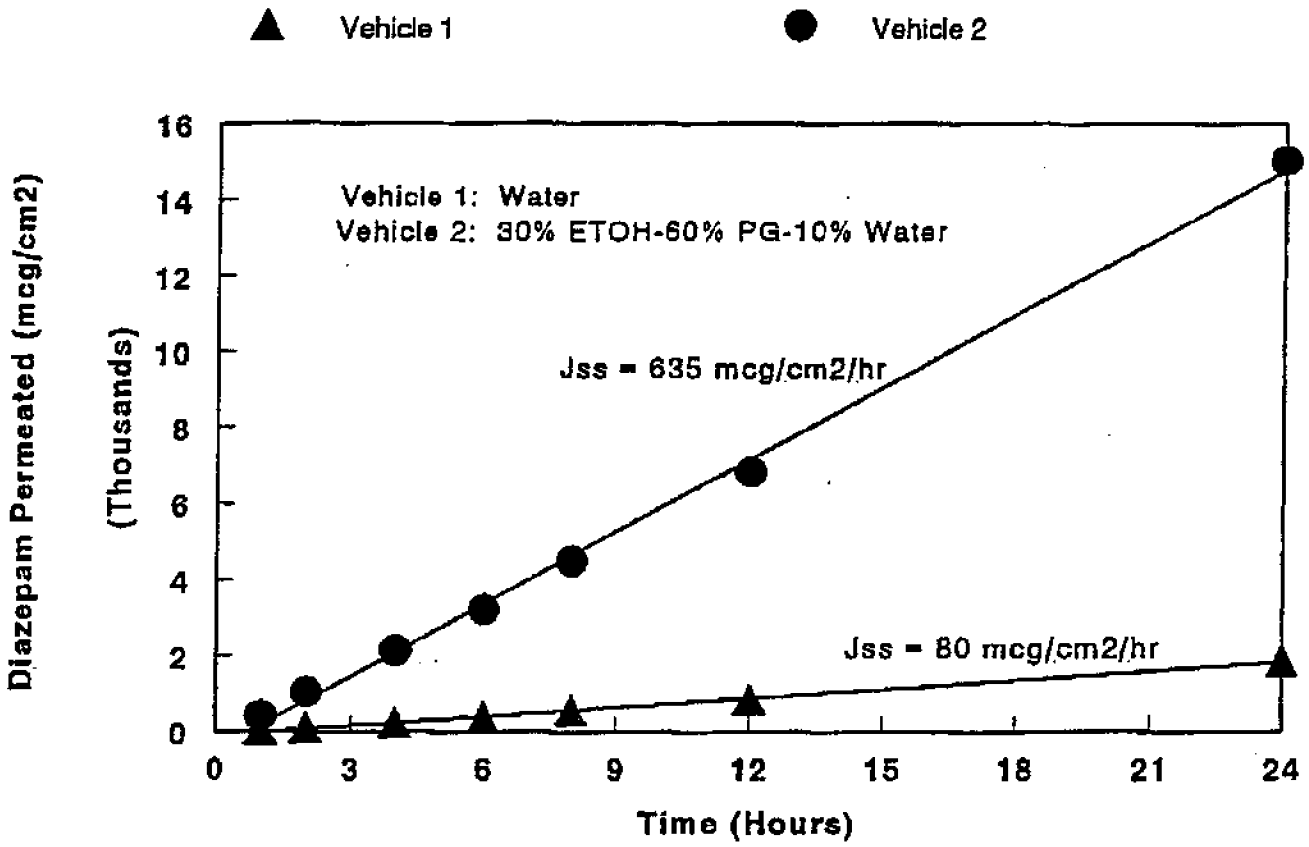


Fig. 2

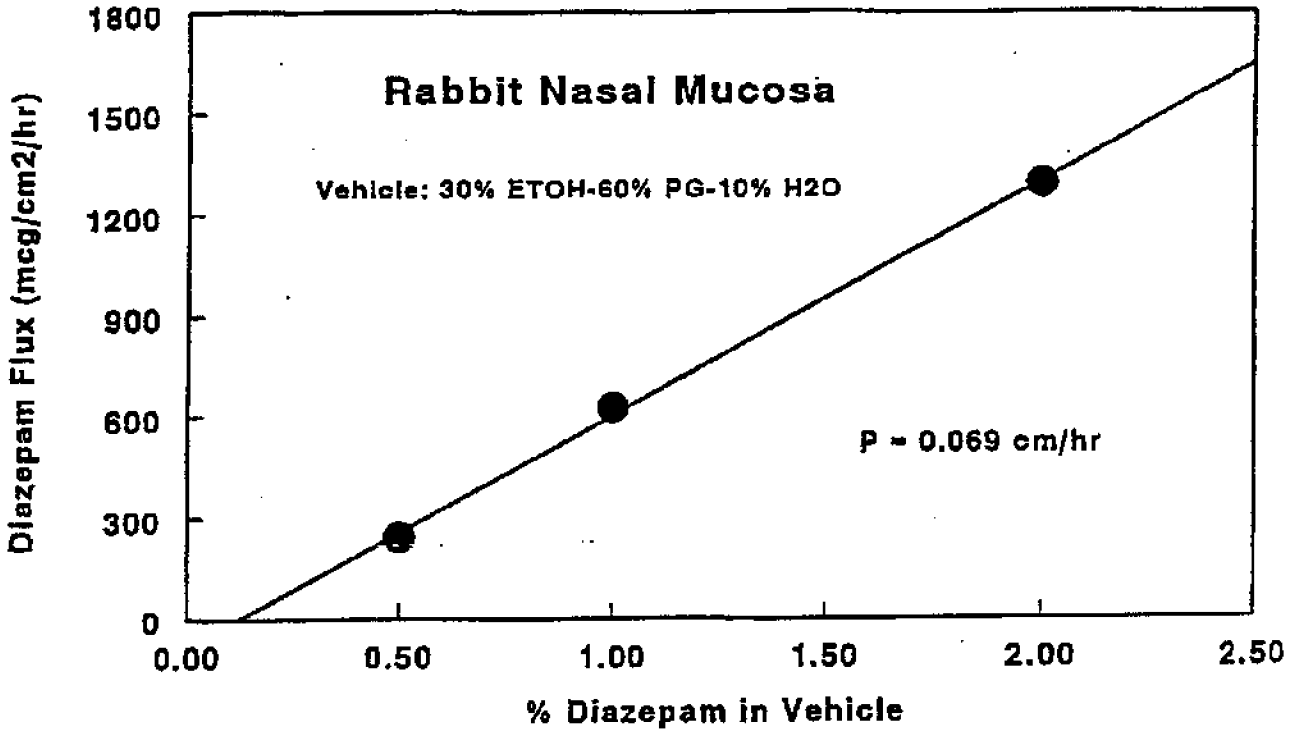

Rabbit Nasal Mucosa
Vehicle: 30% ETOH-60% PG-10% H2O
P = 0.069 cm/hr
Diazepam Flux (mcg/cm2/hr)
1800
1500
1200
900
600
300
0
0.00
0.50
1.00
1.50
2.00
2.50
% Diazepam in Vehicle

Fig.3

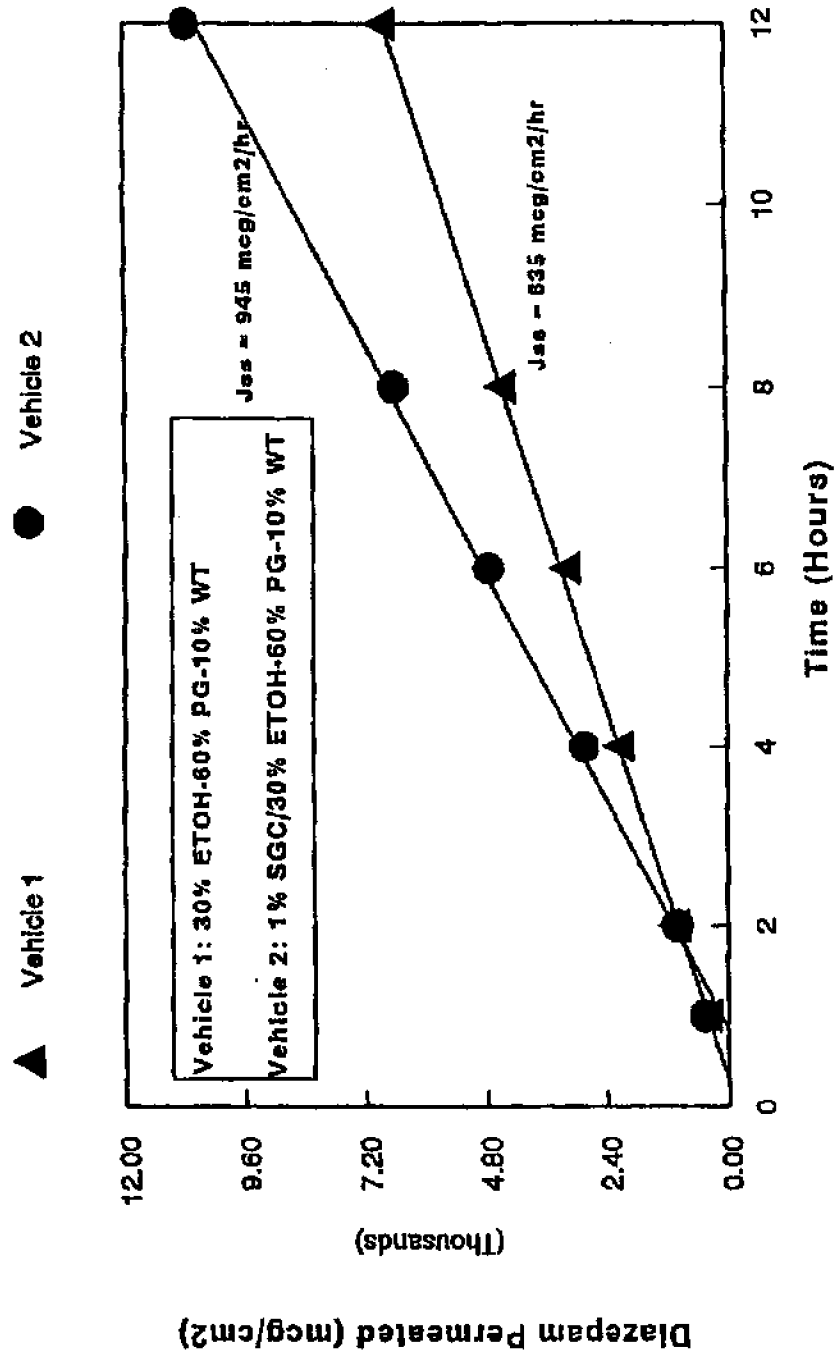


Fig. 4

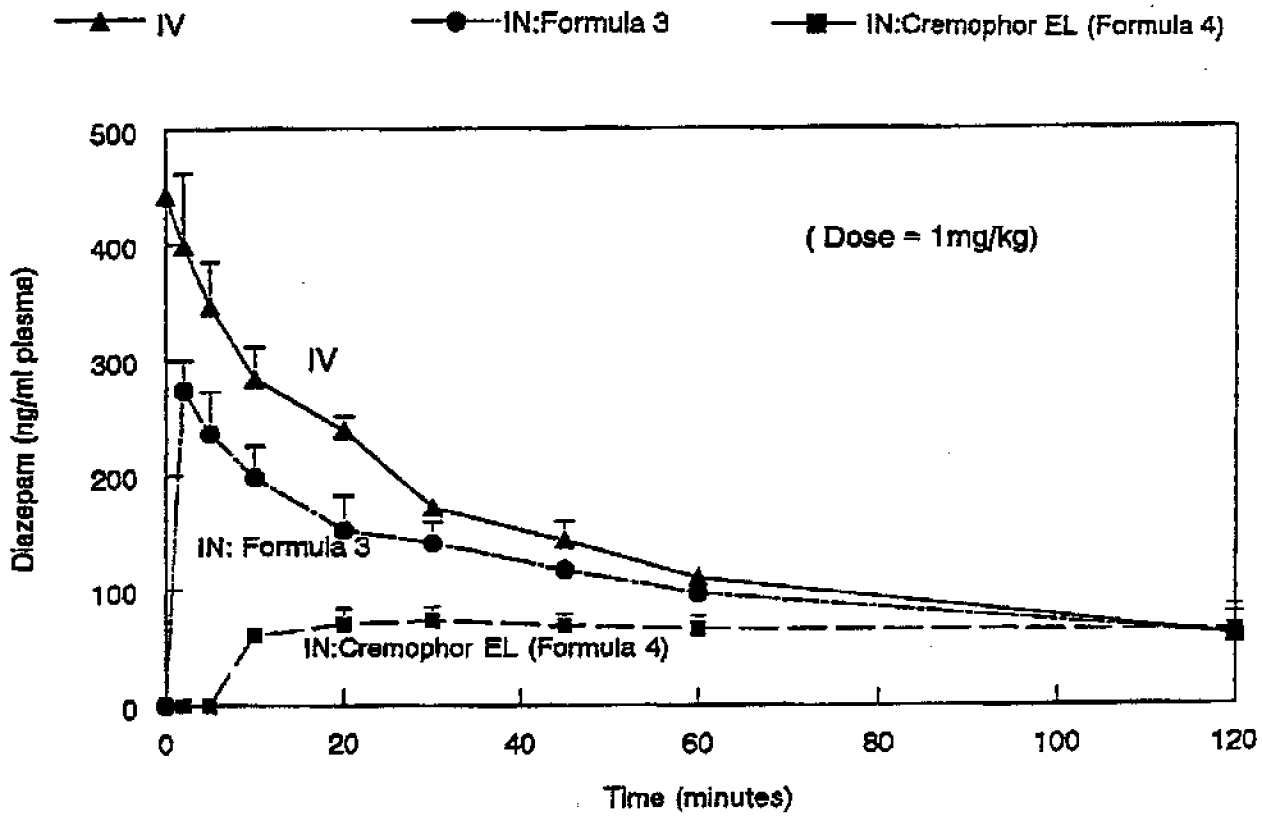

IV
IN:Formula 3
IN:Cremophor EL (Formula 4)
Diazepam (ng/ml plasma)
500
400
300
200
100
0
(Dose = 1mg/kg)
IV
IN: Formula 3
IN:Cremophor EL (Formula 4)
0
20
40
60
80
100
120
Time (minutes)

Fig. 5

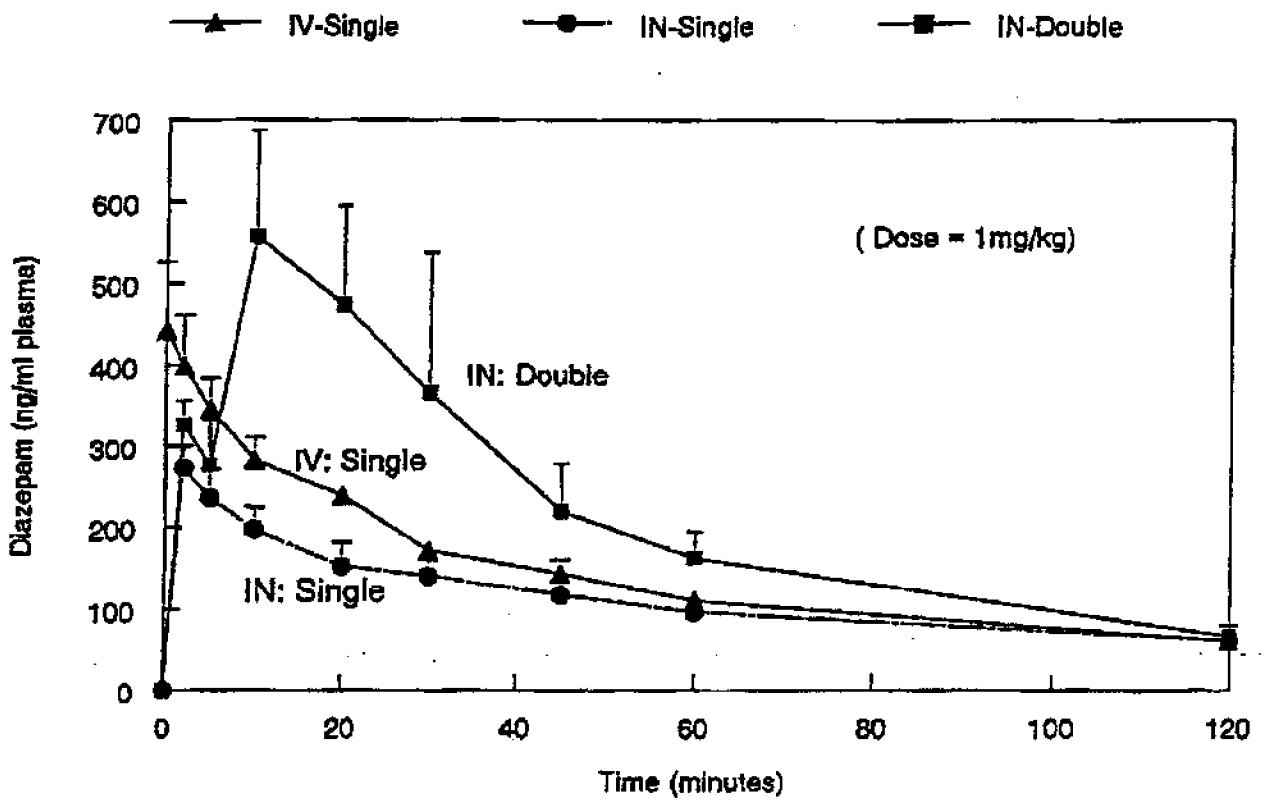


Fig. 6

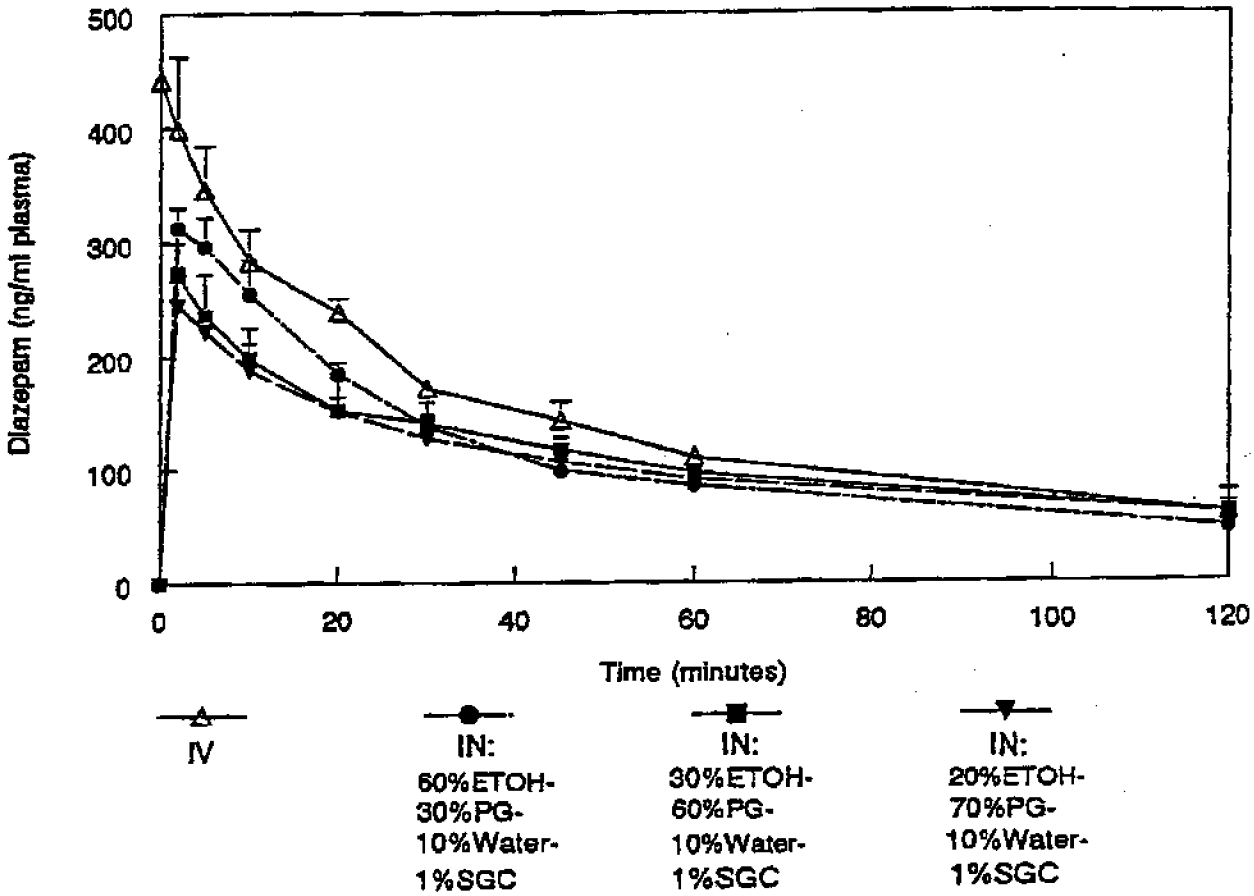


Fig. 7

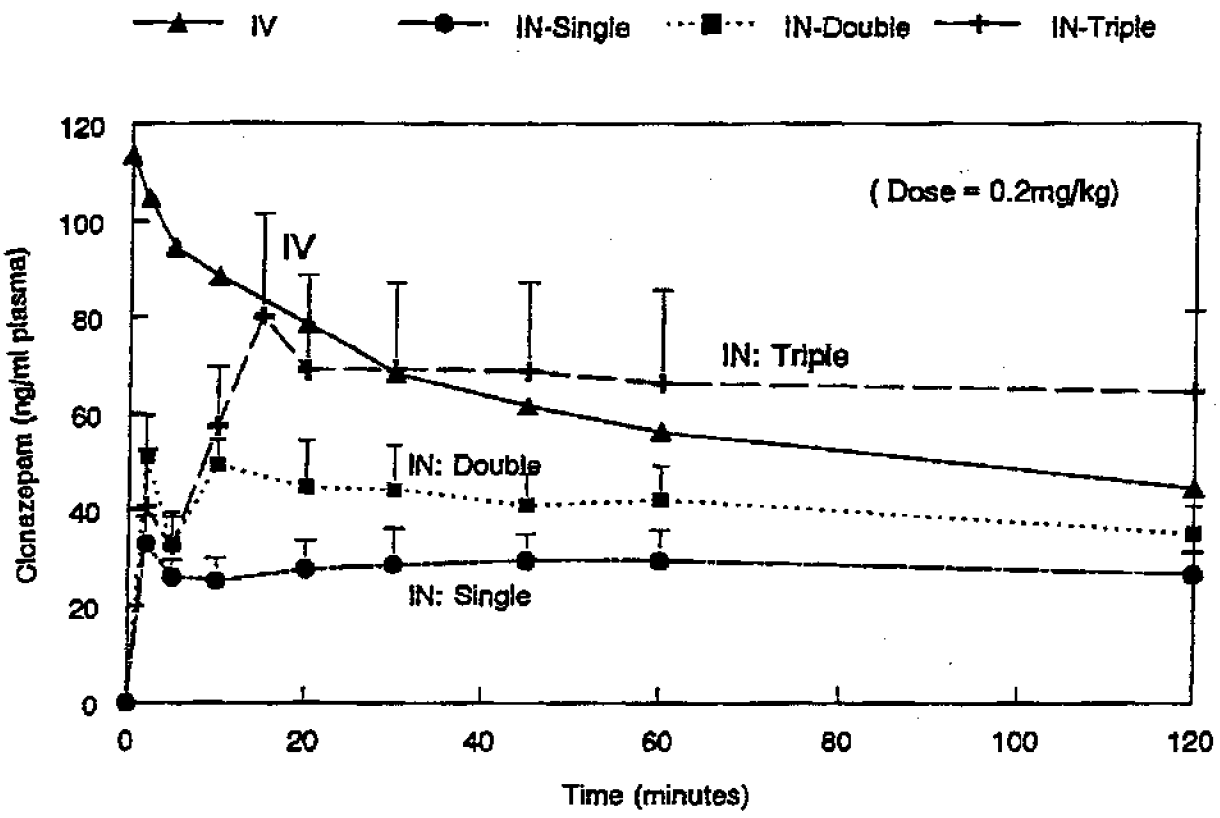

IV
IN-Single
IN-Double
IN-Triple
(Dose = 0.2mg/kg)
IV
IN: Triple
IN: Double
IN: Single
Clonazepam (ng/ml plasma)
Time (minutes)
0
20
40
60
80
100
120

Fig. 8

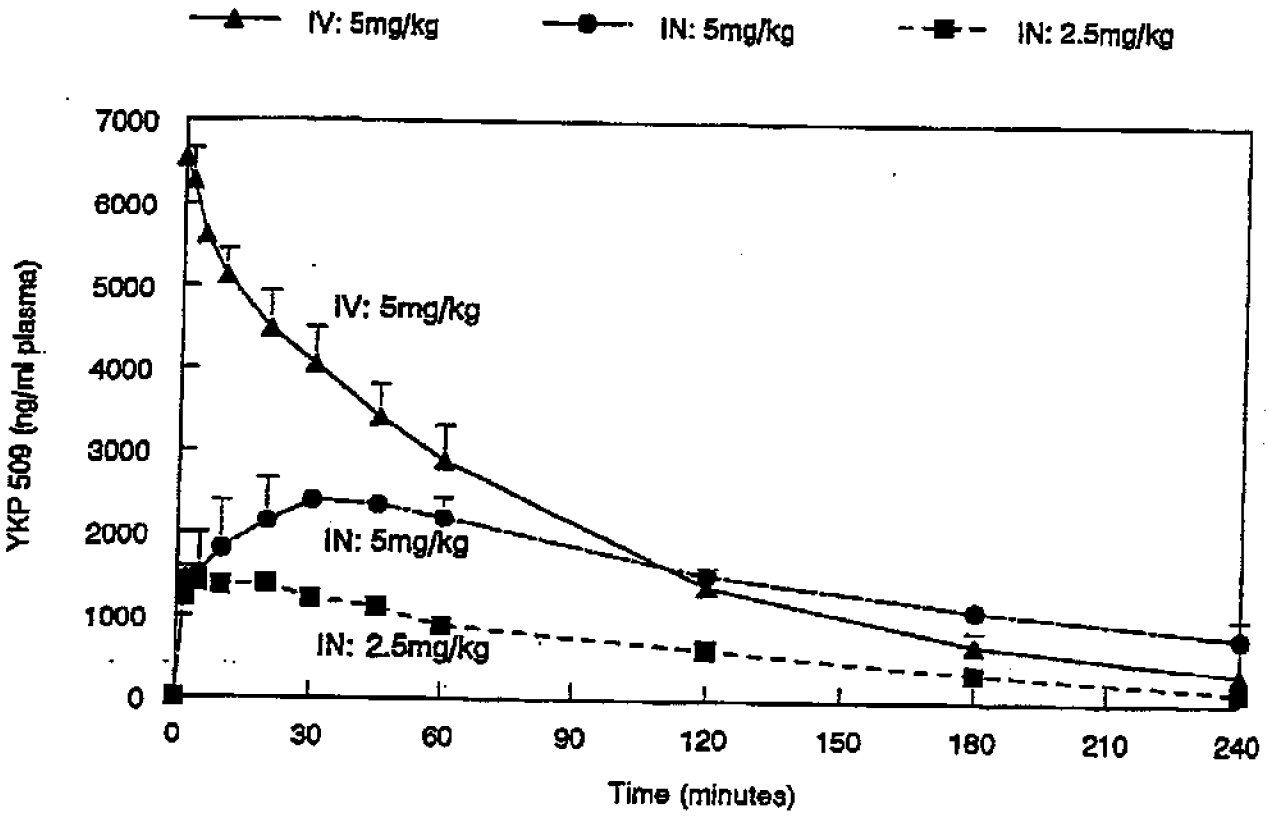
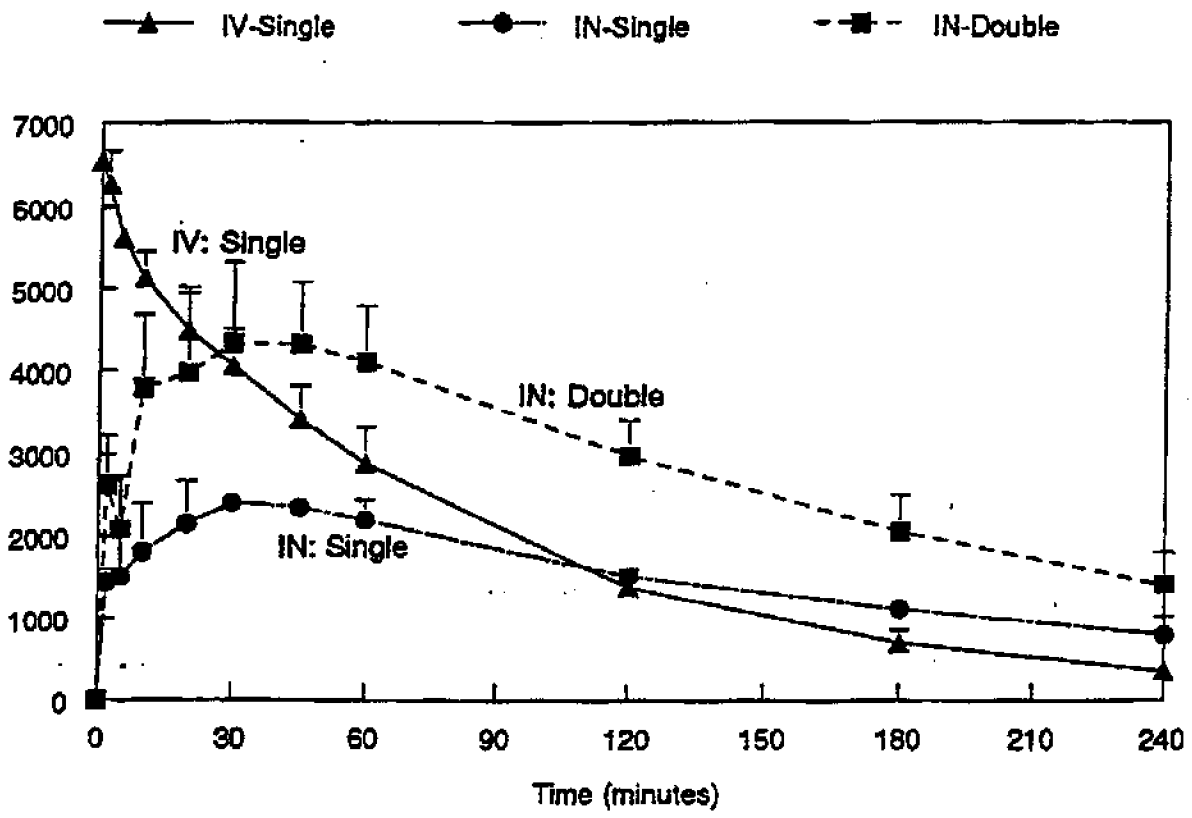


Fig. 9


IV-Single
IN-Single
IN-Double
7000
6000
5000
4000
3000
2000
1000
0
IV: Single
IN: Double
IN: Single
0
30
60
90
120
150
180
210
240
Time (minutes)

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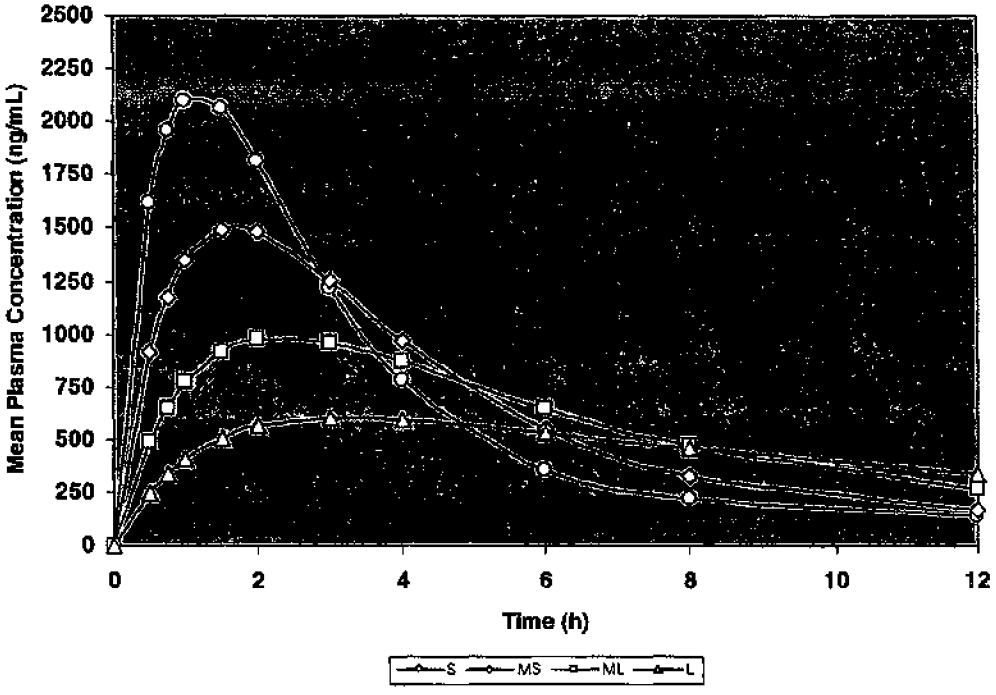
(54) Title: COMPOSITIONS HAVING A COMBINATION OF IMMEDIATE RELEASE AND CONTROLLED RELEASE CHARACTERISTICS



(57) Abstract: Disclosed are compositions exhibiting a combination of immediate release and controlled release characteristics. The compositions comprise at least one poorly soluble active ingredient having a nanoparticulate particle size, at least one surface stabilizer adsorbed onto the surface of the nanoparticulate active agent particles, and at least one active ingredient having a microparticulate particle size.

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COMPOSITIONS HAVING A COMBINATION OF IMMEDIATE RELEASE AND CONTROLLED RELEASE CHARACTERISTICS

FIELD OF THE INVENTION

The present invention relates to compositions exhibiting a combination of immediate release and controlled release characteristics. The compositions comprise at least one poorly soluble active ingredient having a nanoparticulate particle size, at least one surface stabilizer adsorbed onto the surface of the nanoparticulate active agent particles, and at least one poorly soluble active ingredient having a microparticulate particle size.

BACKGROUND OF THE INVENTION

A. Background Regarding Nanoparticulate Compositions

Nanoparticulate compositions, first described in U.S. Patent No. 5,145,684 ("the '684 patent"), are particles consisting of a poorly soluble active agent having adsorbed onto the surface thereof a non-crosslinked surface stabilizer. The '684 patent also describes methods of making such nanoparticulate compositions. Nanoparticulate compositions are desirable because with a decrease in particle size, and a consequent increase in surface area, a composition is rapidly dissolved and absorbed following administration. Methods of making such compositions are described in U.S. Patent Nos. 5,518,187 and 5,862,999, both for "Method of Grinding Pharmaceutical Substances," U.S. Patent No. 5,718,388, for "Continuous Method of Grinding Pharmaceutical Substances;" and U.S. Patent No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles."

Nanoparticulate compositions are also described in, for example, U.S. Patent Nos. 5,298,262 for "Use of Ionic Cloud Point Modifiers to Prevent Particle Aggregation During Sterilization;" 5,302,401 for "Method to Reduce Particle Size Growth During Lyophilization;" 5,318,767 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,326,552 for "Novel Formulation For Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,328,404 for "Method of X-Ray Imaging Using Iodinated Aromatic Propanedioates;" 5,336,507 for

"Use of Charged Phospholipids to Reduce Nanoparticle Aggregation;" 5,340,564 for "Formulations Comprising Olin 10-G to Prevent Particle Aggregation and Increase Stability;" 5,346,702 for "Use of Non-Ionic Cloud Point Modifiers to Minimize Nanoparticulate Aggregation During Sterilization;" 5,349,957 for "Preparation and Magnetic Properties of Very Small Magnetic-Dextran Particles;" 5,352,459 for "Use of Purified Surface Modifiers to Prevent Particle Aggregation During Sterilization;" 5,399,363 and 5,494,683, both for "Surface Modified Anticancer Nanoparticles;" 5,401,492 for "Water Insoluble Non-Magnetic Manganese Particles as Magnetic Resonance Enhancement Agents;" 5,429,824 for "Use of Tyloxapol as a Nanoparticulate Stabilizer;" 5,447,710 for "Method for Making Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,451,393 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,466,440 for "Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents in Combination with Pharmaceutically Acceptable Clays;" 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation;" 5,472,683 for "Nanoparticulate Diagnostic Mixed Carbamic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,500,204 for "Nanoparticulate Diagnostic Dimers as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,518,187 for "Method of Grinding Pharmaceutical Substances;" 5,518,738 for "Nanoparticulate NSAID Formulations;" 5,521,218 for "Nanoparticulate Iododipamide Derivatives for Use as X-Ray Contrast Agents;" 5,525,328 for "Nanoparticulate Diagnostic Diatrizoxy Ester X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" 5,552,160 for "Surface Modified NSAID Nanoparticles;" 5,560,931 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,565,188 for "Polyalkylene Block Copolymers as Surface Modifiers for Nanoparticles;" 5,569,448 for "Sulfated Non-ionic Block Copolymer Surfactant as Stabilizer Coatings for Nanoparticle Compositions;" 5,571,536 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,573,749 for "Nanoparticulate Diagnostic Mixed Carboxylic Anydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,573,750 for "Diagnostic Imaging X-Ray Contrast Agents;" 5,573,783 for "Redispersible Nanoparticulate Film Matrices

With Protective Overcoats;" 5,580,579 for "Site-specific Adhesion Within the GI Tract Using Nanoparticles Stabilized by High Molecular Weight, Linear Poly(ethylene Oxide) Polymers;" 5,585,108 for "Formulations of Oral Gastrointestinal Therapeutic Agents in Combination with Pharmaceutically Acceptable Clays;" 5,587,143 for "Butylene Oxide-Ethylene Oxide Block Copolymers Surfactants as Stabilizer Coatings for Nanoparticulate Compositions;" 5,591,456 for "Milled Naproxen with Hydropropyl Cellulose as Dispersion Stabilizer;" 5,593,657 for "Novel Barium Salt Formulations Stabilized by Non-ionic and Anionic Stabilizers;" 5,622,938 for "Sugar Based Surfactant for Nanocrystals;" 5,628,981 for "Improved Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents and Oral Gastrointestinal Therapeutic Agents;" 5,643,552 for "Nanoparticulate Diagnostic Mixed Carbonic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" 5,718,919 for "Nanoparticles Containing the R(-)Enantiomer of Ibuprofen;" 5,747,001 for "Aerosols Containing Beclomethasone Nanoparticle Dispersions;" 5,834,025 for "Reduction of Intravenously Administered Nanoparticulate Formulation Induced Adverse Physiological Reactions;" 6,045,829 "Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,068,858 for "Methods of Making Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,153,225 for "Injectable Formulations of Nanoparticulate Naproxen;" 6,165,506 for "New Solid Dose Form of Nanoparticulate Naproxen;" 6,221,400 for "Methods of Treating Mammals Using Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors;" 6,264,922 for "Nebulized Aerosols Containing Nanoparticle Dispersions;" 6,267,989 for "Methods for Preventing Crystal Growth and Particle Aggregation in Nanoparticle Compositions;" 6,270,806 for "Use of PEG-Derivatized Lipids as Surface Stabilizers for Nanoparticulate Compositions;" 6,316,029 for "Rapidly Disintegrating Solid Oral Dosage Form," 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate;" 6,428,814 for "Bioadhesive Nanoparticulate Compositions Having Cationic Surface Stabilizers;" and 6,432,381 for "Methods for Targeting Drug Delivery to the Upper and/or Lower Gastrointestinal Tract," all of which are specifically incorporated by

reference. In addition, U.S. Patent Application No. 20020012675 A1, published on January 31, 2002, for "Controlled Release Nanoparticulate Compositions," describes nanoparticulate compositions, and is specifically incorporated by reference.

Amorphous small particle compositions are described in, for example, U.S. Patent Nos. 4,783,484 for "Particulate Composition and Use Thereof as Antimicrobial Agent," 4,826,689 for "Method for Making Uniformly Sized Particles from Water-Insoluble Organic Compounds," 4,997,454 for "Method for Making Uniformly-Sized Particles From Insoluble Compounds," 5,741,522 for "Ultrasmall, Non-aggregated Porous Particles of Uniform Size for Entrapping Gas Bubbles Within and Methods," and 5,776,496, for "Ultrasmall Porous Particles for Enhancing Ultrasound Back Scatter.

None of these references, or any other reference that describes nanoparticulate compositions, relates to a nanoparticulate composition having a combination of immediate release and controlled release characteristics.

B. Background Regarding Immediate Release Compositions

Conventional immediate release dosage forms, also referred to as "fast melt" or "rapidly disintegrating" dosage forms, rely on the composition of the drug matrix to effect the rapid release of the component active agent particles, rather than the particle size of the component active agent particles.

Current manufacturers of rapidly disintegrating or dissolving solid dose oral formulations include Cima Labs, Fuisz Technologies Ltd., Prographarm, R.P. Scherer, and Yamanouchi-Shaklee. All of these manufacturers market different types of rapidly dissolving solid oral dosage forms.

Cima Labs markets OraSolv®, which is an effervescent direct compression tablet having an oral dissolution time of five to thirty seconds, and DuraSolv®, which is a direct compression tablet having a taste-masked active agent and an oral dissolution time of 15 to 45 seconds. Cima's U.S. Patent No. 5,607,697, for "Taste Masking Microparticles for Oral Dosage Forms," describes a solid dosage form consisting of coated microparticles that disintegrate in the mouth. The microparticle core has a pharmaceutical agent and one or more sweet-tasting compounds having a negative heat of solution selected from mannitol, sorbitol, a mixture of an artificial sweetener and menthol, a mixture of sugar and menthol, and methyl salicylate. The microparticle core is coated, at least partially, with a material that retards dissolution in the mouth and

masks the taste of the pharmaceutical agent. The microparticles are then compressed to form a tablet. Other excipients can also be added to the tablet formulation.

WO 98/46215 for "Rapidly Dissolving Robust Dosage Form," assigned to Cima Labs, is directed to a hard, compressed, fast melt formulation having an active ingredient and a matrix of at least a non-direct compression filler and lubricant. A non-direct compression filler is typically not free-flowing, in contrast to a direct compression (DC grade) filler, and usually requires additionally processing to form free-flowing granules.

Cima also has U.S. patents and international patent applications directed to effervescent dosage forms (U.S. Patent Nos. 5,503,846, 5,223,264, and 5,178,878) and tableting aids for rapidly dissolving dosage forms (U.S. Patent Nos. 5,401,513 and 5,219,574), and rapidly dissolving dosage forms for water soluble drugs (WO 98/14179 for "Taste-Masked Microcapsule Composition and Methods of Manufacture").

Fuisz Technologies, now part of BioVail, markets Flash Dose®, which is a direct compression tablet containing a processed excipient called Shearform®. Shearform® is a cotton candy-like substance of mixed polysaccharides converted to amorphous fibers. U.S. patents describing this technology include U.S. Patent No. 5,871,781 for "Apparatus for Making Rapidly Dissolving Dosage Units;" U.S. Patent No. 5,869,098 for "Fast-Dissolving Comestible Units Formed Under High-Speed/High-Pressure Conditions;" U.S. Patent Nos. 5,866,163, 5,851,553, and 5,622,719, all for "Process and Apparatus for Making Rapidly Dissolving Dosage Units and Product Therefrom;" U.S. Patent No. 5,567,439 for "Delivery of Controlled-Release Systems;" and U.S. Patent No. 5,587,172 for "Process for Forming Quickly Dispersing Comestible Unit and Product Therefrom."

Prographarm markets Flashtab®, which is a fast melt tablet having a disintegrating agent such as carboxymethyl cellulose, a swelling agent such as a modified starch, and a taste-masked active agent. The tablets have an oral disintegration time of under one minute (U.S. Patent No. 5,464,632).

R.P. Scherer markets Zydis®, which is a freeze-dried tablet having an oral dissolution time of 2 to 5 seconds. Lyophilized tablets are costly to manufacture and difficult to package because of the tablets sensitivity to moisture and temperature. U.S. Patent No. 4,642,903 (R.P. Scherer Corp.) refers to a fast melt dosage formulation prepared by dispersing a gas throughout a solution or suspension to be freeze-dried.

U.S. Patent No. 5,188,825 (R.P. Scherer Corp.) refers to freeze-dried dosage forms prepared by bonding or complexing a water-soluble active agent to or with an ion exchange resin to form a substantially water insoluble complex, which is then mixed with an appropriate carrier and freeze dried. U.S. Patent No. 5,631,023 (R. P. Scherer Corp.) refers to freeze-dried drug dosage forms made by adding xanthan gum to a suspension of gelatin and active agent. U.S. Patent No. 5,827,541 (R.P. Scherer Corp.) discloses a process for preparing solid pharmaceutical dosage forms of hydrophobic substances. The process involves freeze-drying a dispersion containing a hydrophobic active ingredient and a surfactant in a non-aqueous phase and a carrier material in an aqueous phase.

Yamanouchi-Shaklee markets Wowtab®, which is a tablet having a combination of a low moldability and a high moldability saccharide. U.S. Patents covering this technology include U.S. Patent No. 5,576,014 for "Intrabuccally Dissolving Compressed Moldings and Production Process Thereof," and U.S. Patent No. 5,446,464 for "Intrabuccally Disintegrating Preparation and Production Thereof."

Other companies owning rapidly dissolving technology include Janssen Pharmaceutica. U.S. patents assigned to Janssen describe rapidly dissolving tablets having two polypeptide (or gelatin) components and a bulking agent, wherein the two components have a net charge of the same sign, and the first component is more soluble in aqueous solution than the second component. *See* U.S. Patent No. 5,807,576 for "Rapidly Dissolving Tablet;" U.S. Patent No. 5,635,210 for "Method of Making a Rapidly Dissolving Tablet;" U.S. Patent No. 5,595,761 for "Particulate Support Matrix for Making a Rapidly Dissolving Tablet;" U.S. Patent No. 5,587,180 for "Process for Making a Particulate Support Matrix for Making a Rapidly Dissolving Tablet;" and U.S. Patent No. 5,776,491 for "Rapidly Dissolving Dosage Form."

Eurand America, Inc. has U.S. patents directed to a rapidly dissolving effervescent composition having a mixture of sodium bicarbonate, citric acid, and ethylcellulose (U.S. Patent Nos. 5,639,475 and 5,709,886).

L.A.B. Pharmaceutical Research owns U.S. patents directed to effervescent-based rapidly dissolving formulations having an effervescent couple of an effervescent acid and an effervescent base (U.S. Patent Nos. 5,807,578 and 5,807,577).

Schering Corporation has technology relating to buccal tablets having an active agent, an excipient (which can be a surfactant) or at least one of sucrose, lactose, or

sorbitol, and either magnesium stearate or sodium dodecyl sulfate (U.S. Patent Nos. 5,112,616 and 5,073,374).

Laboratoire L. LaFon owns technology directed to conventional dosage forms made by lyophilization of an oil-in-water emulsion in which at least one of the two phases contains a surfactant (U.S. Patent No. 4,616,047). For this type of formulation, the active ingredient is maintained in a frozen suspension state and is tableted without micronization or compression, as such processes could damage the active agent.

Takeda Chemicals Inc., Ltd. owns technology directed to a method of making a fast dissolving tablet in which an active agent and a moistened, soluble carbohydrate are compression molded into a tablet, followed by drying of the tablets.

None of the described prior art teaches an immediate release dosage form in which a poorly soluble active ingredient is in a nanoparticulate form. This is significant because the prior art immediate release formulations do not address the problems associated with the bioavailability of poorly soluble drugs. While prior art immediate release dosage forms may provide rapid presentation of a drug, frequently there is an undesirable lag in the onset of therapeutic action because of the poor solubility and associated slow dissolution rate of the drug. Thus, while prior art immediate release dosage forms may exhibit rapid disintegration of the drug carrier matrix, this does not result in rapid dissolution and absorption of the poorly soluble drug contained within the dosage form.

C. Background Regarding Controlled Release Compositions

Controlled release refers to the release of an agent such as a drug from a composition or dosage form in which the agent is released according to a desired profile over an extended period of time. Controlled release profiles include, for example, sustained release, prolonged release, pulsatile release, and delayed release profiles. In contrast to immediate release compositions, controlled release compositions allow delivery of an agent to a subject over an extended period of time according to a predetermined profile. Such release rates can provide therapeutically effective levels of agent for an extended period of time and thereby provide a longer period of pharmacologic or diagnostic response as compared to conventional rapid release dosage forms. Such longer periods of response provide for many inherent benefits that are not achieved with the corresponding short acting, immediate release preparations. For

example, in the treatment of chronic pain, controlled release formulations are often highly preferred over conventional short-acting formulations.

Controlled release pharmaceutical compositions and dosage forms are designed to improve the delivery profile of agents, such as drugs, medicaments, active agents, diagnostic agents, or any substance to be internally administered to an animal, including humans. A controlled release composition is typically used to improve the effects of administered substances by optimizing the kinetics of delivery, thereby increasing bioavailability, convenience, and patient compliance, as well as minimizing side effects associated with inappropriate immediate release rates such as a high initial release rate and, if undesired, uneven blood or tissue levels.

Prior art teachings of the preparation and use of compositions providing for controlled release of an active compound provide various methods of extending the release of a drug following administration.

Exemplary controlled release formulations known in the art include specially coated pellets, microparticles, implants, tablets, minitabs, and capsules in which a controlled release of a drug is brought about, for example, through selective breakdown of the coating of the preparation, through release through the coating, through compounding with a special matrix to affect the release of a drug, or through a combination of these techniques. Some controlled release formulations provide for pulsatile release of a single dose of an active compound at predetermined periods after administration.

U.S. Patent No. 5,110,605 to Acharya et al. refers to a calcium polycarbophil-alginate controlled release composition. U.S. Patent No. 5,215,758 to Krishnamurthy et al. refers to a controlled release suppository composition of sodium alginate and calcium salt. U.S. Patent No. 5,811,388 to Friend et al. refers to a solid alginate-based formulation including alginate, a water-swellable polymer, and a digestible hydrocarbon derivative for providing controlled release of orally administered compounds.

WO 91/13612 refers to the sustained release of pharmaceuticals using compositions in which the drug is complexed with an ion-exchange resin. The specific ion-exchange resin described in this published patent application is AMBERLITE IRP 69®, a sodium polystyrene sulphonate resin.

U.S. Patent No. 5,811,425 to Woods et al. refers to injectable depot forms of controlled release drugs made by forming microencapsule matrices of the drug in biodegradable polymers, liposomes, or microemulsions compatible with body tissues. U.S. Patent No. 5,811,422 to Lam et al. refers to controlled release compositions obtained by coupling a class of drugs to biodegradable polymers, such as polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, etc.

U.S. Patent No. 5,811,404 to De Frees et al. refers to the use of liposomes having prolonged circulation half-lives to provide for the sustained release of drug compositions.

Finally, WO 00/18374, for "Controlled Release Nanoparticulate Compositions," describes controlled release formulations comprising nanoparticulate active agents.

* * * *

There is a need in the art for compositions of poorly soluble drugs having a combination of immediate release and controlled release characteristics. The present invention satisfies this need.

SUMMARY OF THE INVENTION

This invention is directed to the surprising and unexpected discovery of formulations of poorly soluble active agents having a combination of immediate active agent release and controlled active agent release characteristics. The formulations comprise a combination of very small active agent particles, *i.e.*, nanoparticulate active agent particles, in combination with larger active agent particles, *i.e.*, micronized active agent particles, which enable obtaining the simultaneous presentation of immediate-release (IR) and controlled-release (CR) active agent components.

The nanoparticulate active agent particles, representing the IR component, afford rapid *in vivo* dissolution, owing to their small size and attendant large specific surface. Alternatively, micronized active agent particles, representing the CR component, afford slower *in vivo* dissolution, owing to a comparatively large particle size and small attendant specific surface.

IR and CR components representing a wide range of *in vivo* dissolution rates (and hence, *in vivo* input rates for absorption) can be engineered through precise control

of active agent particle size. Thus, the compositions can comprise a mixture of nanoparticulate active agent particles, wherein each population of particles has a defined size correlating with a precise release rate, and the compositions can comprise a mixture of microparticulate active agent particles, wherein each population of particles has a defined size correlating with a precise release rate.

The compositions of the invention are highly unexpected, particularly since the controlled delivery of active agents has traditionally been achieved through employment of rate-controlling membranes, swellable and erodible polymers, and ion-exchange resins, rather than solely through the particle size of the active agent component of the dosage form.

In another aspect of the invention there is provided a method of preparing formulations having a combination of IR and CR characteristics. The method comprises: (1) forming a composition comprising particles of at least one nanoparticulate active agent to be administered and at least one surface stabilizer adsorbed onto the surface of the nanoparticulate active agent particles; (2), adding at least one microparticulate active agent, which can be the same or different from the active agent of (1), and (3) forming a dosage form of the mixture of (1) and (2) for administration. Additional pharmaceutically acceptable excipients can also be added to the composition for administration.

Yet another aspect of the present invention provides a method of treating a mammal, including a human, with a composition of the invention.

It is to be understood that both the foregoing general description and the following brief description of the figures and detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1: Shows a simulation, using a mathematical model, of pharmacokinetic profiles following single oral doses of a pharmaceutical formulation containing an active agent having a single defined particle size;

FIGURE 2: Shows a simulation, using a mathematical model, of pharmacokinetic profiles following single oral doses of a pharmaceutical formulation containing mixtures of different sizes of particles; and

FIGURE 3: Shows a simulation, using a mathematical model, of pharmacokinetic profiles following single oral doses of a pharmaceutical formulation containing mixtures of different sizes of particles.

DETAILED DESCRIPTION OF THE INVENTION

A. Compositions

This invention is directed to the surprising and unexpected discovery of new compositions exhibiting a combination of IR and CR characteristics. The compositions do not require the presence of an additional ingredient, such as a rate controlling polymer or membrane, a swellable or erodible polymer, or ion-exchange resin, to obtain the CR characteristics. The IR and CR characteristics are obtained using precisely calibrated particle sizes for the one or more active agents. Smaller particle sizes result in IR profiles and larger particle sizes result in CR profiles.

The compositions of the invention comprise: (1) particles of at least one poorly soluble nanoparticulate active agent; (2) at least one surface stabilizer adsorbed onto the surface of the nanoparticulate active agent particles; and (3) at least one poorly soluble microparticulate active agent, which can be the same as or different from the active agent of (1). As taught in the '684 patent, the surface stabilizer functions to stabilize the nanoparticulate active agent by preventing agglomeration and particle size growth.

Methods of making nanoparticulate active agent compositions, which can comprise mechanical grinding, precipitation, homogenization, or any other suitable size reduction process, are known in the art and are described in, for example, the '684 patent and other prior art references disclosed in the "Background of the Invention."

The nanoparticulate and microparticulate active agent particles can be in a crystalline form, semi-crystalline form, amorphous form, semi-amorphous form, or a combination thereof.

The compositions can be formulated for administration to humans and animals via any conventional means, including but not limited to orally, rectally, parenterally

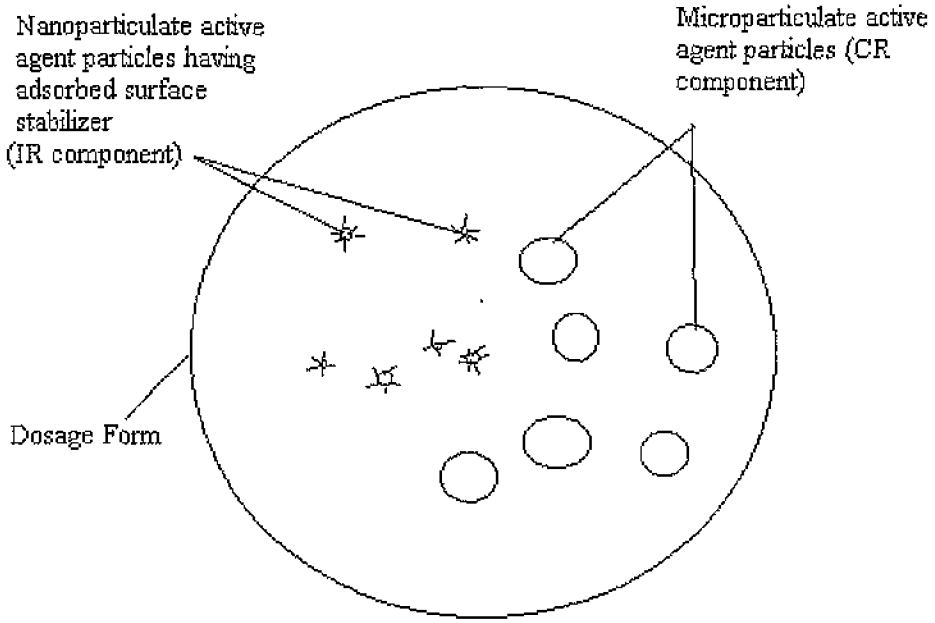
(intravenous, intramuscular, or subcutaneous), intracisternally, pulmonary, intravaginally, intraperitoneally, locally (powders, ointments or drops), ocularly, aurally, or as a buccal or nasal spray.

The present invention also encompasses the compositions of the invention formulated together with one or more non-toxic physiologically acceptable carriers, adjuvants, or vehicles, collectively referred to as carriers. The compositions can be formulated for parenteral injection, oral administration in solid or liquid form, rectal or topical administration, ocular or aural administration, and the like.

For oral dosage forms, the IR component results in rapid dissolution of the poorly soluble active agent in the oral cavity as a result of the nanoparticulate size of the drug. Further, the opportunity for buccal absorption of the poorly soluble active ingredient is enhanced with the present invention. Yet another advantage of the nanoparticulate IR component is that the use of nanoparticulate active agent particles eliminates or minimizes the feeling of grittiness found with prior art IR oral formulations of poorly soluble active agents.

One advantage typically associated with IR dosage forms is a reduction of the time lag between administration of a dose and the physical presentation of the active ingredient. This lag time is usually associated with the break up of the dosage form and the distribution of the active ingredient thereafter. Another advantage of oral IR dosage forms is that the rapid presentation of the active agent in the mouth upon administration may facilitate buccal absorption of the active ingredient directly into the blood stream, thus reducing the first pass effect of the liver on the overall bioavailability of active ingredient from a unit dose. This second advantage is dramatically enhanced for the IR formulations of the invention, as the nanoparticulate size of the active agent enables rapid dissolution in the oral cavity.

It is expected that the CR component of the compositions provides effective blood levels of an incorporated active agent in a patient for an extended period of time. As used herein, "controlled release" means the release of an active agent such as a drug from a composition or dosage form in which the agent is released according to a desired profile over an extended period of time, such as from about 2 to about 24 hours or longer. Release over a longer time period is also contemplated as a "controlled release" dosage form of the present invention. An exemplary formulation is graphically illustrated below:



1. Solid Dosage Forms

In a first embodiment of the invention, both of the IR and CR components are incorporated in a solid, rapidly disintegrating or "waterless tablet" matrix intended for oral administration.

In a second embodiment of the invention, the IR and CR components are combined in a pharmaceutically acceptable tablet intended for oral administration.

In a third embodiment of the invention, the IR and CR components are combined in a pharmaceutically acceptable hard gelatin capsule intended for oral administration.

In a fourth embodiment of the invention, the IR and CR components are combined in a pharmaceutically acceptable soft gelatin capsule intended for oral administration. One variation of this dosage form comprises solubilized drug and microparticulate drug particles, in which the solubilized drug functions as the IR component and the microparticulate drug particles function as the CR component of the dosage form. This variation differs from the other dosage forms described herein in that it does not require the presence of "particulate" nanoparticulate drug particles.

In a fifth embodiment of the invention, the IR and CR components are combined in a pharmaceutically acceptable lozenge or troche intended for oral administration.

In a sixth embodiment of the invention, the IR and CR components are combined in a pharmaceutically acceptable sachet, powder, or "sprinkle" intended for oral administration.

2. Other Dosage Forms

Other suitable dosage forms include, but are not limited to, suppositories for rectal or intravaginally use; injectables, including injectables for intravenous, intramuscular, or subcutaneous administration; aerosols for pulmonary or nasal administration; buccal dosage forms; dosage forms for local application, such as powders, ointments, or drops; ocular and aural dosage forms, and dosage forms for intracisternal and intraperitoneal administration.

The IR and CR compositions of the invention can be combined in any pharmaceutically acceptable dosage form, which is not limited to those specifically described above.

3. Poorly Soluble Active Agent

The compositions of the invention comprise at least one poorly soluble therapeutic agent, diagnostic agent, or other active agent. A therapeutic agent can be a drug or pharmaceutical and a diagnostic agent is typically a contrast agent, such as an x-ray contrast agent, or any other type of diagnostic material.

The invention can be practised with a wide variety of poorly soluble drugs or diagnostic agents. The drug or diagnostic agent is preferably present in an essentially pure form, is poorly soluble, and is dispersible in at least one liquid medium. By "poorly soluble" it is meant that the drug or diagnostic agent has a solubility in a liquid dispersion medium of less than about 30 mg/ml, preferably less than about 10 mg/ml, and more preferably less than about 1 mg/ml. Such a liquid dispersion medium can be, for example, water, aqueous salt solutions, oils such as safflower oil, and solvents such as ethanol, t-butanol, hexane, and glycol.

The poorly soluble active agent can be selected from a variety of known classes of drugs or diagnostic agents, including, for example, proteins, peptides, nucleotides, anti-obesity drugs, nutraceuticals, corticosteroids, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including

penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives (e.g., hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators and xanthines.

Exemplary nutraceuticals and dietary supplements are disclosed, for example, in Roberts et al., *Nutraceuticals: The Complete Encyclopedia of Supplements, Herbs, Vitamins, and Healing Foods* (American Nutraceutical Association, 2001), which is specifically incorporated by reference. A nutraceutical or dietary supplement, also known as phytochemicals or functional foods, is generally any one of a class of dietary supplements, vitamins, minerals, herbs, or healing foods that have medical or pharmaceutical effects on the body. Exemplary nutraceuticals or dietary supplements include, but are not limited to, folic acid, fatty acids (*e.g.*, DHA and ARA), fruit and vegetable extracts, vitamin and mineral supplements, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids (*e.g.*, iso-leucine, leucine, lysine, methionine, phenylanine, threonine, tryptophan, and valine), green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish and marine animal oils, and probiotics. Nutraceuticals and dietary supplements also include bio-engineered foods genetically engineered to have a desired property, also known as "pharmafoods."

The active agents are commercially available and/or can be prepared by techniques known in the art.

The poorly soluble active ingredient may be present in any amount which is sufficient to elicit a therapeutic effect and, where applicable, may be present either substantially in the form of one optically pure enantiomer or as a mixture, racemic or otherwise, of enantiomers.

4. Surface Stabilizers

Useful surface stabilizers, which are known in the art and described, for example, in the '684 patent, are believed to include those which physically adhere to the surface of the active agent but do not chemically bond to or interact with the active agent. The surface stabilizer is adsorbed on the surface of the nanoparticulate active agent in an amount sufficient to maintain an effective average particle size of less than about 1000 nm for the active agent. Furthermore, the individually adsorbed molecules of the surface stabilizer are essentially free of intermolecular cross-linkages. Two or more surface stabilizers can be employed in the compositions and methods of the invention.

Suitable surface stabilizers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products, and surfactants. Preferred surface stabilizers include nonionic and ionic surfactants, including anionic and cationic surfactants.

Representative examples of surface stabilizers include gelatin, casein, lecithin (phosphatides), dextran, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers (*e.g.*, macrogol ethers such as cetomacrogol 1000), polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (*e.g.*, the commercially available Tweens® such as *e.g.*, Tween 20® and Tween 80® (ICI Speciality Chemicals)); polyethylene glycols (*e.g.*, Carbowaxs 3550® and 934® (Union Carbide)), polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethyl-cellulose phthalate, noncrystalline cellulose, magnesium aluminium silicate, triethanolamine, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol, superione, and triton), poloxamers (*e.g.*, Pluronics F68® and F108®, which are block copolymers of ethylene oxide and propylene oxide); poloxamines (*e.g.*, Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Wyandotte Corporation,

Parsippany, N.J.)); Tetronic 1508® (T-1508) (BASF Wyandotte Corporation), dialkylesters of sodium sulfosuccinic acid (*e.g.*, Aerosol OT®, which is a dioctyl ester of sodium sulfosuccinic acid (American Cyanamid)); Duponol P®, which is a sodium lauryl sulfate (DuPont); Tritons X-200®, which is an alkyl aryl polyether sulfonate (Rohm and Haas); Crodestas F-110®, which is a mixture of sucrose stearate and sucrose distearate (Croda Inc.); p-isononylphenoxypoly-(glycidol), also known as Olin-lOG® or Surfactant 10-G® (Olin Chemicals, Stamford, CT); Crodestas SL-40® (Croda, Inc.); and SA9OHCO, which is $C_{18}H_{37}CH_2C(O)N(CH_3)$-$CH_2(CHOH)_4(CH_2OH)_2$ (Eastman Kodak Co.); decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, random copolymers of vinyl pyrrolidone and vinyl acetate, such as Plasdone S630, PEG-derivatized phospholipids, PEG-derivatized cholesterol, PEG-derivatized cholesterol derivatives, PEG-derivatized vitamin A, PEG-derivatized vitamin E, and the like.

Other useful surface stabilizers include sodium lauryl sulfate, dioctyl sodium sulfosuccinate, or a combination thereof.

Examples of useful cationic surface stabilizers include, but are not limited to, polymers, biopolymers, polysaccharides, cellulosics, alginates, phospholipids, and nonpolymeric compounds, such as zwitterionic stabilizers, poly-n-methylpyridinium, anthryul pyridinium chloride, cationic phospholipids, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr), hexyldesyltrimethylammonium bromide (HDMAB), and polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate.

Other useful cationic stabilizers include, but are not limited to, cationic lipids, sulfonium, phosphonium, and quarternary ammonium compounds, such as stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride or bromide, coconut methyl dihydroxyethyl ammonium chloride or bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride or bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride or bromide, coconut dimethyl hydroxyethyl ammonium chloride or bromide,

myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride or bromide, lauryl dimethyl (ethenoxy)$_4$ ammonium chloride or bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts and dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt and/or an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride and dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12}, C_{15}, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride (ALIQUAT 336™), POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters (such as choline esters of fatty acids), benzalkonium chloride, stearalkonium chloride compounds (such as stearyltrimonium chloride and Di-stearyldimonium chloride), cetyl pyridinium bromide or chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™ and ALKAQUAT™ (Alkaril Chemical Company), alkyl pyridinium salts; amines, such as alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, and vinyl pyridine, amine salts, such as lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, and alkylimidazolium salt, and amine oxides; imide azolinium salts; protonated quaternary acrylamides; methylated quaternary polymers, such as poly[diallyl dimethylammonium chloride] and poly-[N-methyl vinyl pyridinium chloride]; and cationic guar.

Such exemplary cationic surface stabilizers and other useful cationic surface stabilizers are described in J. Cross and E. Singer, *Cationic Surfactants: Analytical and Biological Evaluation* (Marcel Dekker, 1994); P. and D. Rubingh (Editor), *Cationic*

Surfactants: Physical Chemistry (Marcel Dekker, 1991); and J. Richmond, *Cationic Surfactants: Organic Chemistry,* (Marcel Dekker, 1990).

Particularly preferred nonpolymeric primary stabilizers are any nonpolymeric compound, such benzalkonium chloride, a carbonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quarternary phosphorous compound, a pyridinium compound, an anilinium compound, an ammonium compound, a hydroxylammonium compound, a primary ammonium compound, a secondary ammonium compound, a tertiary ammonium compound, and quarternary ammonium compounds of the formula $NR_1R_2R_3R_4^{(+)}$. For compounds of the formula $NR_1R_2R_3R_4^{(+)}$:

(i) none of R_1-R_4 are CH_3;

(ii) one of R_1-R_4 is CH_3;

(iii) three of R_1-R_4 are CH_3;

(iv) all of R_1-R_4 are CH_3;

(v) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 is an alkyl chain of seven carbon atoms or less;

(vi) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 is an alkyl chain of nineteen carbon atoms or more;

(vii) two of R_1-R_4 are CH_3 and one of R_1-R_4 is the group $C_6H_5(CH_2)_n$, where $n>1$;

(viii) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one heteroatom;

(ix) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one halogen;

(x) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one cyclic fragment;

(xi) two of R_1-R_4 are CH_3 and one of R_1-R_4 is a phenyl ring; or

(xii) two of R_1-R_4 are CH_3 and two of R_1-R_4 are purely aliphatic fragments.

Such compounds include, but are not limited to, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium

chloride(Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium POE (10) oletyl ether phosphate, diethanolammonium POE (3)oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearalkonium chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, iofetamine hydrochloride, meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procainehydrochloride, cocobetaine, stearalkonium bentonite, stearalkoniumhectonite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

Most of these surface stabilizers are known pharmaceutical excipients and are described in detail in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain (The Pharmaceutical Press, 2000), specifically incorporated by reference. The surface stabilizers are commercially available and/or can be prepared by techniques known in the art.

5. Particle Size

The IR characteristics of the dosage form are obtained by utilizing at least one nanoparticulate active agent having an effective average particle size of less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, or less than about 50 nm.

The CR characteristics of the dosage form are obtained by utilizing at least one microparticulate active agent having an effective average particle size of greater than about 1 micron and less than about 100 microns, less than about 90 microns, less than about 80 microns, less than about 70 microns, less than about 60 microns, less than about 50 microns, less than about 40 microns, less than about 30 microns, less than about 20 microns, less than about 10 microns, less than about 9 microns, less than about

8 microns, less than about 7 microns, less than about 6 microns, less than about 5 microns, less than about 4 microns, less than about 3 microns, or less than about 2 microns.

The compositions can comprise multiple populations of nanoparticulate active agent particles, wherein each population of particles has a defined size correlating with a precise release rate, *i.e.* a first population having an effective average particle size of less than about 1 micron, a second population having an effective average particle size of less than about 800 nm, a third population having an effective average particle size of less than about 500 nm, a fourth population having an effective average particle size of less than about 50 nm, *etc.*, with each population corresponding to a specific release rate.

Similarly, the compositions can comprise multiple populations of microparticulate active agent particles, wherein each population of particles has a defined size correlating with a precise release rate, *i.e.* a first population having an effective average particle size of less than about 100 microns, a second population having an effective average particle size of less than about 60 microns, a third population having an effective average particle size of less than about 40 microns, a fourth population having an effective average particle size of less than about 20 microns, *etc.*, with each population corresponding to a specific release rate.

Each population of particles in the composition, both nanoparticulate and microparticulate, exhibits a baseline resolution of at least 50%, 60%, 70%, 80%, or 90%. This means that the heterogeneous population is characterized by a multi-modal particle size distribution, with a minimum baseline resolution of 50% relative to two adjacent peaks. 50% baseline resolution is defined as half the distance from the baseline of the distribution to the average height of two adjacent peaks.

The baseline resolution of at least 50% distinguishes the claimed invention from a conventional microparticulate composition having a mixture of particle sizes, as in such a conventional composition the particle sizes are randomly distributed and do not have a baseline resolution of at least 50% for two or more particle sizes.

As used herein, particle size is determined on the basis of the weight average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art. Such techniques include, for example, sedimentation

field flow fractionation, photon correlation spectroscopy, light scattering, and disk centrifugation.

By "an effective average particle size of less than about 1000 nm" it is meant that at least 50% of the active agent particles have an average particle size of less than about 1000 nm, when measured by the above techniques. Similarly, by "an effective average particle size of less than about 100 microns, it is meant that at least 50% of the active agent particles have an average particle size of less than about 100 microns, when measured by the above techniques. Preferably, at least 70% of the particles have an average particle size of less than the effective average, more preferably at least about 90% of the particles have an average particle size of less than the effective average.

6. Other Pharmaceutical Excipients

Pharmaceutical compositions according to the invention may also comprise one or more binding agents, filling agents, lubricating agents, suspending agents, sweeteners, flavoring agents, preservatives, buffers, wetting agents, disintegrants, effervescent agents, and other excipients. Such excipients are known in the art.

Examples of filling agents are lactose monohydrate, lactose anhydrous, and various starches; examples of binding agents are various celluloses and cross-linked polyvinylpyrrolidone, microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102, microcrystalline cellulose, and silicified microcrystalline cellulose (SMCC).

Suitable lubricants, including agents that act on the flowability of the powder to be compressed, are colloidal silicon dioxide, such as Aerosil® 200; talc, stearic acid, magnesium stearate, calcium stearate, and silica gel.

Examples of sweeteners are any natural or artificial sweetener, such as sucrose, xylitol, sodium saccharin, cyclamate, aspartame, and acsulfame. Examples of flavoring agents are Magnasweet® (trademark of MAFCO), bubble gum flavor, and fruit flavors, and the like.

Examples of preservatives are potassium sorbate, methylparaben, propylparaben, benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben, alcohols such as ethyl or benzyl alcohol, phenolic compounds such as phenol, or quarternary compounds such as benzalkonium chloride.

Suitable diluents include pharmaceutically acceptable inert fillers, such as microcrystalline cellulose, lactose, dibasic calcium phosphate, saccharides, and/or

mixtures of any of the foregoing. Examples of diluents include microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102; lactose such as lactose monohydrate, lactose anhydrous, and Pharmatose® DCL21; dibasic calcium phosphate such as Emcompress®; mannitol; starch; sorbitol; sucrose; and glucose.

Suitable disintegrants include lightly crosslinked polyvinyl pyrrolidone, corn starch, potato starch, maize starch, and modified starches, croscarmellose sodium, cross-povidone, sodium starch glycolate, and mixtures thereof.

Examples of effervescent agents are effervescent couples such as an organic acid and a carbonate or bicarbonate. Suitable organic acids include, for example, citric, tartaric, malic, fumaric, adipic, succinic, and alginic acids and anhydrides and acid salts. Suitable carbonates and bicarbonates include, for example, sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, magnesium carbonate, sodium glycine carbonate, L-lysine carbonate, and arginine carbonate. Alternatively, only the acid component of the effervescent couple may be present.

7. Quantities of Nanoparticulate Active Agent, Surface Stabilizer, and Microparticulate Active Agent

The relative amount of at least one nanoparticulate active agent, one or more surface stabilizers, and at least one microparticulate active agent can vary widely. The optimal amount of the surface stabilizers can depend, for example, upon the particular active agent selected, the hydrophilic lipophilic balance (HLB), melting point, and water solubility of the surface stabilizer, and the surface tension of water solutions of the stabilizer, *etc.*

The concentration of at least one nanoparticulate active agent can vary from about 99.5% to about 0.001%, from about 95% to about 0.1%, or from about 90% to about 0.5%, by weight, based on the total combined weight of the at least one active agent and at least one surface stabilizer, not including other excipients.

The concentration of at least one surface stabilizer can vary from about 0.5% to about 99.999%, from about 5% to about 99.9%, and from about 10% to about 99.5%, by weight, based on the total combined dry weight of at least one active agent and at least one surface stabilizer, not including other excipients.

The concentration of the microparticulate active agent can vary from about 5% to about 85%, by weight, based on the total combined weight of the nanoparticulate

active agent, surface stabilizer, and microparticulate active agent, not including other excipients.

B. Methods of Making Compositions of the Invention

In another aspect of the invention there is provided a method of preparing formulations having combined CR and IR characteristics. The method comprises: (1) forming a nanoparticulate composition comprising at least one nanoparticulate active agent to be administered and at least one surface stabilizer; (2) adding at least one microparticulate active agent, which is the same or different from the nanoparticulate active agent of (1), and (3) forming a suitable dosage form of the composition for administration. Pharmaceutically acceptable excipients can also be added to the composition for administration.

1. Methods of Making Nanoparticulate Compositions

Methods of making nanoparticulate compositions, which can comprise mechanical grinding, precipitation, homogenization, or any other suitable size reduction process, are known in the art and are described in, for example, the '684 patent.

Methods of making nanoparticulate compositions are also described in U.S. Patent Nos. 5,518,187 and 5,862,999, both for "Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,718,388, for "Continuous Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,665,331, for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Patent No. 5,662,883, for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Patent No. 5,560,932, for "Microprecipitation of Nanoparticulate Pharmaceutical Agents;" U.S. Patent No. 5,543,133, for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" U.S. Patent No. 5,534,270, for "Method of Preparing Stable Drug Nanoparticles;" U.S. Patent No. 5,510,118, for "Process of Preparing Therapeutic Compositions Containing Nanoparticles;" and U.S. Patent No. 5,470,583, for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation," all of which are specifically incorporated by reference.

a. Milling to obtain Nanoparticulate Active Agent Dispersions

Milling of aqueous active agent dispersions to obtain a nanoparticulate dispersion comprises dispersing at least one active agent in a liquid dispersion medium in which the active agent is poorly soluble. By "poorly soluble" it is meant that the active agent has a solubility in a liquid dispersion medium of less than about 30 mg/ml, preferably less than about 10 mg/ml, and more preferably less than about 1 mg/ml. Such a liquid dispersion medium can be, for example, water, aqueous salt solutions, oils such as safflower oil, and solvents such as ethanol, t-butanol, hexane, and glycol.

This is followed by applying mechanical means in the presence of grinding media to reduce the particle size of the active agent to the desired effective average particle size. The active agent particles can be reduced in size in the presence of at least one surface stabilizer. Alternatively, the active agent particles may be contacted with one or more surface stabilizers after attrition. Other compounds, such as a diluent, can be added to the active agent/surface stabilizer composition during the size reduction process. Dispersions can be manufactured continuously or in a batch mode. The resultant nanoparticulate active agent dispersion can be used directly in formulating a dosage form, or the dispersion can be formulated into a powder followed by dosage formulation.

b. Precipitation to Obtain Nanoparticulate Active Agent Compositions

Another method of forming the desired nanoparticulate composition is by microprecipitation. This is a method of preparing stable dispersions of poorly soluble active agents in the presence of one or more surface stabilizers and one or more colloid stability enhancing surface active agents free of any trace toxic solvents or solubilized heavy metal impurities. Such a method comprises, for example: (1) dissolving the poorly water-soluble active agent in a suitable solvent; (2) adding the formulation from step (1) to a solution comprising at least one surface stabilizer to form a solution; and (3) precipitating the formulation from step (2) using an appropriate non-solvent. The method can be followed by removal of any formed salt, if present, by dialysis or diafiltration and concentration of the dispersion by conventional means. The resultant nanoparticulate active agent dispersion can be used directly in formulating a dosage

form, or the dispersion can be formulated into a powder followed by dosage formulation.

c. Homogenization to Obtain Nanoparticulate Active Agent Compositions

Exemplary homogenization methods of preparing active agent nanoparticulate compositions are described in U.S. Patent No. 5,510,118, for "Process of Preparing Therapeutic Compositions Containing Nanoparticles."

Such a method comprises dispersing active agent particles in a liquid dispersion medium, followed by subjecting the dispersion to homogenization to reduce the particle size of the active agent particles to the desired effective average particle size. The active agent particles can be reduced in size in the presence of at least one surface stabilizer. Alternatively, the active agent particles can be contacted with one or more surface stabilizers either before or after particle size reduction. It is preferred, however, to disperse the active agent particles in the liquid dispersion medium in the presence of the at least one surface stabilizer as an aid to wetting of the active agent particles. Other compounds, such as a diluent, can be added to the active agent/surface stabilizer composition either before, during, or after the size reduction process. Dispersions can be manufactured continuously or in a batch mode. The resultant nanoparticulate active agent dispersion can be used directly in formulating a dosage form, or the dispersion can be formulated into a powder followed by dosage formulation.

2. Dosage Formulation

Methods of making oral, injectable, transdermal, aerosol, buccal, topical, ocular, aural, *etc.* pharmaceutical formulations are known in the art, and such methods can be employed in the present invention.

In one embodiment of the invention, nanoparticulate active agent particles having at least one surface stabilizer adsorbed on to the surface of the particles can be incorporated into a dry powder matrix (*e.g.*, through spray drying, spray granulation, or a related pharmaceutically acceptable drying process), and combined with bulk micronized active agent particles by dry blending or a similar mixing process.

In a second embodiment of the invention, nanoparticulate active agent particles can be incorporated into a dry powder matrix (*e.g.*, through spray drying, spray

granulation, or a related pharmaceutically acceptable drying process). Separately, micronized active agent particles can be incorporated into a dry powder matrix using a similar approach, and the resulting matrices can then be combined by dry blending or a similar mixing process.

In a third embodiment of the invention, micronized active agent particles can be prepared by dry milling (*e.g.*, jet milling or pin milling).

In a fourth embodiment of the invention, micronized active agent particles can be prepared by wet milling, similar to the approach employed in the preparation of nanoparticulate active agent particles described in the '684 patent.

In an fifth embodiment of the invention, micronized active agent particles can be employed as the substrate (or a portion thereof) in a spray granulation, rotogranulation, spray coating, or related pharmaceutical process, upon which nanoparticulate active agent particles can be dispersed or deposited to form an outer layer. This particular approach facilitates the release of nanoparticulate active agent particles from the outer layer of the matrix upon exposure to biological fluids, followed by exposure and subsequent dissolution of micronized active agent particles.

In a sixth embodiment of the invention, micronized active agent particles can be employed as the substrate (or a portion thereof) in a high-shear granulation or related pharmaceutical wet-mixing process, upon which nanoparticulate active agent particles can be applied in the form of a granulating fluid. Upon drying, this particular approach enable nanoparticulate active agent particles and micronized active agent particles to be homogeneously distributed in the resulting solid matrix.

In a seventh embodiment of the invention, nanoparticulate active agent particles and micronized active agent particles can exist in the form of a dry powder or powder blend suitable for incorporation into a solid, rapidly disintegrating or "waterless tablet" matrix, the latter being attained upon compression of the dry powder or powder blend using a tablet press or similar pharmaceutically acceptable compression machine.

Exemplary spray drying, lyophilization, granulation, and tableting methods are described below.

a. <u>Spray Drying of Nanoparticulate Dispersions</u>

Dosage forms of nanoparticulate dispersions can be prepared by drying the nanoparticulate formulation following size reduction. A preferred drying method is

spray drying. The spray drying process is used to obtain a nanoparticulate powder following the size reduction process used to transform the active agent into nanoparticulate sized particles.

In an exemplary spray drying process, the nanoparticulate active agent suspension is fed to an atomizer using a peristaltic pump and atomized into a fine spray of droplets. The spray is contacted with hot air in the drying chamber resulting in the evaporation of moisture from the droplets. The resulting spray is passed into a cyclone where the powder is separated and collected. The nanoparticulate dispersion can be spray-dried in the presence or absence of excipients to give the spray-dried intermediate powder.

The powder can be formulated, for example, into a tablet, suppository, or other solid dosage form, or the powder can be formulated into an aerosol for nasal or pulmonary administration. The powder can also be reconstituted into a liquid, and used, for example, for injectable, ocular, ear, or oral dosage forms.

b. Lyophilization

Solid dose forms of nanoparticulate dispersions can also be prepared by lyophilizing the nanoparticulate formulation following size reduction. Suitable lyophilization conditions include, for example, those described in EP 0,363,365 (McNeil-PPC Inc.), U.S. Patent No. 4,178,695 (A. Erbeia), and U.S. Patent No. 5,384,124 (Farmalyoc), all of which are incorporated herein by reference. Typically, the nanoparticulate dispersion is placed in a suitable vessel and frozen to a temperature of between about -5°C to about -100°C. The frozen dispersion is then subjected to reduced pressure for a period of up to about 48 hours. The combination of parameters such as temperature, pressure, dispersion medium, and batch size will impact the time required for the lyophilization process. Under conditions of reduced temperature and pressure, the frozen solvent is removed by sublimation yielding a solid, porous, IR solid dosage form having the active ingredient distributed throughout.

The powder can be formulated, for example, into a tablet, suppository, or other solid dosage form, or the powder can be formulated into an aerosol for nasal or pulmonary administration. The powder can also be reconstituted into a liquid, and used, for example, for injectable, ocular, ear, or oral dosage forms.

c. Granulation

Alternatively, a solid oral dosage form of the invention can be prepared by granulating in a fluidized bed an admixture comprising a nanoparticulate dispersion of active agent and at least one surface stabilizer with a solution of at least one pharmaceutically acceptable water-soluble or water-dispersible excipient, to form a granulate. This is followed by tableting of the granulate to form a solid dosage form.

d. Tableting

The dosage formulations of the invention can be in the form of tablets. Preparation of such tablets can be by pharmaceutical compression or molding techniques known in the art. The tablets of the invention may take any appropriate shape, such as discoid, round, oval, oblong, cylindrical, triangular, hexagonal, and the like.

Powders for tableting can be formulated into tablets by any method known in the art. Suitable methods include, but are not limited to, milling, fluid bed granulation, dry granulation, direct compression, spheronization, spray congealing, and spray-dying. Detailed descriptions of tableting methods are provided in *Remington: The Science and Practice of Pharmacy*, 19th ed. Vol. 11 (1995) (Mack Publishing Co., Pennsylvania); and *Remington's Pharmaceutical Sciences*, Chapter 89, pp. 1633-1658 (Mach Publishing Company, 1990), both of which are specifically incorporated by reference.

The tablets may be coated or uncoated. If coated they may be sugar-coated (to cover objectionable tastes or odors and to protect against oxidation) or film coated (a thin film of water soluble matter for similar purposes).

C. Administration of the Compositions of the Invention

The present invention provides a method of treating a mammal, including a human, requiring the rapid availability of at least one poorly soluble active ingredient in combination with controlled release of the same or a different poorly soluble active ingredient. The administered compositions of the invention rapidly release an incorporated active agent resulting in fast onset of activity, and simultaneously slowly release the same or a different active agent for a prolonged pharmacological effect.

In general, the compositions of the invention will be administered to a mammalian subject in need thereof using a level of drug or active agent that is sufficient

to provide the desired physiological effect. The mammalian subject may be a domestic animal or pet but preferably is a human subject. The level of drug or active agent needed to give the desired physiological result is readily determined by one of ordinary skill in the art by referring to standard texts, such as *Goodman and Gillman* and the *Physician's Desk Reference*.

* * * * *

The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available documents are specifically incorporated into this patent application by reference.

Example 1

The purpose of this example was to simulate, using a mathematical model, pharmacokinetic profiles following single oral doses of a pharmaceutical formulation containing an active agent having a single defined particle size.

The software used for the simulation was MicroMath PKAnalyst for Windows, version 1.1, MicroMath, Inc. The following assumptions were made: the active agent conforms to a 2-compartment (central and peripheral compartments) pharmacokinetic model with 1st-order absorption and 1st-order elimination from the central compartment.

D/V (dose/volume) = 5000;

$K_a > 1.000\ h^{-1}$ (rate constant corresponding to the rate of dissolution);

$K_e = 0.50\ h^{-1}$ (elimination rate constant);

$K_{12} = 0.25\ h^{-1}$ (constant representing the rate of diffusion from the central compartment to the peripheral compartment); and

$K_{21} = 0.125\ h^{-1}$ (constant representing the rate of diffusion from the peripheral compartment to the central compartment).

Four samples were designed, (S) small particles, (MS) medium small particles, (ML) medium large particles, and (L) particles, having the following dissolution rate constants:

dissolution rate constant for small (S) particles	$= 1.000\ h^{-1}$
dissolution rate constant for medium small (MS) particles	$= 0.500\ h^{-1}$
dissolution rate constant for medium large (ML) particles	$= 0.250\ h^{-1}$

dissolution rate constant for large (L) particles $= 0.125\ h^{-1}$

The results of the simulation are shown in Figure 1.

The small particle population (S) showed rapid onset of activity, peaking at a plasma concentration level of about 2100 mg/mL at a little over 1 hour following administration, with plasma levels of over 250 mg/mL several minutes following administration. However, this sample also exhibited the lowest plasma levels at 12 hours after administration.

In contrast, the large particle population (L) showed slow onset of activity, peaking at a plasma concentration level of a little over 500 mg/mL at about 2 hours after administration. However, this sample also exhibited the highest plasma levels at 12 hours after administration.

The results of the simulation demonstrate that small particles dissolve faster than larger particles, but that they also decay more rapidly. As a consequence, larger drug particles provide the longest blood plasma levels, although these same particles exhibit slow dissolution.

Example 2

The purpose of this example was to simulate, using a mathematical model, pharmacokinetic profiles following single oral doses of a pharmaceutical formulation containing mixtures of different sizes of particles.

The assumptions described in Example 1 are applicable to Example 2.

Simulated pharmacokinetic profiles following single oral doses of a pharmaceutical formulation containing 50:50 mixtures of small (S) particles plus medium-small (MS), medium-large (ML), or large (L) particles of an active pharmaceutical ingredient are shown in Figure 2.

The results show, in particular, that the mixture of (S) small and (L) large particles exhibits a significantly greater maximum mean plasma concentration (almost 1000 mg/mL) as compared to the (L) large particles administered in Example 1, and the formulation also exhibits prolonged blood plasma levels, in contrast to the to the (S) small particles administered in Example 1.

Example 3

The purpose of this example was to simulate, using a mathematical model, pharmacokinetic profiles following single oral doses of a pharmaceutical formulation containing mixtures of different sizes of particles.

The assumptions described in Example 1 are applicable to Example 3.

Simulated pharmacokinetic profiles following single oral doses of a pharmaceutical formulation containing 25:75 mixtures of small (S) plus medium-small (MS), medium-large (ML), or large (L) particles of an active pharmaceutical ingredient are shown in Figure 3.

The results show, in particular, that the mixture of (S) small and (L) large particles exhibits a significantly greater maximum mean plasma concentration (almost 900 mg/mL) as compared to the (L) large particles administered in Example 1, and the formulation also exhibits prolonged blood plasma levels, in contrast to the to the (S) small particles administered in Example 1.

* * * *

It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

WE CLAIM:

1. A composition comprising:

(a) particles of at least one poorly soluble active agent having an effective average particle size of less than about 1 micron;

(b) at least one surface stabilizer adsorbed onto the surface of the nanoparticulate active agent particles; and

(c) at least one micronized active agent, which is either the same as or different from the active agent of (a), and having an effective average particle size of greater than about 1 micron and less than about 100 microns.

2. The composition of claim 1, wherein the effective average particle size of the nanoparticulate active agent particles is selected from the group consisting of less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, and less than about 50 nm.

3. The composition of claim 1 or 2, wherein the effective average particle size of the microparticulate active agent particles is greater than about 1 micron and less than the size selected from the group consisting of less than about 90 microns, less than about 80 microns, less than about 70 microns, less than about 60 microns, less than about 50 microns, less than about 40 microns, less than about 30 microns, less than about 20 microns, less than about 10 microns, less than about 9 microns, less than about 8 microns, less than about 7 microns, less than about 6 microns, less than about 5 microns, less than about 4 microns, less than about 3 microns, and less than about 2 microns.

4. The composition of any of claims 1-3 comprising more than one population of nanoparticulate active agent particles, wherein each population of particles has an effective average particle size which is less than about 1 micron.

5. The composition of any of claims 1-4 comprising more than one population of microparticulate active agent particles, wherein each population of particles has an effective average particle size which is greater than about 1 micron and less than about 100 microns.

6. The composition of any of claims 1-5, wherein the concentration of the nanoparticulate active agent is from about 99.5% to about 0.001%, based upon the total weight of the nanoparticulate active agent and the surface stabilizer.

7. The composition of any of claims 1-6, wherein the concentration of the nanoparticulate active agent is from about 95% to about 0.1% (w/w), based upon the total weight of the nanoparticulate active agent and the surface stabilizer.

8. The composition of any of claims 1-7, wherein the concentration of the nanoparticulate active agent is from about 90% to about 0.5% (w/w), based upon the total weight of the nanoparticulate active agent and the surface stabilizer.

9. The composition of any of claims 1-8, wherein the concentration of the surface stabilizer is from about 0.5% to about 99.999% (w/w), based upon the total weight of the nanoparticulate active agent and the surface stabilizer.

10. The composition of any of claims 1-9, wherein the concentration of the surface stabilizer is from about 5% to about 99.9% (w/w), based upon the total weight of the nanoparticulate active agent and the surface stabilizer.

11. The composition of any of claims 1-10, wherein the concentration of the surface stabilizer is from about 10% to about 99.5% (w/w), based upon the total weight of the nanoparticulate active agent and the surface stabilizer.

12. The composition of any of claims 1-11, wherein the concentration of the microparticulate agent is from about 5% to about 85%, based upon the total weight of the microparticulate active agent, nanoparticulate active agent, and surface stabilizer.

13. The composition of any of claims 1-12 formulated into a solid, rapidly disintegrating, "waterless tablet" matrix.

14. The composition of any of claims 1-12 formulated into a pharmaceutically acceptable tablet.

15. The composition of any of claims 1-12 formulated into a pharmaceutically acceptable hard gelatin capsule.

16. The composition of any of claims 1-12 formulated into a pharmaceutically acceptable soft gelatin capsule.

17. The composition of claim 16, wherein the composition comprises solubilized active agent in place of the nanoparticulate active agent particles.

18. The composition of any of claims 1-12 formulated into a pharmaceutically acceptable lozenge or troche.

19. The composition of any of claims 1-12 formulated into a pharmaceutically acceptable sachet, powder, or "sprinkle".

20. The composition of any of claims 1-19 formulated into a dosage form for oral, rectal, intravaginal, injectable, pulmonary, nasal, buccal, topical, local, intracisternal, intraperitoneal, ocular, aural, buccal spray, or nasal spray administration.

21. The composition of any of claims 1-20, wherein the formulation is made utilizing at least one method selected from the group consisting of spray drying, spray granulation, fluid bed granulation, high shear granulation, fluid bed drying, lyophilization, tableting, jet milling, pin milling, wet milling, rotogranulation, and spray coating.

22. The composition of any of claims 1-21, wherein the nanoparticulate and microparticulate active agents are selected from the group consisting of proteins,

peptides, nucleotides, anti-obesity drugs, nutraceuticals, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones, anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, and xanthines.

23. The composition of any of claims 1-22, wherein the at least one surface stabilizer is selected from the group consisting of a nonionic surface stabilizer, an anionic surface stabilizer, a cationic surface stabilizer, and an ionic surface stabilizer.

24. The composition of any of claims 1-23, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, stearic acid esters and salts, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hydroxypropyl methylcellulose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylmethyl-cellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers, poloxamines, a charged phospholipid, dimyristoyl phophatidyl glycerol, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate,

alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, triblock copolymers of the structure: -(-PEO)--(-PBO-)--(-PEO-)-, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside, n-decyl β-D-maltopyranoside, n-dodecyl β-D-glucopyranoside, n-dodecyl β-D-maltoside, heptanoyl-N-methylglucamide, n-heptyl-β-D-glucopyranoside, n-heptyl β-D-thioglucoside, n-hexyl β-D-glucopyranoside, nonanoyl-N-methylglucamide, n-noyl β-D-glucopyranoside, octanoyl-N-methylglucamide, n-octyl-β-D-glucopyranoside, octyl β-D-thioglucopyranoside, lysozyme, a PEG derivatized phospholipid, PEG derivatized cholesterol, a PEG derivatized cholesterol derivative, PEG derivatized vitamin A, PEG derivatized vitamin E, and random copolymers of vinyl acetate and vinyl pyrrolidone.

25. The composition of any of claims 1-23, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, and a phospholipid.

26. The composition of any of claims 1-23, wherein the at least one surface stabilizer is selected from the group consisting of cationic lipids, benzalkonium chloride, sulfonium compounds, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14})

dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride; N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™, ALKAQUAT™, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, cationic guar, polymethylmethacrylate trimethylammonium bromide, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, poly (2-methacryloxyethyltrimethylammonium bromide) (S1001), poly(N-vinylpyrrolidone/2-dimethylaminoethyl methacrylate) di methylsulphate quarternary (S1002), and poly(2-methylacryloxyamidopropyltrimethylammonium chloride) (S1004).

27. The composition of any of claims 1-26, comprising two or more surface stabilizers.

28. The composition of any of claims 1-27, comprising sodium lauryl sulfate, dioctyl sodium sulfosuccinate, or a combination thereof, as surface stabilizers.

29. A method of preparing a formulation comprising:

(a) combining (i) particles of at least one nanoparticulate poorly soluble active agent and at least one surface stabilizer adsorbed to the surface thereof, wherein the nanoparticulate active agent has an effective average particle size of less than about 1000 nm, and (ii) at least one microparticulate active agent having an effective average particle size of greater than about 1 micron and less than about 10 microns; and

(b) forming a suitable dosage formulation.

30. The method of claims 29, wherein the microparticulate active agent particles are prepared by dry milling.

31. The method of claim 29 or 30, wherein the microparticulate, nanoparticulate, or both active agent particles are prepared by wet milling.

32. The method of any of claims 29-31, wherein the nanoparticulate active agent particles having at least one surface stabilizer adsorbed onto the surface of the particles are incorporated into a dry powder matrix by spray drying, spray granulation, lyophilization, or a related pharmaceutically acceptable drying process, and then combined with bulk micronized active agent particles by dry blending or a similar mixing process.

33. The method of any of claim 29-32, wherein:

(a) the nanoparticulate active agent particles having at least one surface stabilizer adsorbed on to the surface of the particles are incorporated into a dry powder matrix by spray drying, spray granulation, lyophilization, or a related pharmaceutically acceptable drying process;

(b) the micronized active agent particles are incorporated into a dry powder matrix by spray drying, spray granulation, lyophilization, or a related pharmaceutically acceptable drying process; and

(c) the resulting matrices from (a) and (b) are combined by dry blending or a similar mixing process.

34. The method of any of claims 29-31, wherein the microparticulate active agent particles are coated with the nanoparticulate active agent/surface stabilizer particles.

35. The method of claim 34, wherein the coating is accomplished by a method selected from the group consisting of spray granulation, rotogranulation, spray coating, or a related pharmaceutical process.

36. The method of any of claims 29-31, wherein the microparticulate active agent particles are employed as a substrate in a high-shear granulation or related pharmaceutical wet-mixing process, upon which nanoparticulate active agent/surface stabilizer particles are applied in the form of a granulating fluid, wherein upon drying the nanoparticulate active agent particles and microparticulate active agent particles are homogeneously distributed in the resulting solid matrix.

37. The method of any of claims 29-36, wherein the nanoparticulate active agent/surface stabilizer particles and microparticulate active agent particles are formulated into a dry powder or powder blend for incorporation into a solid, rapidly disintegrating matrix, followed by compressing the dry powder or powder blend using a tablet press or similar pharmaceutically acceptable compression machine to form tablets.

38. Use of the composition of claim 1 for making a pharmaceutical which is useful in treating a mammal.

FIGURE 1

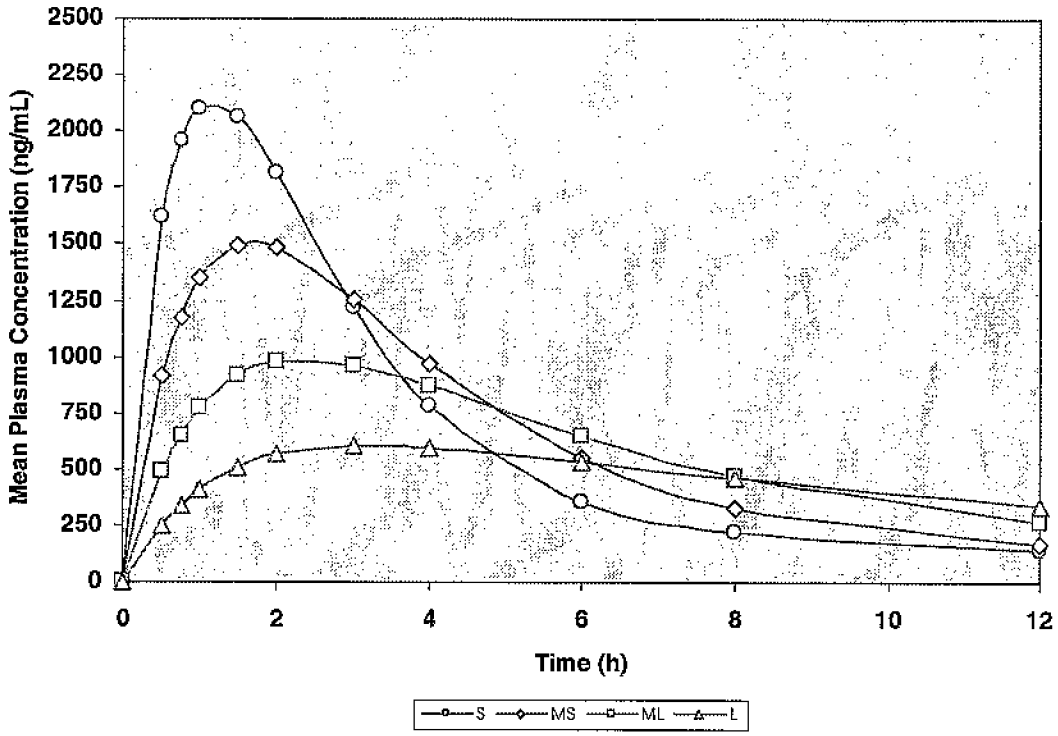


FIGURE 2

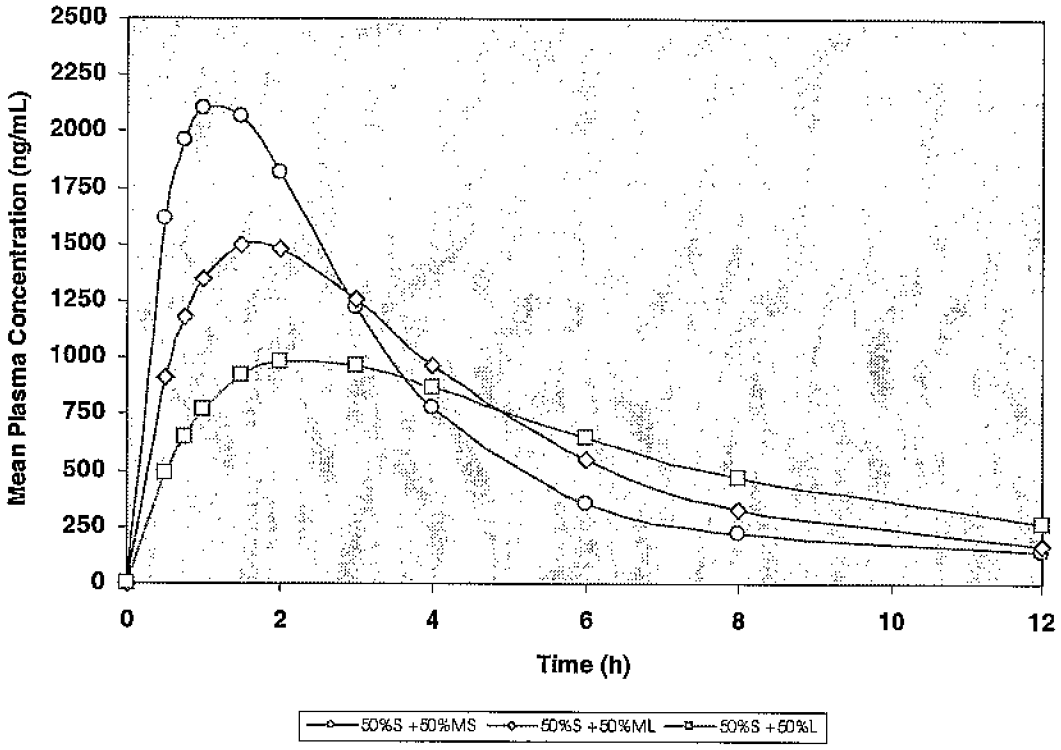
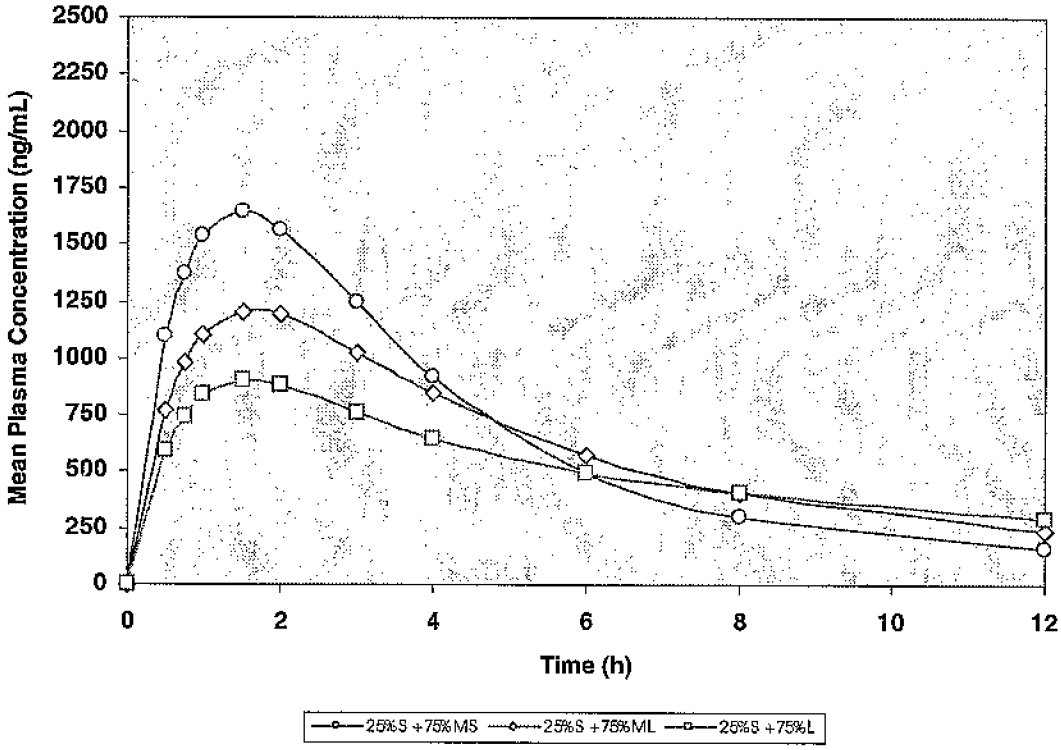


FIGURE 3



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(54) Title: NANOPARTICULATE COMPOSITIONS HAVING A PEPTIDE AS A SURFACE STABILIZER

A B

(57) Abstract: The present invention is directed to nanoparticulate active agent compositions comprising at least one peptide as a surface stabilizer. Also encompassed by the invention are pharmaceutical compositions comprising a nanoparticulate active agent composition of the invention and methods of making and using such nanoparticulate and pharmaceutical compositions.

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NANOPARTICULATE COMPOSITIONS HAVING A PEPTIDE AS A SURFACE STABILIZER

FIELD OF THE INVENTION

The present invention is directed to nanoparticulate active agent compositions having a peptide adsorbed onto or associated with the surface of the active agent as a surface stabilizer, and methods of making and using such compositions.

BACKGROUND OF THE INVENTION

Nanoparticulate active agent compositions, first described in U.S. Patent No. 5,145,684 ("the '684 patent"), are particles consisting of a poorly soluble therapeutic or diagnostic agent having adsorbed onto, or associated with, the surface thereof a non-crosslinked surface stabilizer. The '684 patent describes the use of a variety of surface stabilizers for nanoparticulate compositions. The use of a peptide as a surface stabilizer for nanoparticulate active agent compositions is not described by the '684 patent.

The '684 patent describes a method of screening active agents to identify useful surface stabilizers that enable the production of a nanoparticulate composition. Not all surface stabilizers will function to produce a stable, non-agglomerated nanoparticulate composition for all active agents. Moreover, known surface stabilizers may be unable to produce a stable, non-agglomerated nanoparticulate composition for certain active agents. Thus, there is a need in the art to identify new surface stabilizers useful in making nanoparticulate active agent compositions. Additionally, such new surface stabilizers may have superior properties over prior known surface stabilizers.

Methods of making nanoparticulate active agent compositions are described, for example, in U.S. Patent Nos. 5,518,187 and 5,862,999, both for "Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,718,388, for "Continuous Method of Grinding Pharmaceutical Substances;" and U.S. Patent No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles."

Nanoparticulate active agent compositions are also described, for example, in U.S. Patent Nos. 5,298,262 for "Use of Ionic Cloud Point Modifiers to Prevent Particle Aggregation During Sterilization;" 5,302,401 for "Method to Reduce Particle Size Growth During Lyophilization;" 5,318,767 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,326,552 for "Novel Formulation For Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,328,404 for "Method of X-Ray Imaging Using Iodinated Aromatic Propanedioates;" 5,336,507 for "Use of Charged Phospholipids to Reduce Nanoparticle Aggregation;" 5,340,564 for "Formulations Comprising Olin 10-G to Prevent Particle Aggregation and Increase Stability;" 5,346,702 for "Use of Non-Ionic Cloud Point Modifiers to Minimize Nanoparticulate Aggregation During Sterilization;" 5,349,957 for "Preparation and Magnetic Properties of Very Small Magnetic-Dextran Particles;" 5,352,459 for "Use of Purified Surface Modifiers to Prevent Particle Aggregation During Sterilization;" 5,399,363 and 5,494,683, both for "Surface Modified Anticancer Nanoparticles;" 5,401,492 for "Water Insoluble Non-Magnetic Manganese Particles as Magnetic Resonance Enhancement Agents;" 5,429,824 for "Use of Tyloxapol as a Nanoparticulate Stabilizer;" 5,447,710 for "Method for Making Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,451,393 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,466,440 for "Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents in Combination with Pharmaceutically Acceptable Clays;" 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation;" 5,472,683 for "Nanoparticulate Diagnostic Mixed Carbamic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,500,204 for "Nanoparticulate Diagnostic Dimers as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,518,738 for "Nanoparticulate NSAID Formulations;" 5,521,218 for "Nanoparticulate Iododipamide Derivatives for Use as X-Ray Contrast Agents;" 5,525,328 for "Nanoparticulate Diagnostic Diatrizoxy Ester X-Ray Contrast Agents for Blood Pool

and Lymphatic System Imaging;" 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" 5,552,160 for "Surface Modified NSAID Nanoparticles;" 5,560,931 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,565,188 for "Polyalkylene Block Copolymers as Surface Modifiers for Nanoparticles;" 5,569,448 for "Sulfated Non-ionic Block Copolymer Surfactant as Stabilizer Coatings for Nanoparticle Compositions;" 5,571,536 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,573,749 for "Nanoparticulate Diagnostic Mixed Carboxylic Anydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,573,750 for "Diagnostic Imaging X-Ray Contrast Agents;" 5,573,783 for "Redispersible Nanoparticulate Film Matrices With Protective Overcoats;" 5,580,579 for "Site-specific Adhesion Within the GI Tract Using Nanoparticles Stabilized by High Molecular Weight, Linear Poly(ethylene Oxide) Polymers;" 5,585,108 for "Formulations of Oral Gastrointestinal Therapeutic Agents in Combination with Pharmaceutically Acceptable Clays;" 5,587,143 for "Butylene Oxide-Ethylene Oxide Block Copolymers Surfactants as Stabilizer Coatings for Nanoparticulate Compositions;" 5,591,456 for "Milled Naproxen with Hydroxypropyl Cellulose as Dispersion Stabilizer;" 5,593,657 for "Novel Barium Salt Formulations Stabilized by Non-ionic and Anionic Stabilizers;" 5,622,938 for "Sugar Based Surfactant for Nanocrystals;" 5,628,981 for "Improved Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents and Oral Gastrointestinal Therapeutic Agents;" 5,643,552 for "Nanoparticulate Diagnostic Mixed Carbonic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" 5,718,919 for "Nanoparticles Containing the R(-)Enantiomer of Ibuprofen;" 5,747,001 for "Aerosols Containing Beclomethasone Nanoparticle Dispersions;" 5,834,025 for "Reduction of Intravenously Administered Nanoparticulate Formulation Induced Adverse Physiological Reactions;" 6,045,829 "Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,068,858 for "Methods of Making

Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,153,225 for "Injectable Formulations of Nanoparticulate Naproxen;" 6,165,506 for "New Solid Dose Form of Nanoparticulate Naproxen;" 6,221,400 for "Methods of Treating Mammals Using Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors;" 6,264,922 for "Nebulized Aerosols Containing Nanoparticle Dispersions;" 6,267,989 for "Methods for Preventing Crystal Growth and Particle Aggregation in Nanoparticle Compositions;" 6,270,806 for "Use of PEG-Derivatized Lipids as Surface Stabilizers for Nanoparticulate Compositions;" 6,316,029 for "Rapidly Disintegrating Solid Oral Dosage Form," 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate," 6,428,814 for "Bioadhesive nanoparticulate compositions having cationic surface stabilizers;" 6,431,478 for "Small Scale Mill;" 6,432,381 for "Methods for Targeting Drug Delivery to the Upper and/or Lower Gastrointestinal Tract," Patent No. 6,582,285 for "Apparatus for Sanitary Wet Milling;" 6,592,903 for "Nanoparticulate Dispersions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate," 6,742,734 for "System and Method for Milling Materials," and 6,745,962 for "Small Scale Mill and Method Thereof," all of which are specifically incorporated by reference. In addition, U.S. Patent Application No. 20020012675 A1, published on January 31, 2002, for "Controlled Release Nanoparticulate Compositions," and WO 02/098565 for "System and Method for Milling Materials," describe nanoparticulate active agent compositions, and are specifically incorporated by reference. None of these references describe nanoparticulate active agent compositions comprising a peptide surface stabilizer.

Amorphous small particle compositions are described, for example, in U.S. Patent Nos. 4,783,484 for "Particulate Composition and Use Thereof as Antimicrobial Agent;" 4,826,689 for "Method for Making Uniformly Sized Particles from Water-Insoluble Organic Compounds;" 4,997,454 for "Method for Making Uniformly-Sized Particles From Insoluble Compounds;" 5,741,522 for "Ultrasmall, Non-aggregated

Porous Particles of Uniform Size for Entrapping Gas Bubbles Within and Methods;" and 5,776,496, for "Ultrasmall Porous Particles for Enhancing Ultrasound Back Scatter."

There is a need in the art for new surface stabilizers useful in preparing nanoparticulate active agent compositions. The present invention satisfies this need.

SUMMARY OF THE INVENTION

The present invention is directed to nanoparticulate compositions comprising at least one active agent and at least one peptide as a surface stabilizer adsorbed on to, or associated with, the surface of the active agent.

Another aspect of the invention is directed to pharmaceutical compositions comprising a nanoparticulate active agent composition of the invention. The pharmaceutical compositions preferably comprise at least one active agent, at least one peptide, and a pharmaceutically acceptable carrier, as well as any desired excipients.

In yet another embodiment, the invention is directed to bioadhesive nanoparticulate active agent compositions comprising at least one cationic peptide as a surface stabilizer, or at least one non-cationic peptide surface stabilizer in combination with at least one secondary cationic surface stabilizer. Such compositions can coat the gut, or the desired site of application, and be retained for a period of time, thereby increasing the efficacy of the active agent as well as eliminating or decreasing the frequency of dosing.

This invention further discloses a method of making a nanoparticulate active agent composition having a peptide surface stabilizer adsorbed on or associated with the surface of the active agent. Such a method comprises contacting an active agent with at least one peptide for a time and under conditions sufficient to provide a Nanoparticle active agent/peptide composition. The peptide surface stabilizer can be contacted with the active agent either before, preferably during, or after size reduction of the active agent.

The present invention is further directed to a method of treatment comprising administering to a mammal a therapeutically effective amount of a nanoparticulate active agent/peptide composition according to the invention.

Both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1: Shows representative photomicrographs of nystatin crystals before (Fig. 1A) and after (Fig. 1B) milling;

FIGURE 2: Shows the results of monitoring the particle size stability over time at 5°C (solid line), 25°C (dashed line), and 40°C (dotted line) for a nanoparticulate nystatin composition comprising the peptide poly(Lysine, Tryptophan) 4:1 hydrobromide as a surface stabilizer; and

FIGURE 3: Shows representative micrographs of cells with anionic particles (Fig. 3A) and cationic particles (Fig. 3B).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to compositions comprising nanoparticulate active agents having at least one peptide as a surface stabilizer adsorbed on or associated with the surface thereof, and methods of making and using such nanoparticulate compositions.

As taught in the '684 patent, not every combination of surface stabilizer and active agent will result in a stable nanoparticulate composition. The discovery of the present invention is surprising in that peptides are biological compounds having secondary and tertiary structures which are critical to the activity of the peptide. It was surprising that such a compound could be successfully used to stabilize a

nanoparticulate active agent. Moreover, it was even more surprising that milling of a peptide surface stabilizer did not change the activity or function of the peptide.

A "peptide" is defined as any compound consisting of two or more amino acids where the alpha carboxyl group of one is bound to the alpha amino group of another. A polypeptide is a long peptide chain. A protein is a large macromolecule composed of one or more polypeptide chains. In the context of the present invention, "peptide" refers to a peptide or a polypeptide, but not a protein.

A striking characteristic of peptides is that they have well-defined three dimensional structures. Peptides fold into compact structures with nominal bond lengths. The strong tendency of hydrophobic amino acid residues to flee from water drives the folding of soluble peptides.

A stretched-out or randomly arranged polypeptide chain is devoid of biological activity. This is because the function of a peptide arises from conformation, which is the three dimensional arrangement of atoms in a structure. *See e.g.*, L. Stryer, *Biochemistry*, 3rd Edition, p. 1-41 (W.H. Freeman & Co., NY, 1988). Amino acid sequences are important because they specify the conformation of peptides. *Id.*

Peptides have several different defined structures, including a primary, secondary, and tertiary structure. The primary structure of a peptide is generally the amino acid sequence of the peptide and the location of disulfides. *See e.g.*, L. Stryer, *Biochemistry*, 3rd Edition, p. 31 (W.H. Freeman & Co., NY, 1988). Secondary structure refers to the spatial arrangement of amino acid residues that are near one another in the linear sequence. Examples of these steric relationships are structures known as an alpha helix, a beta pleated sheet, and a collagen helix. *Id.* Tertiary structure refers to the spatial arrangement of amino acid residues in a peptide or polypeptide that are far apart in the linear sequence.

Proteins, comprising multiple polypeptide chains, also have a quaternary structure, which refers to the spatial arrangement of the polypeptide subunits and the nature of their contacts. *Id.*

It was very surprising that such complex compounds as peptides and polypeptides could be successfully utilized as a surface stabilizer for a nanoparticulate active agent. In addition to enabling the use of a new class of surface stabilizers for nanoparticulate active agents, this discovery is significant as the peptide surface stabilizer in the compositions of the invention may also have therapeutic or diagnostic properties. This is in contrast to prior art nanoparticulate active agent compositions, in which the surface stabilizer is generally a surfactant, which lacks such therapeutic or diagnostic properties.

The nanoparticulate active agent compositions of the invention may also offer the following advantages as compared to prior conventional or non-nanoparticulate active agent compositions: (1) faster onset of action; (2) a potential decrease in the frequency of dosing; (3) smaller doses of active agent required to obtain the same pharmacological effect; (4) increased bioavailability; (5) an increased rate of dissolution; (6) improved performance characteristics for oral, intravenous, subcutaneous, or intramuscular injection, such as higher active agent dose loading and smaller tablet or liquid dose volumes; (7) improved pharmacokinetic profiles, such as improved T_{max}, C_{max}, and AUC profiles; (8) substantially similar or bioequivalent pharmacokinetic profiles of the nanoparticulate active agent compositions when administered in the fed versus the fasted state; (9) bioadhesive active agent compositions, which can coat the gut or the desired site of application and be retained for a period of time, thereby increasing the efficacy of the active agent as well as eliminating or decreasing the frequency of dosing; (10) high redispersibility of the nanoparticulate active agent particles present in the compositions of the invention following administration; (11) the nanoparticulate active agent compositions can be formulated in a dried form which readily redisperses; (12) low viscosity liquid nanoparticulate active agent dosage forms can be made; (13) for liquid nanoparticulate active agent compositions having a low viscosity - better subject compliance due to the perception of a lighter formulation which is easier to consume and digest; (14) for liquid nanoparticulate active agent compositions having a low viscosity - ease of dispensing because one can use a cup or a syringe; (15) the nanoparticulate active

agent compositions can be used in conjunction with other active agents; (16) the nanoparticulate active agent compositions can be sterile filtered; (17) the nanoparticulate active agent compositions are suitable for parenteral administration; and (18) the nanoparticulate active agent compositions do not require organic solvents or pH extremes.

A preferred dosage form of the invention is a solid dosage form, although any pharmaceutically acceptable dosage form can be utilized. Exemplary dosage forms include, but are not limited to, tablets, capsules, sachets, lozenges, powders, pills, granules, liquid dispersions, oral suspensions, gels, aerosols (including nasal and pulmonary), ointments, and creams.

The dosage form of the invention can be, for example, a fast melt dosage form, controlled release dosage form, lyophilized dosage form, delayed release dosage form, extended release dosage form, pulsatile release dosage form, mixed immediate release and controlled release dosage form, or a combination thereof.

In addition, the compositions of the invention can be formulated for any suitable administration route, such as oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, or topical administration.

The present invention is described herein using several definitions, as set forth below and throughout the application.

As used herein, "about" will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

"Conventional" or "non-nanoparticulate active agent" shall mean an active agent which is solubilized or which has an effective average particle size of greater than about 2 microns. Nanoparticulate active agents as defined herein have an effective average particle size of less than about 2 microns.

"Pharmaceutically acceptable" as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

"Pharmaceutically acceptable salts" as used herein refers to derivatives wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

"Poorly water soluble drugs" as used herein means those having a solubility of less than about 30 mg/ml, preferably less than about 20 mg/ml, preferably less than about 10 mg/ml, or preferably less than about 1 mg/ml. Such drugs tend to be eliminated from the gastrointestinal tract before being absorbed into the circulation.

As used herein with reference to stable drug particles, "stable" includes, but is not limited to, one or more of the following parameters: (1) that the active agent particles do not appreciably flocculate or agglomerate due to interparticle attractive forces, or otherwise significantly increase in particle size over time; (2) that the physical structure of the active agent particles is not altered over time, such as by conversion from an amorphous phase to crystalline phase; (3) that the active agent

particles are chemically stable; and/or (4) where the active agent has not been subject to a heating step at or above the melting point of the active agent in the preparation of the nanoparticles of the invention.

"Therapeutically effective amount" as used herein with respect to an active agent dosage, shall mean that dosage that provides the specific pharmacological response for which the active agent is administered in a significant number of subjects in need of such treatment. It is emphasized that "therapeutically effective amount," administered to a particular subject in a particular instance will not always be effective in treating the diseases described herein, even though such dosage is deemed a 'therapeutically effective amount' by those skilled in the art. It is to be further understood that active agent dosages are, in particular instances, measured as oral dosages, or with reference to active agent levels as measured in blood.

I. Preferred Characteristics of the Nanoparticulate Active Agent Compositions of the Invention

A. Increased Bioavailability, Frequency of Dosing, and Dosage Quantity

The nanoparticulate active agent compositions of the invention, having at least one peptide as a surface stabilizer, may preferably exhibit increased bioavailability and require smaller doses as compared to prior non-nanoparticulate compositions of the same active agent administered at the same dose.

Any active agent can have adverse side effects. Thus, lower doses of an active agent that can achieve the same or better therapeutic effects as those observed with larger doses of a non-nanoparticulate composition of the same active agent are desired. Such lower doses may be realized with the nanoparticulate active agent compositions of the invention because the nanoparticulate active agent compositions may exhibit greater bioavailability as compared to non-nanoparticulate compositions of the same active agent, which means that smaller doses of the active agent are likely required to obtain the desired therapeutic effect.

The nanoparticulate active agent compositions of the invention may be administered less frequently and at lower doses, as compared to conventional non-nanoparticulate compositions of the same active agent, in dosage forms such as liquid dispersions, powders, sprays, aerosols (pulmonary and nasal), solid re-dispersable dosage forms, gels, ointments, creams, *etc.* of the nanoparticulate active agent. Lower dosages can be used because the small particle size of the active agent particles ensure greater absorption, and in the case of bioadhesive nanoparticulate active agent compositions, the active agent is retained at the desired site of application for a longer period of time as compared to conventional, non-nanoparticulate active agent dosage forms.

In one embodiment of the invention, the therapeutically effective amount of the nanoparticulate active agent compositions is 1/6, 1/5, ¼, 1/3rd, or ½ of the therapeutically effective amount of a non-nanoparticulate composition of the same active agent.

Such lower doses are preferred as they may decrease or eliminate adverse effects of the active agent. In addition, such lower doses decrease the cost of the dosage form and may increase patient compliance.

B. Pharmacokinetic Profiles of the Nanoparticulate Active Agent Compositions of the Invention

The invention also preferably provides nanoparticulate active agent compositions, having at least one peptide as a surface stabilizer, and having a desirable pharmacokinetic profile when administered to mammalian subjects. The desirable pharmacokinetic profile of the active agent compositions preferably includes, but is not limited to: (1) a T_{max} for an active agent, when assayed in the plasma of a mammalian subject following administration, that is preferably less than the T_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; (2) a C_{max} for an active agent, when assayed in the plasma of a mammalian subject following administration, that is preferably greater than the C_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; and/or (3) an AUC for an active agent, when assayed in the plasma of a

mammalian subject following administration, that is preferably greater than the AUC for a non-nanoparticulate composition of the same active agent, administered at the same dosage.

The desirable pharmacokinetic profile, as used herein, is the pharmacokinetic profile measured after the initial dose of the active agent. The compositions can be formulated in any way as described herein and as known to those of skill in the art.

A preferred active agent composition of the invention, comprising at least one peptide as a surface stabilizer, exhibits in comparative pharmacokinetic testing with a non-nanoparticulate composition of the same active agent, administered at the same dosage, a T_{max} not greater than about 100%, not greater than about 90%, not greater than about 80%, not greater than about 70%, not greater than about 60%, not greater than about 50%, not greater than about 40%, not greater than about 30%, not greater than about 25%, not greater than about 20%, not greater than about 15%, not greater than about 10%, or not greater than about 5% of the T_{max} exhibited by the non-nanoparticulate active agent composition. This shorter T_{max} translates into a faster onset of therapeutic activity.

A preferred active agent composition of the invention, comprising at least one peptide as a surface stabilizer, exhibits in comparative pharmacokinetic testing with a non-nanoparticulate composition of the same active agent, administered at the same dosage, a C_{max} which is at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, or at least about 200% greater than the C_{max} exhibited by the non-nanoparticulate active agent composition.

A preferred active agent composition of the invention, comprising at least one peptide as a surface stabilizer, exhibits in comparative pharmacokinetic testing with a non-nanoparticulate composition of the same active agent, administered at the same

dosage, an AUC which is at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, or at least about 200% greater than the AUC exhibited by the non-nanoparticulate active agent formulation.

Any formulation giving the desired pharmacokinetic profile is suitable for administration according to the present methods.

C. The Pharmacokinetic Profiles of the Nanoparticulate Active Agent Compositions of the Invention are Preferably not Substantially Affected by the Fed or Fasted State of the Subject Ingesting the Compositions

The invention encompasses nanoparticulate active agent compositions, comprising at least one peptide as a surface stabilizer, wherein preferably the pharmacokinetic profile of the active agent is not substantially affected by the fed or fasted state of a subject ingesting the composition. This means that there is no substantial difference in the quantity of active agent absorbed or the rate of active agent absorption when the nanoparticulate active agent compositions are administered in the fed versus the fasted state. Thus, the nanoparticulate active agent compositions of the invention can preferably substantially eliminate the effect of food on the pharmacokinetics of the active agent.

In another embodiment of the invention, the pharmacokinetic profile of the active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, when administered to a mammal in a fasted state, is bioequivalent to the pharmacokinetic profile of the same nanoparticulate active agent composition administered at the same dosage, when administered to a mammal in a fed state.

"Bioequivalency" is preferably established by a 90% Confidence Interval (CI) of between 0.80 and 1.25 for both C_{max} and AUC under U.S. Food and Drug Administration (USFDA) regulatory guidelines, or a 90% CI for AUC of between

0.80 to 1.25 and a 90% CI for C_{max} of between 0.70 to 1.43 under the European Medicines Evaluation Agency (EMEA) regulatory guidelines (T_{max} is not relevant for bioequivalency determinations under USFDA and EMEA regulatory guidelines).

Preferably the difference in AUC (*e.g.*, absorption) of the nanoparticulate active agent composition of the invention, comprising at least one peptide as a surface stabilizer, when administered in the fed versus the fasted state, is less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 3%.

In addition, preferably the difference in C_{max} of the nanoparticulate active agent composition of the invention, comprising at least one peptide as a surface stabilizer, when administered in the fed versus the fasted state, is less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 3%.

Finally, preferably the difference in the T_{max} of the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, when administered in the fed versus the fasted state, is less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 3%, or essentially no difference.

Benefits of a dosage form that substantially eliminates the effect of food include an increase in subject convenience, thereby increasing subject compliance, as the subject does not need to ensure that they are taking a dose either with or without food.

D. Redispersibility Profiles of the Nanoparticulate Active Agent Compositions of the Invention

An additional feature of the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, comprising at least one peptide as a surface stabilizer, is that the compositions redisperse such that the effective average particle size of the redispersed active agent particles is less than about 2 microns. This is significant, as if upon administration the nanoparticulate active agent particles present in the compositions of the invention did not redisperse to a substantially nanoparticulate particle size, then the dosage form may lose the benefits afforded by formulating the active agent into a nanoparticulate particle size.

This is because the nanoparticulate active agent compositions of the invention benefit from the small particle size of the active agent; if the nanoparticulate active agent particles do not redisperse into the small particle sizes upon administration, then "clumps" or agglomerated active agent particles are formed. With the formation of such agglomerated particles, the bioavailability of the dosage form may fall.

Moreover, the nanoparticulate active agent compositions of the invention exhibit dramatic redispersion of the active agent particles upon administration to a mammal, such as a human or animal, as demonstrated by reconstitution in a biorelevant aqueous media. Such biorelevant aqueous media can be any aqueous media that exhibit the desired ionic strength and pH, which form the basis for the biorelevance of the media. The desired pH and ionic strength are those that are representative of physiological conditions found in the human body. Such biorelevant aqueous media can be, for example, aqueous electrolyte solutions or aqueous solutions of any salt, acid, or base, or a combination thereof, which exhibit the desired pH and ionic strength.

Biorelevant pH is well known in the art. For example, in the stomach, the pH ranges from slightly less than 2 (but typically greater than 1) up to 4 or 5. In the small intestine the pH can range from 4 to 6, and in the colon it can range from 6 to 8. Biorelevant ionic strength is also well known in the art. Fasted state gastric fluid has an ionic strength of about 0.1M while fasted state intestinal fluid has an ionic strength

of about 0.14. *See e.g.*, Lindahl et al., "Characterization of Fluids from the Stomach and Proximal Jejunum in Men and Women," *Pharm. Res., 14* (4): 497-502 (1997).

It is believed that the pH and ionic strength of the test solution is more critical than the specific chemical content. Accordingly, appropriate pH and ionic strength values can be obtained through numerous combinations of strong acids, strong bases, salts, single or multiple conjugate acid-base pairs (*i.e.*, weak acids and corresponding salts of that acid), monoprotic and polyprotic electrolytes, *etc.*

Representative electrolyte solutions can be, but are not limited to, HCl solutions, ranging in concentration from about 0.001 to about 0.1 M, and NaCl solutions, ranging in concentration from about 0.001 to about 0.1 M, and mixtures thereof. For example, electrolyte solutions can be, but are not limited to, about 0.1 M HCl or less, about 0.01 M HCl or less, about 0.001 M HCl or less, about 0.1 M NaCl or less, about 0.01 M NaCl or less, about 0.001 M NaCl or less, and mixtures thereof. Of these electrolyte solutions, 0.01 M HCl and/or 0.1 M NaCl, are most representative of fasted human physiological conditions, owing to the pH and ionic strength conditions of the proximal gastrointestinal tract.

Electrolyte concentrations of 0.001 M HCl, 0.01 M HCl, and 0.1 M HCl correspond to pH 3, pH 2, and pH 1, respectively. Thus, a 0.01 M HCl solution simulates typical acidic conditions found in the stomach. A solution of 0.1 M NaCl provides a reasonable approximation of the ionic strength conditions found throughout the body, including the gastrointestinal fluids, although concentrations higher than 0.1 M may be employed to simulate fed conditions within the human GI tract.

Exemplary solutions of salts, acids, bases or combinations thereof, which exhibit the desired pH and ionic strength, include but are not limited to phosphoric acid/phosphate salts + sodium, potassium and calcium salts of chloride, acetic acid/acetate salts + sodium, potassium and calcium salts of chloride, carbonic acid/bicarbonate salts + sodium, potassium and calcium salts of chloride, and citric acid/citrate salts + sodium, potassium and calcium salts of chloride.

In other embodiments of the invention, the redispersed active agent particles of the invention (redispersed in an aqueous, biorelevant, or any other suitable media) have an effective average particle size of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods.

Redispersibility can be tested using any suitable means known in the art. *See e.g.,* the example sections of U.S. Patent No. 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate."

E. Bioadhesive Nanoparticulate Active Agent Compositions

Bioadhesive nanoparticulate active agent compositions of the invention comprise at least one cationic peptide surface stabilizer, or in addition to at least one non-cationic peptide as a surface stabilizer, at least one secondary non-peptide cationic surface stabilizer. Exemplary non-peptide cationic surface stabilizers are described in more detail below. Bioadhesive formulations of active agents exhibit exceptional bioadhesion to biological surfaces, such as mucous and skin.

Cationic surface stabilizers generally confer relatively large, positive zeta potentials to particles on which they adsorb or associate. To increase the bioadhesive properties of a nanoparticulate composition, two or more cationic surface stabilizers can be utilized.

In the case of bioadhesive nanoparticulate active agent compositions, the term "bioadhesion" is used to describe the adhesion between the nanoparticulate active agent compositions and a biological substrate (*i.e.*, gastrointestinal mucin, lung tissue,

nasal mucosa, *etc.*). *See e.g.*, U.S. Patent No. 6,428,814 for "Bioadhesive Nanoparticulate Compositions Having Cationic Surface Stabilizers," which is specifically incorporated by reference.

There are basically two mechanisms which may be responsible for this bioadhesion phenomena: mechanical or physical interactions and chemical interactions. The first of these, mechanical or physical mechanisms, involves the physical interlocking or interpenetration between a bioadhesive entity and the receptor tissue, resulting from a good wetting of the bioadhesive surface, swelling of the bioadhesive polymer, penetration of the bioadhesive entity into a crevice of the tissue surface, or interpenetration of bioadhesive composition chains with those of the mucous or other such related tissues. The second possible mechanism of bioadhesion incorporates forces such as ionic attraction, dipolar forces, van der Waals interactions, and hydrogen bonds. It is this form of bioadhesion which is primarily responsible for the bioadhesive properties of the nanoparticulate active agent compositions of the invention. However, physical and mechanical interactions may also play a secondary role in the bioadhesion of such nanoparticulate active agent compositions.

The bioadhesive active agent compositions of the invention are useful in any situation in which it is desirable to apply the compositions to a biological surface. The bioadhesive active agent compositions preferably coat the targeted surface in a continuous and uniform film that is invisible to the naked human eye.

A bioadhesive nanoparticulate active agent composition slows the transit of the composition, and some active agent particles would also most likely adhere to tissue other than the mucous cells and therefore give a prolonged exposure to the active agent, thereby increasing absorption and the bioavailability of the administered dosage.

The adhesion exhibited by the inventive compositions means that nanoparticulate active agent particles are not easily washed off, rubbed off, or otherwise removed from the biological surface for an extended period of time. The period of time in which a biological cell surface is replaced is the factor that limits

retention of the bioadhesive nanoparticulate active agent particles to that biological surface.

F. Low Viscosity Active Agent Dosage Forms

A liquid dosage form of a conventional microcrystalline or non-nanoparticulate active agent composition would be expected to be a relatively large volume, highly viscous substance which would not be well accepted by patient populations. Moreover, viscous solutions can be problematic in parenteral administration because these solutions require a slow syringe push and can stick to tubing. In addition, conventional formulations of poorly water-soluble active agents tend to be unsafe for intravenous administration techniques, which are used primarily in conjunction with highly water-soluble substances.

Liquid dosage forms of the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, provide significant advantages over a liquid dosage form of a conventional microcrystalline or solubilized active agent composition. The low viscosity and silky texture of liquid dosage forms of the nanoparticulate active agent compositions of the invention result in advantages in both preparation and use. These advantages include, for example: (1) better subject compliance due to the perception of a lighter formulation which is easier to consume and digest; (2) ease of dispensing because one can use a cup or a syringe; (3) potential for formulating a higher concentration of active agent resulting in a smaller dosage volume and thus less volume for the subject to consume; and (4) easier overall formulation concerns.

Liquid active agent dosage forms that are easier to consume are especially important when considering juvenile patients, terminally ill patients, and elderly patients. Viscous or gritty formulations, and those that require a relatively large dosage volume, are not well tolerated by these patient populations. Liquid oral dosage forms can be particularly preferably for patient populations who have difficulty consuming tablets, such as infants and the elderly.

The viscosities of liquid dosage forms of a nanoparticulate active agent according to the invention are preferably less than about 1/200, less than about 1/175, less than about 1/150, less than about 1/125, less than about 1/100, less than about 1/75, less than about 1/50, or less than about 1/25 of a liquid oral dosage form of a non-nanoparticulate composition of the same active agent, at about the same concentration per ml of active agent.

Typically liquid nanoparticulate active agent dosage forms of the invention, comprising at least one peptide as a surface stabilizer, have a viscosity at a shear rate of 0.1 (1/s) measured at 20°C, is from about 2000 mPa s to about 1 mPa s, from about 1900 mPa·s to about 1 mPa·s, from about 1800 mPa·s to about 1 mPa·s, from about 1700 mPa·s to about 1 mPa·s, from about 1600 mPa·s to about 1 mPa·s, from about 1500 mPa·s to about 1 mPa·s, from about 1400 mPa·s to about 1 mPa·s, from about 1300 mPa·s to about 1 mPa·s, from about 1200 mPa·s to about 1 mPa·s, from about 1100 mPa·s to about 1 mPa·s, from about 1000 mPa·s to about 1 mPa·s, from about 900 mPa·s to about 1 mPa·s, from about 800 mPa·s to about 1 mPa·s, from about 700 mPa·s to about 1 mPa·s, from about 600 mPa·s to about 1 mPa·s, from about 500 mPa·s to about 1 mPa·s, from about 400 mPa·s to about 1 mPa·s, from about 300 mPa·s to about 1 mPa·s, from about 200 mPa·s to about 1 mPa·s, from about 175 mPa·s to about 1 mPa·s, from about 150 mPa·s to about 1 mPa·s, from about 125 mPa·s to about 1 mPa·s, from about 100 mPa·s to about 1 mPa·s, from about 75 mPa·s to about 1 mPa·s, from about 50 mPa·s to about 1 mPa·s, from about 25 mPa·s to about 1 mPa·s, from about 15 mPa·s to about 1 mPa·s, from about 10 mPa·s to about 1 mPa·s, or from about 5 mPa·s to about 1 mPa·s. Such a viscosity is much more attractive for subject consumption and may lead to better overall subject compliance.

Viscosity is concentration and temperature dependent. Typically, a higher concentration results in a higher viscosity, while a higher temperature results in a lower viscosity. Viscosity as defined above refers to measurements taken at about 20°C. (The viscosity of water at 20°C is 1 mPa s.) The invention encompasses equivalent viscosities measured at different temperatures.

Another important aspect of the invention is that the nanoparticulate active agent compositions of the invention, formulated into a liquid dosage form, are not turbid. "Turbid," as used herein refers to the property of particulate matter that can be seen with the naked eye or that which can be felt as "gritty." The nanoparticulate active agent compositions of the invention, formulated into a liquid dosage form, can be poured out of or extracted from a container as easily as water, whereas a liquid dosage form of a non-nanoparticulate or solubilized composition of the same active agent is expected to exhibit notably more "sluggish" characteristics.

The liquid formulations of this invention can be formulated for dosages in any volume but preferably equivalent or smaller volumes than a liquid dosage form of a non-nanoparticulate composition of the same active agent.

G. Sterile Filtered Nanoparticulate Active Agent Compositions

The nanoparticulate active agent compositions of the invention can be sterile filtered. This obviates the need for heat sterilization, which can harm or degrade an active agent, as well as result in crystal growth and particle aggregation of the active agent.

Sterile filtration can be difficult because of the required small particle size of the composition. Filtration is an effective method for sterilizing homogeneous solutions when the membrane filter pore size is less than or equal to about 0.2 microns (200 nm) because a 0.2 micron filter is sufficient to remove essentially all bacteria. Sterile filtration is normally not used to sterilize suspensions of micron-sized active agents because the active agent particles are too large to pass through the membrane pores.

A sterile nanoparticulate active agent dosage form is particularly useful in treating immunocompromised patients, infants or juvenile patients, and the elderly, as these patient groups are the most susceptible to infection caused by a non-sterile liquid dosage form.

Because the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer and formulated into a liquid dosage form, can be sterile filtered, and because the compositions can have a very small active agent effective average particle size, the compositions are suitable for parenteral administration.

H. Combination Pharmacokinetic Profile Compositions

In yet another embodiment of the invention, a first nanoparticulate active agent composition providing a desired pharmacokinetic profile is co-administered, sequentially administered, or combined with at least one other active agent composition that generates a desired different pharmacokinetic profile. More than two active agent compositions can be co-administered, sequentially administered, or combined. While the first active agent composition has a nanoparticulate particle size, the additional one or more active agent compositions can be nanoparticulate, solubilized, or have a microparticulate particle size.

The second, third, fourth, *etc.*, active agent compositions can differ from the first, and from each other, for example: (1) in the identity of the active agent; (2) in the effective average particle sizes of the active agent; or (3) in the dosage of the active agent. Such a combination composition can reduce the dose frequency required.

For example, a first active agent composition can have a nanoparticulate particle size, conferring a short T_{max} and typically a higher C_{max}. This first active agent composition can be combined, co-administered, or sequentially administered with a second composition comprising: (1) the same active agent having a larger (but still nanoparticulate as defined herein) particle size, and therefore exhibiting slower absorption, a longer T_{max}, and typically a lower C_{max}; or (2) a microparticulate or solubilized composition of the same active agent, exhibiting a longer T_{max}, and typically a lower C_{max}.

If the second active agent composition has a nanoparticulate particle size, then preferably the active agent particles of the second composition have at least one

surface stabilizer associated with the surface of the active agent particles. The one or more surface stabilizers can be the same as or different from the surface stabilizer(s) present in the first active agent composition.

Preferably where co-administration of a "fast-acting" formulation and a "longer-lasting" formulation is desired, the two formulations are combined within a single composition, for example a dual-release composition.

I. Miscellaneous Benefits of the Nanoparticulate Active Agent Compositions of the Invention

The nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, preferably exhibit an increased rate of dissolution as compared to microcrystalline or non-nanoparticulate forms of the same active agent. In addition, the nanoparticulate active agent compositions preferably exhibit improved performance characteristics for oral, intravenous, subcutaneous, or intramuscular injection, such as higher dose loading and smaller tablet or liquid dose volumes. Moreover, the nanoparticulate active agent compositions of the invention do not require organic solvents or pH extremes.

II. Compositions

The compositions of the invention comprise a nanoparticulate active agent and at least one peptide as a surface stabilizer adsorbed to or associated with the surface of the active agent. In addition, the compositions can comprise one or more secondary surface stabilizers. Surface stabilizers useful herein physically adhere to or associate with the surface of the nanoparticulate active agent but do not chemically react with the active agent or itself. Individual molecules of the surface stabilizer are essentially free of intermolecular cross-linkages.

The present invention also includes nanoparticulate active agent compositions, having at least one peptide as a surface stabilizer, formulated into compositions together with one or more non-toxic physiologically acceptable carriers, adjuvants, or vehicles, collectively referred to as carriers.

A. Peptide Surface Stabilizer

The choice of a surface stabilizer is non-trivial and usually requires extensive experimentation to realize a desirable formulation. Accordingly, the present invention is directed to the surprising discovery that a peptide, used as a nanoparticulate surface stabilizer, yields stable nanoparticulate active agent compositions that exhibit low degrees of aggregation.

A "peptide" is defined as any compound consisting of two or more amino acids, which are the basic structural units or "building blocks" of peptides. All peptides in all species, from bacteria to humans, are constructed from the same set of twenty commonly occurring, genetically encoded amino acids, as shown in the table below.

Each amino acid contains an "amine" group (NH_3), a "carboxy" group (COOH), a hydrogen atom, and a distinctive R group, or sidechain, bonded to a carbon atom. The amino acids vary in their sidechains, with variations in size, shape, charge, hydrogen-bonding capacity, and chemical reactivity. *See e.g.*, L. Stryer, *Biochemistry,* 3rd Edition, 1-40 (W.H. Freeman & Co., NY, 1988).

Amino Acid	3 Letter Abbreviation	1 Letter Abbreviation
alanine	ALA	A
asparagine	ASN	N
aspartic acid	ASP	D
arginine	ARG	R
cysteine	CYS	C
glutamic acid	GLU	E
glutamine	GLN	Q
glycine	GLY	G
histidine	HIS	H
isoleucine	ILE	I
leucine	LEU	L

Amino Acid	3 Letter Abbreviation	1 Letter Abbreviation
lysine	LYS	K
methionine	MET	M
phenylalanine	PHE	F
proline	PRO	P
serine	SER	S
threonine	THR	T
tryptophan	TRP	W
tyrosine	TYR	Y
valine	VAL	V
aspartic acid or asparagines	ASX	
glutamic acid or glutamine	GLX	
Unknown or other	Xaa	X

Peptides useful in the present invention can also comprise substituents other than amino acids. There are also naturally occurring chemical modifications of these twenty genetically encoded amino acids, such as hydroxylation of proline, addition of carbohydrates and lipids, and phosphorylation of serine and tyrosine. In addition, D-isomers of the amino acids, as opposed to the L-isomers found in naturally-occuring peptides and proteins, have been synthesized.

The amino acids of a peptide are connected by a amide, covalent linkage between the alpha carboxyl group of one amino acid and the alpha amino group of another amino acid. Many amino acids are joined by peptide bonds to form a polypeptide chain, which is unbranched. A polypeptide chain is a long peptide chain, consisting of a regularly repeating part, called the main chain, and a variable part, comprising the distinctive sidechains. Disulfide cross-links can be formed by cysteine residues in polypeptides. Most natural polypeptide chains contain between 50 and

2000 amino acids residues. The mean molecular weight of an amino acid residue is about 110 daltons, and so the molecular weights of most polypeptide chains are between 5500 and 220,000. *See e.g.*, L. Stryer, *Biochemistry*, 3rd Edition, p. 22 (W.H. Freeman & Co., NY, 1988).

A protein is a large macromolecule composed of one or more polypeptide chains. In the context of the present invention, a "peptide" refers to a peptide or a polypeptide, but not a protein.

Preferably, the peptide surface stabilizers of the invention are water soluble. By "water soluble," it is meant that the peptide has a water solubility of greater than about 1 mg/mL, greater than about 10 mg/mL, greater than about 20 mg/mL, or greater than about 30 mg/mL. This is in contrast to prior art compositions teaching the use of a peptide as an active agent in a nanoparticulate active agent composition. *See e.g.*, U.S. Patent Nos. 6,270,806; 6,592,903; 6,428,814; and 6,375,986. In such prior art references, when a peptide is utilized as an active agent in a nanoparticulate composition, the peptide is *poorly water soluble.*

There is an extensive catalog of commercially available peptides that can be used in the compositions of the invention. For example, the on-line peptide catalog http://www.peptide-catalog.com/PC/Peptides provides a list of hundreds of commercially available peptides, along with their structure and molecular weight. In addition, to the many commercially available peptides, custom peptides can be made and utilized in the compositions of the invention.

A preferred peptide surface stabilizer is poly(Lysine, Tryptophan)) 4:1 hydrobromide.

B. Secondary or Auxiliary Surface Stabilizers

The compositions of the invention can also include one or more auxiliary non-peptide surface stabilizers in addition to the at least one peptide surface stabilizer.

The auxiliary surface stabilizers of the invention are preferably adsorbed on, or associated with, the surface of the active agent particles. The auxiliary surface

stabilizers especially useful herein preferably do not chemically react with the active agent particles or itself. Preferably, individual molecules of the auxiliary surface stabilizer are essentially free of intermolecular cross-linkages.

Two or more auxiliary surface stabilizers can be employed in the compositions and methods of the invention.

Suitable surface stabilizers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products, and surfactants. Preferred auxiliary surface stabilizers include nonionic, anionic, cationic, zwitterionic, and ionic surfactants.

Representative examples of secondary surface stabilizers include gelatin, casein, lecithin (phosphatides), dextran, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers (*e.g.*, macrogol ethers such as cetomacrogol 1000), polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (*e.g.*, the commercially available Tweens® such as *e.g.*, Tween 20® and Tween 80® (ICI Speciality Chemicals)); polyethylene glycols (*e.g.*, Carbowaxs 3550® and 934® (Union Carbide)), polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropyl celluloses (e.g., HPC, HPC-SL, and HPC-L), hydroxypropyl methylcellulose (HPMC), hydroxypropylmethyl-cellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol, superione, and triton), poloxamers (*e.g.*, Pluronics F68® and F108®, which are block copolymers of ethylene oxide and propylene oxide); poloxamines (*e.g.*, Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to

ethylenediamine (BASF Wyandotte Corporation, Parsippany, N.J.)); Tetronic 1508® (T-1508) (BASF Wyandotte Corporation), dialkylesters of sodium sulfosuccinic acid (*e.g.*, Aerosol OT®, which is a dioctyl ester of sodium sulfosuccinic acid (DOSS) (American Cyanamid)); Duponol P®, which is a sodium lauryl sulfate (DuPont); Tritons X-200®, which is an alkyl aryl polyether sulfonate (Rohm and Haas); Crodestas F-110®, which is a mixture of sucrose stearate and sucrose distearate (Croda Inc.); p-isononylphenoxypoly-(glycidol), also known as Olin-10G® or Surfactant 10-G® (Olin Chemicals, Stamford, CT); Crodestas SL-40® (Croda, Inc.); and SA9OHCO, which is $C_{18}H_{37}CH_2C(O)N(CH_3)\text{-}CH_2(CHOH)_4(CH_2OH)_2$ (Eastman Kodak Co.); decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-derivatized phospholipid, PEG-derivatized cholesterol, PEG- derivatized cholesterol derivative, PEG-derivatized vitamin A, PEG-derivatized vitamin E, random copolymers of vinyl pyrrolidone and vinyl acetate, and the like.

Examples of useful cationic surface stabilizers include but are not limited to polymers, biopolymers, polysaccharides, cellulosics, alginates, phospholipids, and nonpolymeric compounds, such as zwitterionic stabilizers, poly-n-methylpyridinium, anthryul pyridinium chloride, cationic phospholipids, a charged phospholipid such as dimyristoyl phophatidyl glycerol, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr), hexyldesyltrimethylammonium bromide (HDMAB), and polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate.

Other useful cationic stabilizers include, but are not limited to, cationic lipids, sulfonium, phosphonium, and quarternary ammonium compounds, such as stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride or bromide, coconut methyl

dihydroxyethyl ammonium chloride or bromide, dodecyl trimethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride or bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride or bromide, coconut dimethyl hydroxyethyl ammonium chloride or bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride or bromide, lauryl dimethyl (ethenoxy)$_4$ ammonium chloride or bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts and dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt and/or an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride and dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12}, C_{15}, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride (ALIQUAT 336™), POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters (such as choline esters of fatty acids), benzalkonium chloride, stearalkonium chloride compounds (such as stearyltrimonium chloride and Di-stearyldimonium chloride), cetyl pyridinium bromide or chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™ and ALKAQUAT™ (Alkaril Chemical Company), alkyl pyridinium salts; amines, such as alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, and vinyl pyridine, amine salts, such as lauryl amine

acetate, stearyl amine acetate, alkylpyridinium salt, and alkylimidazolium salt, and amine oxides; imide azolinium salts; protonated quaternary acrylamides; methylated quaternary polymers, such as poly[diallyl dimethylammonium chloride] and poly-[N-methyl vinyl pyridinium chloride]; and cationic guar.

Such exemplary cationic surface stabilizers and other useful cationic surface stabilizers are described in J. Cross and E. Singer, *Cationic Surfactants: Analytical and Biological Evaluation* (Marcel Dekker, 1994); P. and D. Rubingh (Editor), *Cationic Surfactants: Physical Chemistry* (Marcel Dekker, 1991); and J. Richmond, *Cationic Surfactants: Organic Chemistry,* (Marcel Dekker, 1990).

Particularly preferred nonpolymeric primary stabilizers are any nonpolymeric compound, such benzalkonium chloride, a carbonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quarternary phosphorous compound, a pyridinium compound, an anilinium compound, an immonium compound, a hydroxylammonium compound, a primary ammonium compound, a secondary ammonium compound, a tertiary ammonium compound, and quarternary ammonium compounds of the formula $NR_1R_2R_3R_4^{(+)}$. For compounds of the formula $NR_1R_2R_3R_4^{(+)}$:

(i) none of R_1-R_4 are CH_3;

(ii) one of R_1-R_4 is CH_3;

(iii) three of R_1-R_4 are CH_3;

(iv) all of R_1-R_4 are CH_3;

(v) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 is an alkyl chain of seven carbon atoms or less;

(vi) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 is an alkyl chain of nineteen carbon atoms or more;

(vii) two of R_1-R_4 are CH_3 and one of R_1-R_4 is the group $C_6H_5(CH_2)_n$, where $n>1$;

(viii) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one heteroatom;

(ix) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one halogen;

(x) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one cyclic fragment;

(xi) two of R_1-R_4 are CH_3 and one of R_1-R_4 is a phenyl ring; or

(xii) two of R_1-R_4 are CH_3 and two of R_1-R_4 are purely aliphatic fragments.

Such compounds include, but are not limited to, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium chloride(Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium POE (10) oletyl ether phosphate, diethanolammonium POE (3)oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearalkonium chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, iofetamine hydrochloride, meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procainehydrochloride, cocobetaine, stearalkonium bentonite, stearalkoniumhectonite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

Most of these surface stabilizers are known pharmaceutical excipients and are described in detail in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain (The Pharmaceutical Press, 1986), specifically incorporated by reference. The surface stabilizers are commercially available and/or can be prepared by techniques known in the art.

C. Active Agents

The nanoparticles of the invention comprise at least one active, therapeutic, or diagnostic agent, collectively referred to as a "drug." A therapeutic agent can be a pharmaceutical agent, including biologics such as proteins, peptides, and nucleotides, or a diagnostic agent, such as a contrast agent, including x-ray contrast agents.

The active agent exists as a crystalline phase, an amorphous phase, a semi-amorphous phase, a semi-crystalline phase, or mixtures thereof. The crystalline phase differs from a non-crystalline or amorphous phase which results from precipitation techniques, such as those described in EP Patent No. 275,796.

The invention can be practiced with a wide variety of active agents. The active agent is preferably present in an essentially pure form, is poorly soluble, and is dispersible in at least one liquid dispersion media. By "poorly soluble" it is meant that the active agent has a solubility in a liquid dispersion media of less than about 30 mg/mL, less than about 20 mg/mL, less than about 10 mg/mL, or less than about 1 mg/mL. Useful liquid dispersion medias include, but are not limited to, water, aqueous salt solutions, safflower oil, and solvents such as ethanol, t-butanol, hexane, and glycol. A preferred liquid dispersion media is water.

Two or more active agents can be used in combination.

1. Active Agents Generally

The active agent can be selected from a variety of known classes of drugs, including, for example, nutraceuticals, COX-2 inhibitors, retinoids, anticancer agents, NSAIDS, proteins, peptides, nucleotides, anti-obesity drugs, nutraceuticals, dietary supplements, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents,

anxiolytics, sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, and xanthines.

Examples of representative active agents useful in this invention include, but are not limited to, acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole, loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

Exemplary nutraceuticals and dietary supplements are disclosed, for example, in Roberts et al., *Nutraceuticals: The Complete Encyclopedia of Supplements, Herbs, Vitamins, and Healing Foods* (American Nutraceutical Association, 2001), which is specifically incorporated by reference. A nutraceutical or dietary supplement, also known as a phytochemical or functional food, is generally any one of a class of dietary supplements, vitamins, minerals, herbs, or healing foods that have medical or

pharmaceutical effects on the body. Exemplary nutraceuticals or dietary supplements include, but are not limited to, lutein, folic acid, fatty acids (*e.g.*, DHA and ARA), fruit and vegetable extracts, vitamin and mineral supplements, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids (*e.g.*, iso-leucine, leucine, lysine, methionine, phenylanine, threonine, tryptophan, and valine), green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish and marine animal oils, and probiotics. Nutraceuticals and dietary supplements also include bio-engineered foods genetically engineered to have a desired property, also known as "pharmafoods."

Active agents to be administered in an aerosol formulation are preferably selected from the group consisting of proteins, peptide, bronchodilators, corticosteroids, elastase inhibitors, analgesics, anti-fungals, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, an analgesic, and a cardiovascular agent.

2. Anticancer Active Agents

Useful anticancer agents are preferably selected from alkylating agents, antimetabolites, natural products, hormones and antagonists, and miscellaneous agents, such as radiosensitizers.

Examples of alkylating agents include: (1) alkylating agents having the bis-(2-chloroethyl)-amine group such as, for example, chlormethine, chlorambucile, melphalan, uramustine, mannomustine, extramustinephoshate, mechlore-thaminoxide, cyclophosphamide, ifosfamide, and trifosfamide; (2) alkylating agents having a substituted aziridine group such as, for example, tretamine, thiotepa, triaziquone, and mitomycine; (3) alkylating agents of the alkyl sulfonate type, such as, for example,

busulfan, piposulfan, and piposulfam; (4) alkylating N-alkyl-N-nitrosourea derivatives, such as, for example, carmustine, lomustine, semustine, or streptozotocine; and (5) alkylating agents of the mitobronitole, dacarbazine and procarbazine type.

Examples of antimetabolites include: (1) folic acid analogs, such as, for example, methotrexate; (2) pyrimidine analogs such as, for example, fluorouracil, floxuridine, tegafur, cytarabine, idoxuridine, and flucytosine; and (3) purine derivatives such as, for example, mercaptopurine, thioguanine, azathioprine, tiamiprine, vidarabine, pentostatin, and puromycine.

Examples of natural products include: (1) vinca alkaloids, such as, for example, vinblastine and vincristine; (2) epipodophylotoxins, such as, for example, etoposide and teniposide; (3) antibiotics, such as, for example, adriamycine, daunomycine, doctinomycin, daunorubicin, doxorubicin, mithramycin, bleomycin, and mitomycin; (4) enzymes, such as, for example, L-asparaginase; (5) biological response modifiers, such as, for example, alpha-interferon; (6) camptothecin; (7) taxol; and (8) retinoids, such as retinoic acid.

Examples of hormones and antagonists include: (1) adrenocorticosteroids, such as, for example, prednisone; (2) progestins, such as, for example, hydroxyprogesterone caproate, medroxyprogesterone acetate, and megestrol acetate; (3) estrogens, such as, for example, diethylstilbestrol and ethinyl estradiol; (4) antiestrogens, such as, for example, tamoxifen; (5) androgens, such as, for example, testosterone propionate and fluoxymesterone; (6) antiandrogens, such as, for example, flutamide; and (7) gonadotropin-releasing hormone analogs, such as, for example, leuprolide.

Examples of miscellaneous agents include: (1) radiosensitizers, such as, for example, 1,2,4-benzotriazin-3-amine 1,4-dioxide (SR 4889) and 1,2,4-benzotriazine-7-amine 1,4-dioxide (WIN 59075); (2) platinum coordination complexes such as cisplatin and carboplatin; (3) anthracenediones, such as, for example, mitoxantrone;

(4) substituted ureas, such as, for example, hydroxyurea; and (5) adrenocortical suppressants, such as, for example, mitotane and aminoglutethimide.

In addition, the anticancer agent can be an immunosuppressive drug, such as, for example, cyclosporine, azathioprine, sulfasalazine, methoxsalen, and thalidomide.

The anticancer agent can also be a COX-2 inhibitor.

3. Analgesic Active Agents

An analgesic can be, for example, an NSAID or a COX-2 inhibitor.

Exemplary NSAIDS that can be formulated in compositions of the invention include, but are not limited to, suitable nonacidic and acidic compounds. Suitable nonacidic compounds include, for example, nabumetone, tiaramide, proquazone, bufexamac, flumizole, epirazole, tinoridine, timegadine, and dapsone. Suitable acidic compounds include, for example, carboxylic acids and enolic acids. Suitable carboxylic acid NSAIDs include, for example: (1) salicylic acids and esters thereof, such as aspirin, diflunisal, benorylate, and fosfosal; (2) acetic acids, such as phenylacetic acids, including diclofenac, alclofenac, and fenclofenac; (3) carbo- and heterocyclic acetic acids such as etodolac, indomethacin, sulindac, tolmetin, fentiazac, and tilomisole; (4) propionic acids, such as carprofen, fenbufen, flurbiprofen, ketoprofen, oxaprozin, suprofen, tiaprofenic acid, ibuprofen, naproxen, fenoprofen, indoprofen, and pirprofen; and (5) fenamic acids, such as flufenamic, mefenamic, meclofenamic, and niflumic. Suitable enolic acid NSAIDs include, for example: (1) pyrazolones such as oxyphenbutazone, phenylbutazone, apazone, and feprazone; and (2) oxicams such as piroxicam, sudoxicam, isoxicam, and tenoxicam.

Exemplary COX-2 inhibitors that can be formulated in combination with the nanoparticulate nimesulide composition of the invention include, but are not limited to, celecoxib (SC-58635, CELEBREX®, Pharmacia/Searle & Co.), rofecoxib (MK-966, L-748731, VIOXX®, Merck & Co.), meloxicam (MOBIC®, co-marketed by Abbott Laboratories, Chicago, IL, and Boehringer Ingelheim Pharmaceuticals), valdecoxib (BEXTRA®, G.D. Searle & Co.), parecoxib (G.D. Searle & Co.),

etoricoxib (MK-663; Merck), SC-236 (chemical name of 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)] benzenesulfonamide; G.D. Searle & Co., Skokie, IL); NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl)methane sulfonamide; Taisho Pharmaceutical Co., Ltd., Japan); SC-58125 (methyl sulfone spiro(2.4)hept-5-ene I; Pharmacia/Searle & Co.); SC-57666 (Pharmacia/Searle & Co.); SC-558 (Pharmacia/Searle & Co.); SC-560 (Pharmacia/Searle & Co.); etodolac (Lodine®, Wyeth-Ayerst Laboratories, Inc.); DFU (5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl 2(5H)-furanone); monteleukast (MK-476), L-745337 ((5-methanesulphonamide-6-(2,4-difluorothio-phenyl)-1-indanone), L-761066, L-761000, L-748780 (all Merck & Co.); DUP-697 (5-Bromo-2-(4-fluorophenyl)-3-(4-(methylsulfonyl)phenyl; DuPont Merck Pharmaceutical Co.); PGV 20229 (1-(7-tert.-butyl-2,3-dihydro-3,3-dimethylbenzo(b)furan-5-yl)-4-cyclopropylbutan-1-one; Procter & Gamble Pharmaceuticals); iguratimod (T-614; 3-formylamino-7-methylsulfonylamino-6-phenoxy-4H-1- benzopyran-4-one; Toyama Corp., Japan); BF 389 (Biofor, USA); CL 1004 (PD 136095), PD 136005, PD 142893, PD 138387, and PD 145065 (all Parke-Davis/Warner-Lambert Co.); flurbiprofen (ANSAID®; Pharmacia & Upjohn); nabumetone (FELAFEN®; SmithKline Beecham, plc); flosulide (CGP 28238; Novartis/Ciba Geigy); piroxicam (FELDANE®; Pfizer); diclofenac (VOLTAREN® and CATAFLAM®, Novartis); lumiracoxib (COX-189; Novartis); D 1367 (Celltech Chiroscience, plc); R 807 (3 benzoyldifluoromethane sulfonanilide, diflumidone); JTE-522 (Japan Tobacco, Japan); FK-3311 (4'-Acetyl-2'-(2,4-difluorophenoxy)methanesulfonanilide), FK 867, FR 140423, and FR 115068 (all Fujisawa, Japan); GR 253035 (Glaxo Wellcome); RWJ 63556 (Johnson & Johnson); RWJ 20485 (Johnson & Johnson); ZK 38997 (Schering); S 2474 ((E)-(5)-(3,5-di-tert-butyl-4-hydroxybenzylidene)-2-ethyl-1,2-isothiazolidine-1,1-dioxide indomethacin; Shionogi & Co., Ltd., Japan); zomepirac analogs, such as RS 57067 and RS 104897 (Hoffmann La Roche); RS 104894 (Hoffmann La Roche); SC 41930 (Monsanto); pranlukast (SB 205312, Ono-1078, ONON®, ULTAIR®; SmithKline Beecham); SB 209670 (SmithKline Beecham); and APHS (heptinylsulfide).

D. Nanoparticulate Active Agent Particle Size

The compositions of the invention contain nanoparticulate active agent particles which have an effective average particle size of less than about 2000 nm (*i.e.*, 2 microns). In other embodiments of the invention, the active agent particles have a size of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods.

By "an effective average particle size of less than about 2000 nm" it is meant that at least 50% by weight of the active agent particles have a particle size less than the effective average, *i.e.*, less than about 2000 nm, 1900 nm, 1800 nm, *etc.*, when measured by the above-noted techniques. In other embodiments of the invention, at least about 70%, at least about 90%, at least about 95%, or at least about 99% of the active agent particles have a particle size less than the effective average, *i.e.*, less than about 2000 nm, 1900 nm, 1800 nm, *etc.*

If the nanoparticulate active agent composition is combined with a conventional active agent composition, then such a composition is either solubilized or has an effective average particle size greater than about 2 microns. By "an effective average particle size of greater than about 2 microns" it is meant that at least 50% of the microparticulate active agent particles have a particle size greater than about 2 microns, by weight, when measured by the above-noted techniques. In other embodiments of the invention, at least about 70%, at least about 90%, at least about 95%, or at least about 99%, by weight, of the microparticulate active agent particles have a particle size greater than about 2 microns.

In the present invention, the value for D50 of a nanoparticulate active agent composition is the particle size below which 50% of the active agent particles fall, by weight. Similarly, D90 and D99 are the particle sizes below which 90% and 99%, respectively, of the active agent particles fall, by weight.

5. Concentration of Nanoparticulate Active Agent and Peptide Stabilizer

The relative amounts of active agent and peptide surface stabilizer, and optionally one or more secondary surface stabilizers, can vary widely. The optimal amount of the individual components can depend, for example, upon the particular active agent selected, the hydrophilic lipophilic balance (HLB), melting point, and the surface tension of water solutions of the stabilizer, *etc.*

The concentration of the peptide surface stabilizer can vary from about 0.5% to about 99.999%, from about 5.0% to about 99.9%, or from about 10% to about 99.5%, by weight, based on the total combined dry weight of the at least one active agent and at least one peptide surface stabilizer, not including other excipients.

The concentration of the at least one active agent can vary from about 99.5% to about 0.001%, from about 95% to about 0.1%, or from about 90% to about 0.5%, by weight, based on the total combined dry weight of the active agent and at least one peptide surface stabilizer, not including other excipients.

B. Methods of Making Nanoparticulate Active Agent Formulations

The nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, can be made using, for example, milling, homogenization, or precipitation techniques. Exemplary methods of making nanoparticulate compositions are described in the '684 patent. Methods of making nanoparticulate active agent compositions are also described in U.S. Patent No. 5,518,187 for "Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,862,999 for "Method of Grinding Pharmaceutical Substances;" U.S.

Patent No. 5,665,331 for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Patent No. 5,662,883 for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Patent No. 5,560,932 for "Microprecipitation of Nanoparticulate Pharmaceutical Agents;" U.S. Patent No. 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" U.S. Patent No. 5,534,270 for "Method of Preparing Stable Drug Nanoparticles;" U.S. Patent No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles;" and U.S. Patent No. 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation," all of which are specifically incorporated by reference.

The resultant nanoparticulate active agent compositions can be utilized in any desired dosage form.

1. Milling to obtain Nanoparticulate Active Agent Dispersions

Milling the active agent to obtain a nanoparticulate dispersion comprises dispersing active agent particles in a liquid dispersion media in which the active agent is poorly soluble, followed by applying mechanical means in the presence of grinding media to reduce the particle size of the active agent to the desired effective average particle size. The dispersion media can be, for example, water, safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, or glycol. Water is a preferred dispersion media.

The active agent particles are preferably reduced in size in the presence of at least one peptide surface stabilizer. Alternatively, the active agent particles can be contacted with at least one peptide surface stabilizer either during or after attrition. One or more secondary surface stabilizers may also be added before, during, or after attrition. Other compounds, such as a diluent, can be added to the active agent/peptide surface stabilizer composition before, during, or after the size reduction process. Dispersions can be manufactured continuously or in a batch mode.

2. Precipitation to Obtain Nanoparticulate Active Agent Compositions

Another method of forming the desired nanoparticulate active agent composition is by microprecipitation. This is a method of preparing stable dispersions of poorly soluble active agents in the presence of one or more peptide surface stabilizers and one or more colloid stability enhancing surface active agents free of any trace toxic solvents or solubilized heavy metal impurities. Such a method comprises, for example: (1) dissolving the poorly soluble active agent in a suitable solvent; (2) adding the formulation from step (1) to a solution comprising at least one peptide surface stabilizer and optionally one or more secondary surface stabilizers, to form a clear solution; and (3) precipitating the formulation from step (2) using an appropriate non-solvent. The method can be followed by removal of any formed salt, if present, by dialysis or diafiltration and concentration of the dispersion by conventional means.

3. Homogenization to Obtain Nanoparticulate Active Agent Compositions

Exemplary homogenization methods of preparing active agent nanoparticulate compositions are described in U.S. Patent No. 5,510,118, for "Process of Preparing Therapeutic Compositions Containing Nanoparticles."

Such a method comprises dispersing active agent particles in a liquid dispersion media in which the active agent is poorly soluble, followed by subjecting the dispersion to homogenization to reduce the particle size of the active agent to the desired effective average particle size. The active agent particles can be reduced in size in the presence of at least one peptide surface stabilizer and, if desired, one or more additional surface stabilizers. Alternatively, the active agent particles can be contacted with at least one peptide surface stabilizer and, if desired, one or more additional surface stabilizers, either during or after attrition. Other compounds, such as a diluent, can be added to the active agent/peptide surface stabilizer composition either before, during, or after the size reduction process. Dispersions can be manufactured continuously or in a batch mode.

C. Methods of Using Nanoparticulate Active Agent Formulations

The nanoparticulate active agent compositions of the present invention can be administered to humans and animals via any conventional means including, but not limited to, orally, rectally, ocularly, parenterally (intravenous, intramuscular, or subcutaneous), intracisternally, pulmonary, intravaginally, intraperitoneally, locally (powders, ointments or drops), or as a buccal or nasal spray.

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles including water, ethanol, polyols (propyleneglycol, polyethylene-glycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The nanoparticulate active agent compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the growth of microorganisms can be ensured by various antibacterial and antifungal agents, such as parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, such as aluminum monostearate and gelatin.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active agent is admixed with at least one of the following: (a) one or more inert excipients (or carrier), such as sodium citrate or dicalcium phosphate; (b) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (c) binders, such as carboxymethylcellulose, alignates, gelatin, polyvinylpyrrolidone, sucrose and acacia; (d) humectants, such as glycerol; (e) disintegrating agents, such as agar-agar, calcium

carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; (f) solution retarders, such as paraffin; (g) absorption accelerators, such as quaternary ammonium compounds; (h) wetting agents, such as cetyl alcohol and glycerol monostearate; (i) adsorbents, such as kaolin and bentonite; and (j) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. For capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active agent, the liquid dosage forms may comprise inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers. Exemplary emulsifiers are ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, such as cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, fatty acid esters of sorbitan, or mixtures of these substances, and the like.

Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Actual dosage levels of active agent in the nanoparticulate compositions of the invention may be varied to obtain an amount of active agent that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired therapeutic effect, the route of administration, the potency of the administered active agent, the desired duration of treatment, and other factors.

Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors

including the body weight, general health, sex, diet, time and route of administration, potency of the administered active agent, rates of absorption and excretion, combination with other active agents, and the severity of the particular disease being treated.

* * * * *

The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available document, including a U.S. patent, are specifically incorporated by reference.

The formulations in the examples that follow were also investigated using a light microscope. Here, "stable" nanoparticulate dispersions (uniform Brownian motion) were readily distinguishable from "aggregated" dispersions (relatively large, nonuniform particles without motion).

Example 1

The purpose of this example was to prepare a nanoparticulate nystatin composition having a peptide surface stabilizer.

Nystatin is a poorly water-soluble antimycotic polyene antibiotic obtained from *Streptomyces noursei.* It is an antifungal agent indicated for oral, gastrointestinal, and vaginal candidiasis. Oral candidiasis, in particular, is a common affliction of immunocompromised patients. Nystatin is indicated in the therapy of all infections caused by susceptible microorganisms in those patients in whom candidal (monilial) infections are most likely to complicate therapy.

A slurry of 2% (w/w) nystatin (Sigma-Aldrich Co.) and 1% (w/w) poly(Lysine, Tryptophan) 4:1 hydrobromide ("Poly(Lys,Trp)") (Sigma; St. Louris, MO), which is a cationic random co-polyamino acid having a molecular weight of 38,000, in water was

milled for 1 day using low energy (ball milling) techniques in the presence of ceramic YTZ grinding media.

The mean size of the nystatin particles following milling was 149 nm, with a D90 of 270 nm, as determined by static light scattering using a Horiba LA-910 light-scattering particle size analyzer (Horiba Instruments, Irvine, CA). The composition had a zeta potential of 47.7 mV, as measured by electrophoresis in $5x10^{-4}$ M NaCl (Malvern ZetaSizer). Dispersibility was verified by phase contrast microscopy.

Figure 1 shows representative photomicrographs of the nystatin crystals before (Fig. 1A) and after (Fig. 1B) milling.

Particle size stability under controlled conditions was monitored over time. Figure 2 shows the results of monitoring the nystatin particle size stability over time at 5°C (solid line), 25°C (dashed line), and 40°C (dotted line) for the nanoparticulate nystatin/peptide composition.

These results demonstrate that a peptide surface stabilizer can be successfully used to stabilize an active agent at a nanoparticulate particle size. Moreover, such a peptide surface stabilizer may confer additional therapeutic advantages to the final formulation. For example, the peptide surface stabilizer Poly(Lys,Trp) is cationic and, therefore, nanoparticulate active agent compositions utilizing this surface stabilizer will be bioadhesive.

The resultant composition exhibited a mean particle sizes of 149 nm and were free of agglomeration. Moreover, the nanoparticulate nystatin/peptide composition exhibited virtually no particle size growth at all three temperatures tested.

Example 2

The purpose of this example was to determine whether a cationic surface charge, such as that obtained with the use of a cationic peptide surface stabilizer, enhances the adhesion of small particles to cells.

Cell-binding experiments were performed with polystyrene latex microspheres as a model. A positive surface charge would be expected to enhance the interaction of particles with cell-surface macromolecules, which have a net negative charge.

Cationic microspheres with a mean zeta-potential (51.5 mV) comparable to the nanoparticulate nystatin/peptide composition of Example 1 were tested against anionic microspheres (mean zeta-potential = -50.9 mV). The microspheres were incubated with NIH/3T3 fibroblasts, washed thoroughly, fixed, and subjected to SEM analysis.

Figure 3 shows representative micrographs of cells with anionic particles (Fig. 3A) and cationic particles (Fig. 3B).

The results indicate that positively-charged particles interact more strongly with the cell surface than negatively-charged particles, and it is believed that nanoparticulate active agent compositions having a cationic peptide as a surface stabilizer with comparable zeta potentials will follow the same trend.

Example 3

The purpose of this example was to determine if milling of an active agent, such as nystatin, having a peptide surface stabilizer affects the active agent's activity.

The minimum inhibitory concentration (MIC) of a milled nystatin composition having as a peptide surface stabilizer Poly(Lys, Trp) was compared to the MIC of two unmilled nystatin compositions. Nystatin for the milled nanoparticulate composition was obtained from Sigma-Aldrich Co. and the two unmilled nystatin compositions were obtained from Sigma-Aldrich Co. and Paddock Laboratories, Inc. Details regarding the milled and unmilled nystatin compositions are given in Table 1 below, including particle size of the milled nanoparticulate nystatin/Poly(Lys, Trp) composition and the potency (USP U/ml) and MIC for each nystatin composition.

TABLE 1				
Nystatin Concentration	**Surface Stabilizer and Concentration**	**Mean Particle Size (nm)**	**Potency (USP U/ml)**	**MIC**
2% (Sigma)	1% Poly(Lys, Trp)[1]	129	101,200	1:10,000
5% (Sigma)	N/A – unmilled	N/A	253,000	1:10,000
4% (Paddock)	N/A – unmilled	N/A	253,000	1:100,000

[1]Poly(Lysine, Tryptophan) is a cationic random co-polyamino acid.

The nanoparticulate sample was ball milled for 26 hours with ceramic YTZ milling media.

The minimum inhibitory concentration (MIC) of the milled nystatin/peptide composition and the two unmilled samples were determined in cultures of *C. albicans*. MIC as reported here is the maximum dilution of formulation in culture broth which inhibits growth of *C albicans*. As shown in Table 1, above, the milled nystatin/peptide composition did not exhibit any significant differences in MIC, and surprisingly, was more active than at least one of the unmilled nystatin samples. These data confirm that the milling process does not decrease the activity of nystatin.

Example 4

The purpose of this example was to prepare a nanoparticulate composition of a diuretic, Compound A, utilizing a peptide surface stabilizer. Diuretics can be used to reduce the swelling and fluid retention caused by various medical problems, including heart or liver disease. They are also is used to treat high blood pressure.

A slurry of 2% (w/w) Compound A and 1% (w/w) poly(Lysine, Tryptophan) 4:1 hydrobromide as a peptide surface stabilizer in water was milled for 3 days in an aqueous environment in a low energy mill, in the presence of 0.8 mm yttrium-stabilized ceramic media.

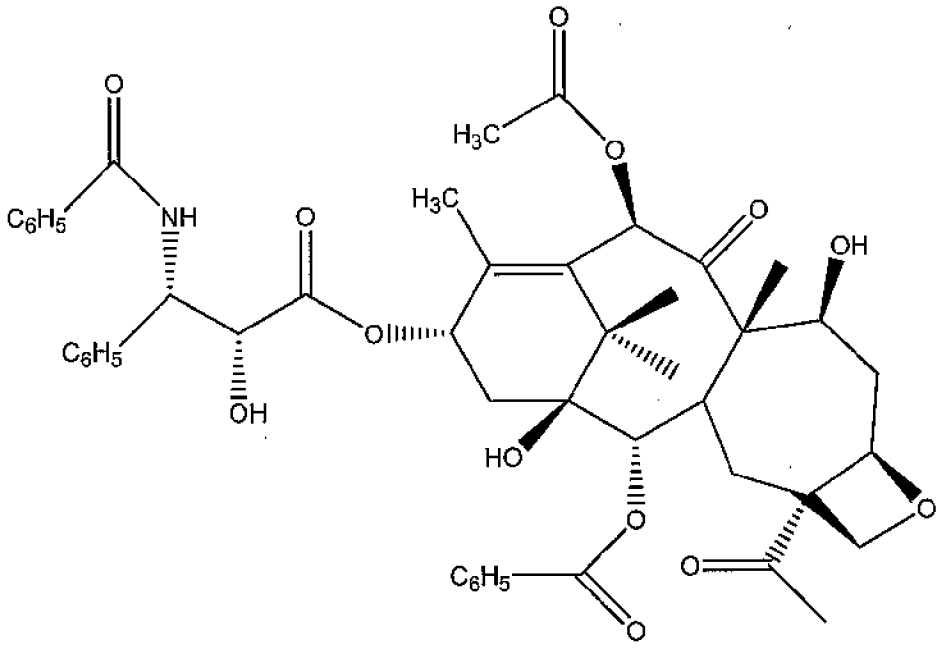
Particle size analysis of the resulting Compound A dispersion was conducted via laser light diffraction using the Horiba LA 910 particle size analyzer (Horiba Instruments, Irvine, CA) and water as a diluent. The mean particle size of the milled

Compound A dispersion was 99 nm, with a D90 of 138 nm. The composition was stable.

Example 5

The purpose of this example was to prepare a nanoparticulate composition of paclitaxel utilizing a peptide surface stabilizer. Paclitaxel belongs to the group of medicines called antineoplastics. It is used to treat cancer of the ovaries, breast, certain types of lung cancer, and a cancer of the skin and mucous membranes more commonly found in patients with acquired immunodeficiency syndrome (AIDS). It may also be used to treat other kinds of cancer.

Paclitaxel has the following chemical structure:



A slurry of 2% (w/w) paclitaxel and 1% (w/w) poly(Lysine, Tryptophan) 4:1 hydrobromide as a peptide surface stabilizer in water was milled for 3 days in an aqueous environment in a low energy mill, in the presence of 0.8 mm yttrium-stabilized ceramic media.

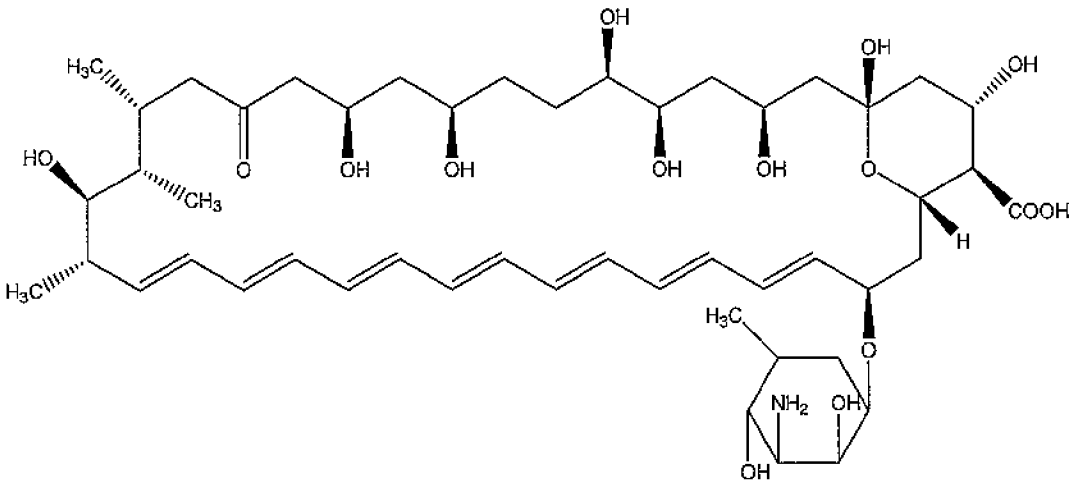
Particle size analysis of the resulting paclitaxel dispersion was conducted via laser light diffraction using the Horiba LA 910 particle size analyzer (Horiba Instruments, Irvine, CA) and water as a diluent. The mean particle size of the milled

paclitaxel dispersion was 139 nm, with a D90 of 185 nm. The composition was stable.

Example 6

The purpose of this example was to prepare a nanoparticulate composition of amphotericin B utilizing a peptide surface stabilizer. Amphotericin B is a poorly water soluble antifungal agent. Topically, it is used to treat skin yeast infections; intravenously, it is used to treat a variety of life-threatening fungal infections.

Amphotericin B has the following chemical structure:



In this experiment, amphotericin B was milled with Poly (Lys, Trp) 4:1 Hydrobromide as a peptide surface stabilizer. A 2% (w/w) slurry of amphotericin B (Sigma) in water was prepared with 1% (w/w) poly (Lys, Trp) (Sigma). The composition was ball-milled for 24 hours with 0.8 mm ceramic YTZ milling media. The particle size of the resulting amphotericin B dispersion was characterized by static laser light scattering on a Horiba LA-910 particle size distribution analyzer. The results are shown in Table 2, below.

TABLE 2

Drug and Concentration	Surface Stabilizer and Concentration	Mean Particle Size (nm)	D50 (nm)	D90 (nm)
2% Amphotericin B	1% Poly(Lys, Trp)	121	96	230

These results demonstrate that amphotericin B dispersions can be successfully stabilized by a peptide surface stabilizer, such as the random copolypeptide poly (Lys, Trp) 4:1 Hydrobromide.

* * * *

It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

We claim:

1. A composition comprising:

(a) particles of at least one active agent having an effective average particle size of less than about 2000 nm; and

(b) at least one water soluble peptide surface stabilizer.

2. The composition of claim 1, wherein the peptide surface stabilizer is poly(Lysine, Tryptophan) 4:1 hydrobromide.

3. The composition of claim 1 or claim 2, further comprising at least one secondary surface stabilizer.

4. The composition of any one of claims 1 to 3, wherein the secondary surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

5. The composition of claim 3 or claim 4, wherein the secondary surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-

phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, and random copolymers of vinyl acetate and vinyl pyrrolidone, a cationic polymer, a cationic biopolymer, a cationic polysaccharide, a cationic cellulosic, a cationic alginate, a cationic nonpolymeric compound, a cationic phospholipids, cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl

ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™, ALKAQUAT™, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

6. The composition of any one of claims 1 to 5, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

7. The composition of any one of claims 1 to 6 formulated into a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, tablets, capsules, sachets, lozenges, powders, pills, granules, controlled release formulations, fast melt formulations, lyophilized

formulations, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

8. The composition of any one of claims 1 to 7, wherein:

(a) the active agent is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined dry weight of the active agent and at least one peptide surface stabilizer, not including other excipients; or

(b) the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999% by weight, from about 5.0% to about 99.9% by weight, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the active agent and at least one peptide surface stabilizer, not including other excipients.

9. The composition of any one of claims 1 to 8, wherein the active agent is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

10. The composition of any one of claims 1 to 10, wherein the effective average particle size of the active agent particles is selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

11. The composition of any one of claims 1 to 10, wherein at least about 70%, at least about 90%, at least about 95%, or at least about 99% of the active agent particles have a particle size less than the effective average particle size.

12. The composition of any one of claims 1 to 11, further comprising at least one additional active agent composition having an effective average particle size which is different that the effective average particle size of the active agent composition of claim 1.

13. The composition of any one of claims 1 to 12, wherein the active agent is selected from the group consisting of nystatin, paclitaxel, amphotericin B, a diuretic, a dermal agent, nutraceuticals, COX-2 inhibitors, retinoids, anticancer agents, NSAIDS, proteins, peptides, nucleotides, anti-obesity drugs, dietary supplements, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants, anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole,

loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

14. The composition of claim 13, wherein the nutraceutical is selected from the group consisting of dietary supplements, vitamins, minerals, herbs, lutein, folic acid, fatty acids, fruit extracts, vegetable extracts, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids, green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish oils, marine animal oils, and probiotics.

15. The composition of claim 13, wherein the anticancer agent is selected from the group consisting of alkylating agents, antimetabolites, anthracenediones, natural products, hormones, antagonists, radiosensitizers, platinum coordination complexes, adrenocortical suppressants, immunosuppressive agent, substituted ureas, and COX-2 inhibitors.

16. The composition of claim 15, wherein:

(a) the alkylating agent is selected from the group consisting of chlormethine, chlorambucile, melphalan, uramustine, mannomustine, extramustinephoshate, mechlore-thaminoxide, cyclophosphamide, ifosfamide, trifosfamide, tretamine, thiotepa, triaziquone, mitomycine, busulfan, piposulfan, piposulfam, carmustine, lomustine, semustine, streptozotocine, mitobronitole, dacarbazine and procarbazinc; or

(b) the antimetabolite is selected from the group consisting of methotrexate, fluorouracil, floxuridine, tegafur, cytarabine, idoxuridine, flucytosine, mercaptopurine, thioguanine, azathioprine, tiamiprine, vidarabine, pentostatin, and puromycine; or

(c) the natural product is selected from the group consisting of vinblastine, vincristine, etoposide, teniposide, adriamycine, daunomycine, doctinomycin, daunorubicin, doxorubicin, mithramycin, bleomycin, mitomycin, L-asparaginase, alpha-interferon, camptothecin, taxol, and retinoic acid; or

(d) the hormone or antagonist is selected from the group consisting of prednisone, hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, ethinyl estradiol, tamoxifen, testosterone propionate, fluoxymesterone, flutamide, leuprolide; or

(e) the anticancer agent is selected from the group consisting of cisplatin, carboplatin, mitoxantrone, hydroxyurea, mitotane, aminoglutethimide, cyclosporine, azathioprine, sulfasalazine, methoxsalen, and thalidomide.

17. The composition of claim 13, wherein the NSAID is selected from the group consisting of nabumetone, tiaramide, proquazone, bufexamac, flumizole, epirazole, tinoridine, timegadine, dapsone, aspirin, diflunisal, benorylate, fosfosal, diclofenac, alclofenac, fenclofenac, etodolac, indomethacin, sulindac, tolmetin, fentiazac, tilomisole, carprofen, fenbufen, flurbiprofen, ketoprofen, oxaprozin, suprofen, tiaprofenic acid, ibuprofen, naproxen, fenoprofen, indoprofen, pirprofen, flufenamic, mefenamic, meclofenamic, niflumic, oxyphenbutazone, phenylbutazone, apazone, feprazone, piroxicam, sudoxicam, isoxicam, and tenoxicam.

18. The composition of claim 13, wherein the COX-2 inhibitor is selected from the group consisting of nimesulide, celecoxib, rofecoxib, meloxicam, valdecoxib, parecoxib, etoricoxib, flurbiprofen, nabumetone, etodolac, iguratimod, flosulide, piroxicam, diclofenac, lumiracoxib, monteleukast, pranlukast, heptinylsulfide, SC-236, SC-58125, SC-57666, SC-558, SC-560, SC 41930, NS-398, DFU, L-745337, L-

761066, L-761000, L-748780, DUP-697, PGV 20229, BF 389, CL 1004, PD 136005, PD 142893, PD 138387, PD 145065, D 1367, R 807, JTE-522, FK-3311, FK 867, FR 140423, FR 115068, GR 253035, RWJ 63556, RWJ 20485, ZK 38997, S 2474, RS 57067, RS 104897, RS 104894, and SB 209670.

19. The composition of any one of claims 1 to 18, wherein upon administration to a mammal the active agent particles redisperse such that the particles have an effective average particle size selected from the group consisting of less than about 2 microns, less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

20. The composition of any one of claims 1 to 19, wherein the composition redisperses in a biorelevant media such that the active agent particles have an effective average particle size selected from the group consisting of less than about 2 microns, less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

21. The composition of claim 20, wherein the biorelevant media is selected from the group consisting of water, aqueous electrolyte solutions, aqueous solutions of a

salt, aqueous solutions of an acid, aqueous solutions of a base, and combinations thereof.

22. The composition of any one of claims 1 to 21, wherein:

(a) the T_{max} of the active agent, when assayed in the plasma of a mammalian subject following administration, is less than the T_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; or

(b) the C_{max} of the active agent, when assayed in the plasma of a mammalian subject following administration, is greater than the C_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; or

(c) the AUC of the active agent, when assayed in the plasma of a mammalian subject following administration, is greater than the AUC for a non-nanoparticulate composition of the same active agent, administered at the same dosage.

23. The composition of claim 22, wherein the T_{max} is selected from the group consisting of not greater than about 90%, not greater than about 80%, not greater than about 70%, not greater than about 60%, not greater than about 50%, not greater than about 30%, not greater than about 25%, not greater than about 20%, not greater than about 15%, not greater than about 10%, and not greater than about 5% of the T_{max} exhibited by a non-nanoparticulate composition of the same active agent, administered at the same dosage.

24. The composition of claim 22, wherein the C_{max} is selected from the group consisting of at least about 50%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, at least about 1000%, at least about 1100%, at least about 1200%, at least about 1300%, at least about 1400%, at

least about 1500%, at least about 1600%, at least about 1700%, at least about 1800%, or at least about 1900% greater than the C_{max} exhibited by a non-nanoparticulate composition of the same active agent, administered at the same dosage.

25. The composition of claim 22, wherein the AUC is selected from the group consisting of at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, at least about 500%, at least about 550%, at least about 600%, at least about 750%, at least about 700%, at least about 750%, at least about 800%, at least about 850%, at least about 900%, at least about 950%, at least about 1000%, at least about 1050%, at least about 1100%, at least about 1150%, or at least about 1200% greater than the AUC exhibited by the non-nanoparticulate composition of the same active agent, administered at the same dosage.

26. The composition of any one of claims 1 to 25 which does not produce significantly different absorption levels when administered under fed as compared to fasting conditions.

27. The composition of claim 26, wherein the difference in absorption of the active agent composition of the invention, when administered in the fed versus the fasted state, is selected from the group consisting of less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, and less than about 3%.

28. The composition of any one of claims 1 to 27, wherein administration of the composition to a human in a fasted state is bioequivalent to administration of the composition to a subject in a fed state.

29. The composition of claim 28, wherein "bioequivalency" is established by:

(a) a 90% Confidence Interval of between 0.80 and 1.25 for both C_{max} and AUC; or

(b) a 90% Confidence Interval of between 0.80 and 1.25 for AUC and a 90% Confidence Interval of between 0.70 to 1.43 for C_{max}.

30. The composition of any one of claims 1 to 29, formulated into a liquid dosage form and having a viscosity at a shear rate of 0.1 (1/s), measured at 20°C, selected from the group consisting of less than about 2000 mPa·s, from about 2000 mPa·s to about 1 mPa·s, from about 1900 mPa·s to about 1 mPa·s, from about 1800 mPa·s to about 1 mPa·s, from about 1700 mPa·s to about 1 mPa·s, from about 1600 mPa·s to about 1 mPa·s, from about 1500 mPa·s to about 1 mPa·s, from about 1400 mPa·s to about 1 mPa·s, from about 1300 mPa·s to about 1 mPa·s, from about 1200 mPa·s to about 1 mPa·s, from about 1100 mPa·s to about 1 mPa·s, from about 1000 mPa·s to about 1 mPa·s, from about 900 mPa·s to about 1 mPa·s, from about 800 mPa·s to about 1 mPa·s, from about 700 mPa·s to about 1 mPa·s, from about 600 mPa·s to about 1 mPa·s, from about 500 mPa·s to about 1 mPa·s, from about 400 mPa·s to about 1 mPa·s, from about 300 mPa·s to about 1 mPa·s, from about 200 mPa·s to about 1 mPa·s, from about 175 mPa·s to about 1 mPa·s, from about 150 mPa·s to about 1 mPa·s, from about 125 mPa·s to about 1 mPa·s, from about 100 mPa·s to about 1 mPa·s, from about 75 mPa·s to about 1 mPa·s, from about 50 mPa·s to about 1 mPa·s, from about 25 mPa·s to about 1 mPa·s, from about 15 mPa·s to about 1 mPa·s, from about 10 mPa·s to about 1 mPa·s, and from about 5 mPa·s to about 1 mPa·s.

31. The composition of claim 30, wherein the viscosity of the dosage form is:

(a) selected from the group consisting of less than about 1/200, less than

about 1/100, less than about 1/50, less than about 1/25, and less than about 1/10 of the viscosity of a liquid dosage form of a non-nanoparticulate composition of the same active agent, at about the same concentration per ml of active agent; or

(b) selected from the group consisting of less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, less than about 50%, less than about 55%, less than about 60%, less than about 65%, less than about 70%, less than about 75%, less than about 80%, less than about 85%, and less than about 90% of the viscosity of a liquid dosage form of a non-nanoparticulate composition of the same active agent, at about the same concentration per ml of active agent.

32. The composition of any one of claims 1 to 31, further comprising one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

33. The composition according to any one of claims 1 to 32, wherein the composition is bioadhesive.

34. The use of a composition according to any one of claims 1 to 33 for the manufacture of a pharmaceutical medicament.

35. A method of making a composition according to any one of claims 1 to 33, comprising contacting particles of at least one active agent with at least one water-soluble peptide surface stabilizer for a time and under conditions sufficient to provide an active agent composition having an effective average particle size of less than about 2000 nm.

FIGURE 1

A B

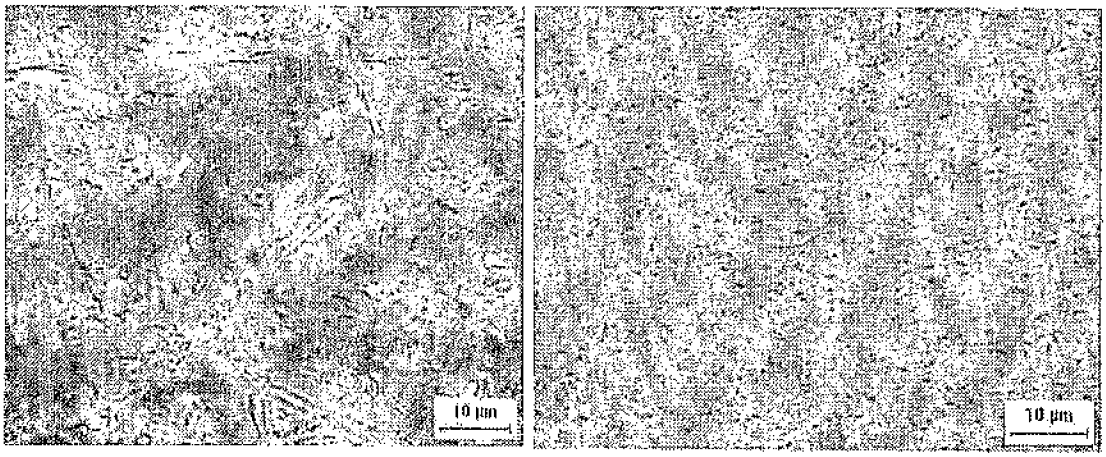


FIGURE 2

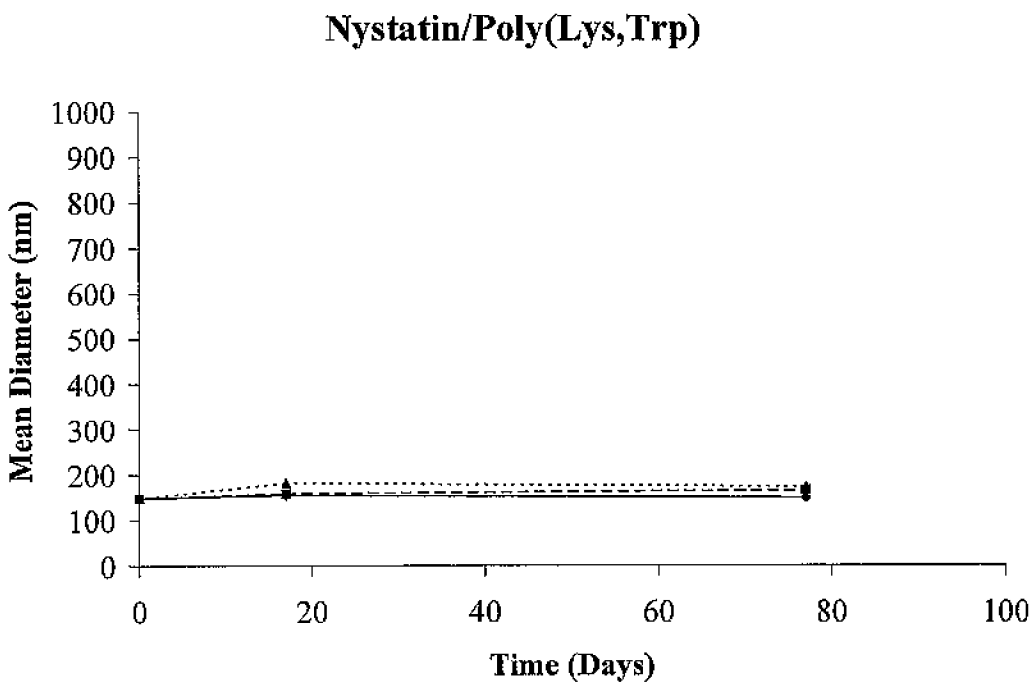
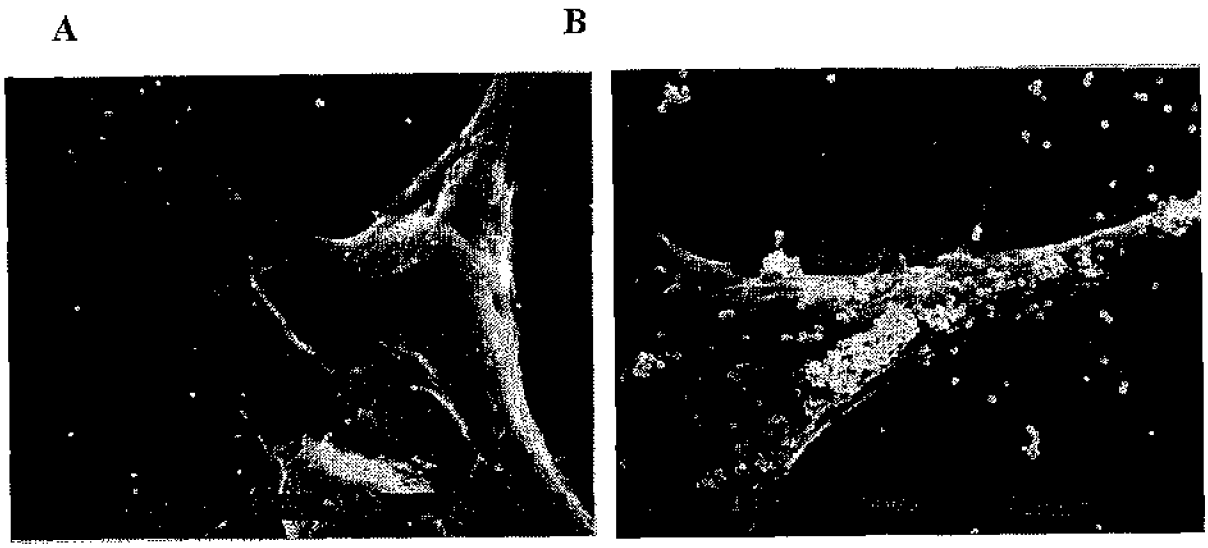

Nystatin/Poly(Lys,Trp)
Mean Diameter (nm)
1000
900
800
700
600
500
400
300
200
100
0
0
20
40
60
80
100
Time (Days)

FIGURE 3



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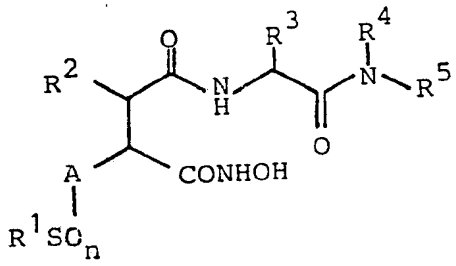

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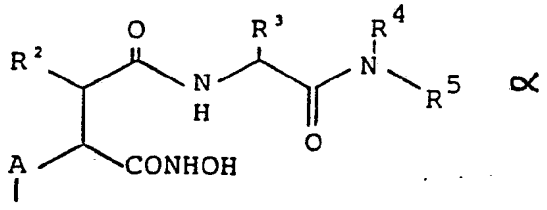

(57) Abstract

Compounds of general formula (I), wherein R^1 represents hydrogen or an alkyl, phenyl, thiophenyl, substituted phenyl, phenylalkyl, heterocyclyl, alkylcarbonyl phenacyl or substituted phenacyl group; or, when n = 0, R^1 represents SR^X, wherein R^X represents a group (α); R^2 represents a hydrogen atom or an alkyl, alkenyl, phenylalkyl, cycloalkylalkyl or cycloalkenylalkyl group; R^3 represents an amino acid residue with R or S stereochemistry or an alkyl, benzyl, (C_1-C_6 alkoxy) benzyl or benzyloxy(C_1-C_6 alkyl) group; R^4 represents a hydrogen atom or an alkyl group; R^5 represents a hydrogen atom or a methyl group; n is an integer having the value 0, 1 or 2; and A represents a hydrocarbon chain optionally substituted with one or more alkyl, phenyl or substituted phenyl groups; and their salts and N-oxides are collagenase inhibitors and are useful in the management of disease involving tissue degradation and/or the promotion of wound healing. Diseases involving tissue degradation include arthropathy (particularly rheumatoid arthritis), inflammation, dermatological diseases, bone resorption diseases and tumour invasion.

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HYDROXAMIC ACID BASED COLLAGENASE INHIBITORS.

This invention relates to pharmaceutically and veterinarily active compounds, which are derivatives of hydroxamic acid.

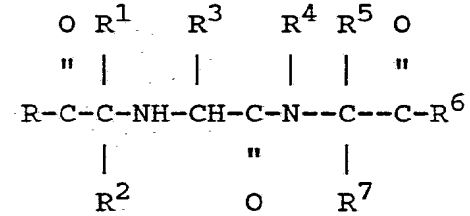
The compounds of the present invention act as inhibitors of metalloproteases involved in tissue degradation, such as collagenase, which initiates collagen breakdown, stromelysin (protoglycanase), gelatinase and collagenase (IV). There is evidence implicating collagenase as one of the key enzymes in the breakdown of articular cartilage and bone in rheumatoid arthritis (Arthritis and Rheumatism, **20**, 1231 - 1239, 1977). Potent inhibitors of collagenase and other metalloproteases involved in tissue degradation are useful in the treatment of rheumatoid arthritis and related diseases in which collagenolytic activity is important. Inhibitors of metalloproteases of this type can therefore be used in treating or preventing conditions which involve tissue breakdown; they are therefore useful in the treatment of arthropathy, dermatological conditions, bone resorption, inflammatory diseases and tumour invasion and in the promotion of wound healing. Specifically, compounds of the present invention may be useful in the treatment of osteopenias such as osteoporosis, rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration and tumour invasion.

A number of small peptide like compounds which inhibit metalloproteases have been described. Perhaps the most notable of these are those relating to the

angiotensin converting enzyme (ACE) where such agents act to block the conversion of the decapeptide angiotensin I to angiotensin II a potent pressor substance. Compounds of this type are described in EP-A-0012401.

Certain hydroxamic acids have been suggested as collagenase inhibitors as in US-A-4599361 and EP-A-0236872. Other hydroxamic acids have been prepared as ACE inhibitors, for example in US-A-4105789, while still others have been described as enkephalinase inhibitors as in US-A-4496540.

EP-A-0012401 discloses antihypertensive compounds of the formula:



wherein

R and R^6 are the same or different and are hydroxy, alkoxy, alkenoxy, dialkylamino alkoxy, acylamino alkoxy, acyloxy alkoxy, aryloxy, alkyloxy, substituted aryloxy or substituted aralkoxy wherein the substituent is methyl, halo, or methoxy, amino, alkylamino, dialkylamino, aralkylamino or hydroxyamino;

R^1 is hydrogen, alkyl of from 1 to 20 carbon atoms, including branched, cyclic and unsaturated alkyl groups;

substituted alkyl wherein the substituent is halo, hydroxy, alkoxy, aryloxy amino, alkylamino, dialkylamino, acrylamino, arylamino, guanidino, imidazolyl, indolyl, mercapto, alkylthio, arylthio, carboxy, carboxamido, carbalkoxy, phenyl, substituted phenyl wherein the substituent is alkyl, alkoxy or halo; aralkyl or heteroaralkyl, aralkenyl or heteroaralkenyl, substituted aralkyl, substituted heteroaralkyl, substituted aralkenyl or substituted hetereoaralkenyl, wherein the substituent is halor or dihalo, alkyl, hydroxy, alkoxy, amino, aminomethyl, acrylamino, dialkylamino, alkylamino, carboxyl, haloalkyl, cyano or sulphonamido, aralkyl or hetereoaralkyl substituted on the alkyl portion by amino or acylamino;

R^2 and R^7 are hydrogen or alkyl;

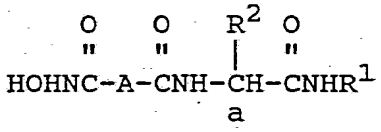
R^3 is hydrogen, alkyl, phenylalkyl, aminomethylphenylalkyl, hydroxyphenylalkyl, hydroxyalkyl, acetylaminoalkyl, acylaminoalkyl, acylaminoalkyl aminoalkyl, dimethylaminoalkyl, haloalkyl, guanidinoalkyl, imidazolylalkyl, indolylalkyl, mercaptoalkyl and alkylthioalkyl;

R^4 is hydrogen or alkyl;

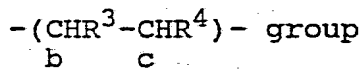
R^5 is hydrogen, alkyl, phenyl, phenylalkyl, hydroxyphenylalkyl, hydroxyalkyl, aminoalkyl, guanidinoalkyl, imidazolylalkyl, indolylalkyl, mercaptoalkyl or alkylthioalkyl;

R^4 and R^5 may be connected together to form an alkylene bridge of from 2 to 4 carbon atoms, an alkylene bridge of from 2 to 3 carbon atoms and one sulphur atom, an alkylene bridge of from 3 to 4 carbon atoms containing a double bond or an alkylene bridge as above, substituted with hydroxy, alkoxy or alkyl and the pharmaceutically acceptable salts thereof.

US-A-4599361 discloses compounds of the formula:



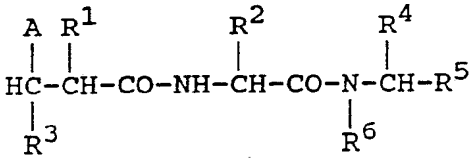
wherein
R^1 is C_1-C_6 alkyl;
R^2 is C_1-C_6 alkyl, benzyl, benzyloxybenzyl, (C_1-C_6 alkoxy)benzyl or benzyloxy(C_1-C_6 alkyl);
a is a chiral centre with optional R or S stereochemistry;
A is a



or a $-(CR^3=CR^4)-$ group wherein b and c are chiral centres with optional R or S stereochemistry;

R^3 is hydrogen, C_1-C_6 alkyl, phenyl or phenyl(C_1-C_6 alkyl) and R^4 is hydrogen, C_1-C_6 alkyl, phenyl(C_1-C_6 alkyl), cycloalkyl or cycloalkyl(C_1-C_6 alkyl).

EP-A-0236872 discloses generically compounds of the formula



wherein

A represents a group of the formula HN(OH)-CO- or HCO-N(OH)-;

R^1 represents a C_2-C_5 alkyl group;

R^2 represents the characterising group of a natural alpha-amino acid in which the functional group can be protected, amino groups may be acylated and carboxyl groups can be amidated, with the proviso that R^2 can not represent hydrogen or a methyl group;

R^3 represents hydrogen or an amino, hydroxy, mercapto, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 acylamino, C_1-C_6-alkylthio, aryl-(C_1-C_6 alkyl)-, amino-(C_1-C_6-alkyl)-, hydroxy(C_1-C_6-alkyl)-, mercapto(C_1-C_6 alkyl) or carboxy(C_1-C_6 alkyl) group,

wherein the amino, hydroxy, mercapto or carboxyl groups can be protected and the amino groups may be acylated or the carboxyl groups may be amidated;

R^4 represents hydrogen or a methyl group;

R^5 represents hydrogen or a C_1-C_6 acyl, C_1-C_6 alkoxy-C_1-C_6 alkyl, di(C_1-C_6-alkoxy)methylene, carboxy, (C_1-C_6 alkyl)carbinyl, (C_1-C_6 alkoxy)carbinyl, arylmethoxy carbinyl, (C_1-C_6 alkyl)amino carbinyl or arylamino carbinyl group; and

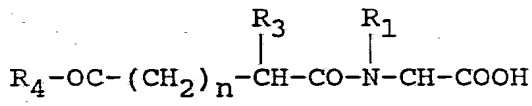
R^6 represents hydroxy or a methylene group; or

R^2 and R^4 together represent a group-$(CH_2)_n$-, wherein n represents a number from 4 to 11; or

R^4 and R^5 together represent a trimethylene group;

and pharmaceutically acceptable salts of such compounds, which are acid or basic.

US-A-4105789 generically discloses compounds which have the general formula



and salts thereof, wherein

R_1 is hydrogen, lower alkyl, phenyl lower alkylene, hydroxy-lower alkylene, hydroxyphenyl lower alkylene, amino-lower alkylene, guanidine lower

alkylene, mercapto-lower alkylene, lower alkyl-mercapto-lower alkylene, imidazolyl lower alkylene, indolyl-lower alkylene or carbamoyl lower alkylene;

R_2 is hydrogen or lower alkyl;

R_3 is lower alkyl or phenyl lower alkylene;

R_4 is hydroxy, lower alkoxy or hydroxyamino; and

n is 1 or 2.

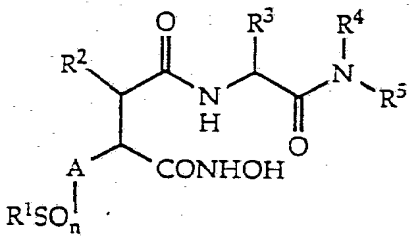
US-A-4496540 discloses compounds of the general formula:

A-B-NHOH

wherein A is one of the aromatic group-containing amino acid residues L-tryptophyl, D-tryptophyl, L-tyrosyl, D-tyrosyl, L-phenylalanyl, or D-phenylalanyl, and B is one of the amino acids glycine, L-alanine, D-alanine, L-leucine, D-leucine, L-isoleucine, or D-isoleucine; and pharmaceutically acceptable salts thereof.

It would however be desirable to improve on the solubility of known collagenase inhibitors and/or stomelysin inhibitors (whether as the free base or the salt) and, furthermore, increases in activity have also been sought. It is not a simple matter, however, to predict what variations in known compounds would be desirable to increase or even retain activity; certain modifications of known hydroxamic acid derivatives have been found to lead to loss of activity.

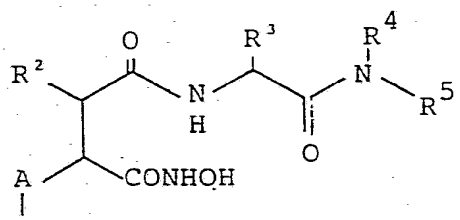
According to a first aspect of the invention, there is provided a compound of general formula I:



(I)

wherein:

R^1 represents a C_1-C_6 alkyl, phenyl, thiophenyl, substituted phenyl, phenyl(C_1-C_6)alkyl, heterocyclyl, (C_1-C_6)alkylcarbonyl, phenacyl or substituted phenacyl group; or, when n = 0, R^1 represents SR^X, wherein R^X represents a group:



;

R^2 represents a hydrogen atom or a C_1-C_6 alkyl, C_1-C_6 alkenyl, phenyl(C_1-C_6)alkyl, cycloalkyl(C_1-C_6)alkyl or cycloalkenyl(C_1-C_6)alkyl group;

R^3 represents an amino acid side chain or a C_1-C_6 alkyl, benzyl, (C_1-C_6 alkoxy)benzyl, benzyloxy(C_1-C_6 alkyl) or benzyloxybenzyl group;

R^4 represents a hydrogen atom or a C_1-C_6 alkyl group;

R^5 represents a hydrogen atom or a methyl group;

n is an integer having the value 0, 1 or 2; and

A represents a C_1-C_6 hydrocarbon chain, optionaly substituted with one or more C_1-C_6 alkyl, phenyl or substituted phenyl groups;

or a salt thereof.

Hereafter in this specification, the term "compound" includes "salt" unless the context requires otherwise.

As used herein the term "C_1-C_6 alkyl" refers to a straight or branched chain alkyl moiety having from one to six carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, t-butyl, pentyl and hexyl, and cognate terms (such as "C^1-C^6 alkoxy") are to be construed accordingly.

The term "C_1-C_6 alkenyl" refers to a straight or branched chain alkyl moiety having one to six carbons and having in addition one double bond, of either E or Z stereochemistry where applicable. This term would include, for example, an alpha, beta-unsaturated methylene group, vinyl, 1-propenyl, 1- and 2-butenyl and 2-methyl-2-propenyl.

The term "cycloalkyl" refers to a saturated alicyclic moiety having from 3 to 8 carbon atoms and includes for example, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The term "cycloalkenyl" refers to an unsaturated alicycle having from 3 to 8 carbon atoms and includes cyclopropenyl, cyclobutenyl and cyclopentenyl, cyclohexenyl.

The term "substituted", as applied to a phenyl or other aromatic ring, means substituted with up to four substituents each of which independently may be C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, thiol, C_1-C_6 alkylthiol, amino, halo (including fluoro, chloro, bromo and iodo), triflouromethyl or nitro.

The term "amino acid side chain" means a characteristic side chain attached to the $-CH(NH_2)(COOH)$ moiety in the following R or S amino acids: glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, serine, threonine, cysteine, methionine, asparagine, glutamine, lysine, histidine, arginine, glutamic acid and aspartic acid.

The term "hydrocarbon chain" includes alkylene, alkenylene and alkynylene chains of from 1 to 6 carbon atoms. Preferably the carbon atom of the hydrocarbon chain nearest to the hydroxamic acid group is a methylene carbon atom.

There are several chiral centres in the compounds according to the invention because of the presence of asymmetric carbon atoms. The presence of several asymmetreic carbon atoms gives rise to a number of diastereomers with the appropriate R or S stereochemistry at each chiral centre. General formula I and, where apprpriate, all other formulae in this

specification are to be understood to include all such stereoisomers and mixtures (for example racemic mixtures) thereof. Compounds in which the chiral centre adjacent the substituent R^3 has S stereochemistry and/or the chiral centre adjacent the substituent R^2 has R stereochemistry are preferred.

Further or other preferred compounds include those in which, independently or in any combination:

R^1 represents a hydrogen atom or a C_1-C_4 alkyl, phenyl, thiophenyl, benzyl, acetyl or benzoyl group;

R^2 represents a C_3-C_6 alkyl (for example isobutyl) group;

R^3 represents a benzyl or 4-(C_1-C_6)alkoxyphenylmethyl or benzyloxybenzyl group;

R^4 represents a C_1-C_4 alkyl (for example methyl) group; and

R^5 represents a hydrogen atom.

Particularly preferred compounds include:

1. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthio-methyl)-succinyl]-L-phenylalanine-N-methylamide,

2. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenyl-thio-methyl)succinyl]-L-phenylalanine-N-methylamide,

3. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzylthiomethyl) succinyl]-L-phenylalanine-N-methylamide,

4. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl)succinyl]-L-phenylalanine-N-methylamide and

5. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiolmethyl) succinyl]-L-phenylalanine-N-methylamide

6. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzoylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

7. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(pivaloylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

8. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

9. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide

10. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-hydroxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

11 [4-(N-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

12. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt

13. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-tert-butylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

14. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(2,4-dimethylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

15. bis-S,S'-{[4(N-Hydroxyamino-2R-isobutyl-3S-(thiomethyl)succinyl]-L-phenylalanine-N-methylamide) disulphide

16. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-bromophenylthio-methyl)succinyl]-L-phenylalanine-N-methylamide

17. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-chlorophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

18. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(3-methylphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

19. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-acetyl)-aminophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

20. [4-(N-Hydroxyamino)-2R-isobutyl-3S-phenyl-sulphinylmethylsuccinyl]-L-phenylalanine-N-methyl-amide

21. [4-(N-Hydroxyamino)-2R-isobutyl-3S-phenyl-sulphonylmethylsuccinyl]-L-phenylalanine-N-methyl-amide

22. [4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenyl-sulphinylmethyl-succinyl]-L-phenylalanine-N-methylamide

23. [4-(N-Hydroxyamino)-2R-isobutyl-3S-thiophenyl-sulphonylmethyl-succinyl]-L-phenylalanine-N-methylamide

24. [4-(N-Hydroxyamino)-2R-isobutyl-3S-phenyl-sulphonylmethyl-succinyl]-L-phenylalanine-N-methylamide sodium salt

25. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(isobutyl-oxycarbonylamino)phenyl)thiomethyl-succinyl]-L-phenylalanine-N-methylamide

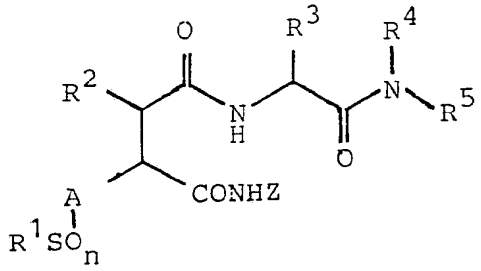
26. [4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-(N-methyl-N-(tert-butoxycarbonyl)-glycylamino)phenyl)thio-methylsuccinyl]-L-phenylalanine-N-methylamide

and, where appropriate, their salts. Compounds 2 and 5 are especially preferred and compound 2 is the most preferred, because of its good collagenase-inhibiting and protoglycanase-inhibiting activities.

Compounds of general formula I may be prepared by any suitable method known in the art and/or by the following process, which itself forms part of the invention.

According to a second aspect of the invention, there is provided a process for preparing a compound of general formula I as defined above, the process comprising:

(a) deprotecting a compound of general formula II

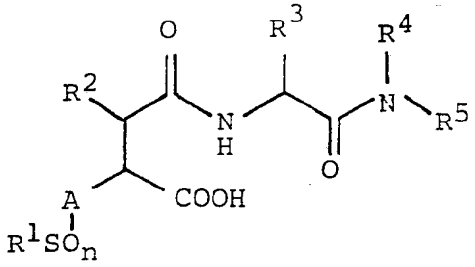


(II)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I and Z represents a protective group such as a benzyl group; or

(b) reacting a compound of general formula III



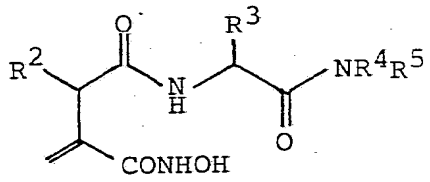
(III)

wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I,

with hydroxylamine or a salt thereof; or

(c) reacting a compound of general formula VIA



(VIA)

wherein

R^2, R^3, R^4 and R^5 are as defined in general formula I,

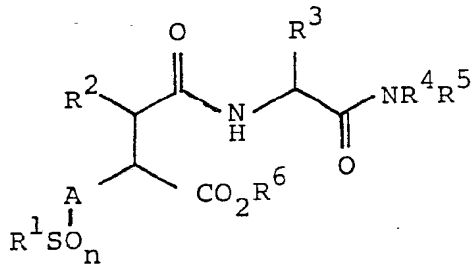
either with a thiol of the general formula R^1S, wherein R^1 is as defined in general formula I to give a compound of general formula I in which A represents a methylene group and n is 0,

or with a cuprate of the general formula $(R^1S\text{-}A^1)_2CuLi$, wherein R^1 is as defined in general formula I and A^1 is such that $-A^1-CH_2-$ is identical to -A-, as defined in general formula I.

(d) optionally after step (a), step (b) or step (c) converting a compound of general formula I into another compound of general formula I.

Compounds of general formula I which are sulphoxides or sulphones can be derived from thiol compounds of general formula I by oxidation. Alternatively, thiols of general formula II or III may be oxidised. Compounds of general formula I which are disulphides (ie compounds wherein R^1 represents SR^X) may be derived from thiol esters of general formula I by milk oxidation, for example in air.

A compound of general formula II may be prepared from a compound of general formula III by reaction with an O-protected (such as benzyl) hydroxylamine. A compound of general formula III may be prepared by desterification (such as hydrolysis) of an ester of the general formula IV

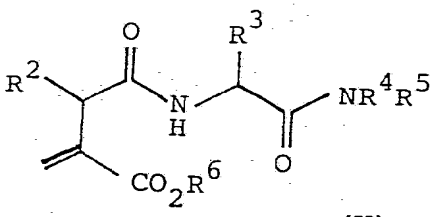


(IV)

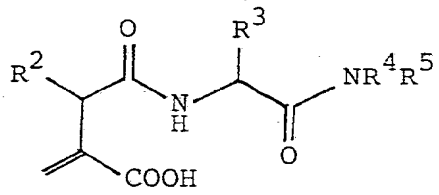
wherein:

R^1, R^2, R^3, R^4, R^5, A and n are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl.

A compound of general formula IV can be prepared from an ester of general formula V or an acid of general formula VI



(V)



(VI)

wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl

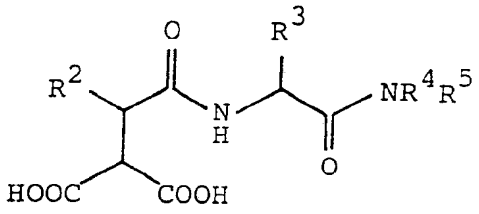
by reaction with a thiol R^1SH, wherein R^1 is as defined in general formula I, to give compounds wherein A represents a methylene group,

or by reaction with a cuprate of the general formula $(R^1S\text{-}A^1)_2CuLi$, wherein R^1 is as defined in general formula I and A^1 is such that $-A^1-CH_2-$ is identical to -A-, as defined in general formula I.

Esters of general formula V can be prepared by esterifying acids of general formula VI with an appropriate alcohol R^6OH or other esterifying agent.

Compounds of general formula VIA can be prepared by reacting compounds of general formula VI with hydroxylamine or a salt thereof.

An acid of general formula VI can be prepared by reacting a malonic acid derivative of general formula VII



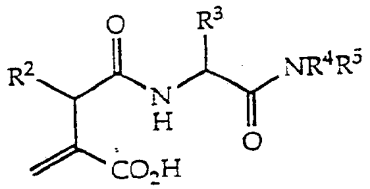
(VII)

wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I

with formaldehyde in the presence of pyridine.

An acid of general formula VII can in turn be prepared by desterifying (for example hydrolysing) a compound of general formula VIII

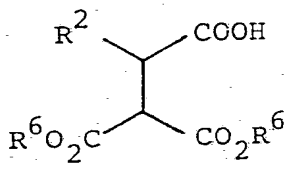


(VIII)

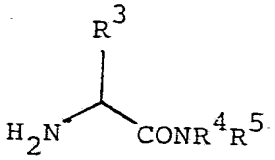
wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl.

A compound of general formula VIII can be prepared by reacting a compound of general formula IX with a compound of general formula X



(IX)



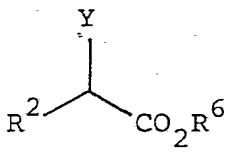
(X)

wherein:

R^2, R^3, R^4 and R^5 are as defined in general formula I and R^6 represents C_1-C_6 alkyl, phenyl C_1-C_6 alkyl or substituted phenyl C_1-C_6 alkyl.

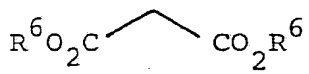
The starting materials and other reagents are either available commercially or can be synthesised by simple chemical procedures.

For example, a substituted acid of general formula IX may be prepared by reacting an ester of the general formula XI



(XI)

wherein Y represents halo and R^5 is as defined above and R^2 and R^6 as defined above, with a malonate derivative of the general formula XII



(XII)

wherein R^6 is as defined above with the proviso that when R^6 is aromatic in general formula XI it is aliphatic in general formula XII or vice versa, and selectively de-esterifying.

Compounds of general formula XI can simply be derived from amino acids, which can be obtained in enantiomerically pure form, enabling a choice of optically active compounds of general formula I to be prepared.

Compounds of general formulae II and III are valuable intermediates in the preparation of compounds of general formula I. According to a third aspect of the invention, there is therefore provided a compound of general formula II. According to a fourth aspect of the invention, there is provided a compound of general formula III.

As mentioned above, compounds of general formula I are useful in human or veterinary medicine as they are active inhibitors, of metalloproteases involved in tissue degradation.

According to a fifth aspect of the invention, there is provided a compound of general formula I for use in human or veterinary medicine, particularly in the management (by which is meant treatment of prophylaxis) of disease involving tissue degradation, in particular rheumatoid arthritis, and/or in the promotion of wound healing.

According to a sixth aspect of the invention, there is provided the use of a compound of general formula I in the preparation of an agent for the management of disease involving tissue degradation, particularly rheumatoid arthritis, and/or in the promotion of wound healing. Compounds of general formula I can therefore be used in a method of treating disease involving tissue degradation, particularly rheumatoid arthritis, and/or in a method of promoting wound healing, the method in either case comprising administering to a human or animal patient an effective amount of a compound of general formula I.

The potency of compounds of general formula I to act as inhibitors of collagenase (a metalloprotease involved in tissue degradation) was determined by the procedure of Cawston and Barrett, (Anal. Biochem., **99**, 340-345, 1979) and their potency to act as inhibitors of stromelysin was determined using the procedure of Cawston et al (Biochem. J., **195**, 159-165 1981), both of which techniques are to be described more fully in the examples and are incorporated by reference herein so far as the law allows.

According to a seventh aspect of the invention, there is provided a pharmaceutical or veterinary formulation comprising a compound of general formula I and a pharmaceutically and/or veterinarily acceptable carrier. One or more compounds of general formula I may be present in association with one or more non-toxic pharmaceutically and/or veterinarily acceptible carriers and/or diluents and/or adjuvents and if desired other active ingredients.

According to an eighth aspect of the invention, there is provided a process for the preparation of a pharmaceutical or veterinary formulation in accordance with the seventh aspect, the process comprising admixing a compound of general formula I and a pharmaceutically and/or veterinarily acceptable carrier.

Compounds of general formula I may be formulated for administration by any route and would depend on the disease being treated. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parental solutions or suspensions.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrollidone; fillers for example lactose, sugar, maize-starch, calcium phosphate,sorbitol or glycine; tabletting lubricant, for example magnesium sterate, talc, polyethylene glycol or silica; disintegrants, for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid

preparations may contain coventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin, hydrogenated edible fats; emulsifiying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqujeous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

The dosage unit involved in oral administration may contain from about 1 to 250 mg, preferably from about 25 to 250 mg of a compound of general formula I. A suitable daily dose for a mammal may vary widely depending on the condition of the patient. However, a dose of a compound of general formula I of about 0.1 to 300mg/kg body weight, particularly from about 1 to 100 mg/kg body weight may be appropriate.

For topical application to the skin the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations that may be used for the drug are conventional fomulations well known in the art, for example, as described in standard text books of pharmaceutics such as the British Pharmacopoeia.

For topical applications to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non-aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edeate; preservatives including bactericidal

and fungicidal agents, such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorohexidine, and thickening agents such as hypromellose may also be included.

The dosage employed for the topical administration will, of course, depend on the size of the area being treated. For the eyes each dose will be typically in the range from 10 to 100 mg of the compound of general formula I.

The active ingredient may also be administered parenterally in a sterile medium. The drug depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anasthetic, preservative and buffering agents can be dissolved in the vehicle.

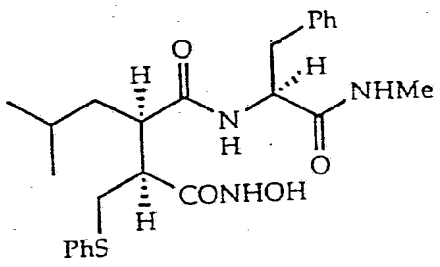
For use in the treatment of rheumatoid arthritis the compounds of this invention can be administered by the oral route or by injection intra-articularly into the affected joint. The daily dosage for a 70 kg mammal will be in the range of 10 mgs to 1 gram of a compound of general formula I.

The following examples illustrate the invention, but are not intended to limit the scope in any way. The following abbreviations have been used in the Examples:-

DCC - Dicyclohexylcarbodiimide
DCM - Dichloromethane
DCU - Dicyclohexylurea
DIPE - Diisopropyl ether
DMF - N,N-dimethylformamide
HOBT - Hydroxybenztriazole
NMM - N-Methylmorpholine
TFA - Trifluoroacetic acid
THF - Tetrahydrofuran
WSCDI - N-(Dimethylaminoethyl)-N'-ethylcarbodiimide

Example 1

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide



a) 2R-Bromo-5-methylpentanoic acid.

D-Leucine (100g, 0.76 mol) and potassium bromide (317.5g, 2.67 mol) were dissolved in aqueous acid (150ml concentrated sulphuric acid in 500ml of water). The solution was cooled to -2° and sodium nitrite (69.6g, 0.95 mol in water) was added over 1h taking care to maintain the temperature between -1 and -2°. After addition was complete the mixture was kept at 0° for a further hour, then DCM was added and the mixture stirred for a few minutes. The layers were separated

and the aqeous phase was washed with further portions of DCM (5 x 250ml). The combined organic layers were dried over magnesium sulphate then the solvent removed to give the acid as a pale yellow oil (123.1g, 0.63 mol, 83%)

$[alpha]_D$ = +38.0° (c = 2, methanol)

$delta_H$ (250 MHz, $CDCl_3$) 4.29 (1H, t, J= 6.5Hz, $BrC\underline{H}CO_2H$), 1.91 (2H, t, J= 7Hz, $CHC\underline{H}_2CH$), 1.83 (1H, m, $Me_2C\underline{H}$), and 0.94 (6H, 2xd, J= 7Hz, $(C\underline{H}_3)_2CH$)

b) tert-Butyl 2R-Bromo-5-methylpentanoate.

2R-Bromo-5-methylpentanoic acid (123g, 0.63 mol) was dissolved in DCM (400ml) and the solution cooled to -40° while isobutene was condensed in to roughly double the volume. Maintaining the temperature at -40° concentrated sulphuric acid (4ml) was added dropwise. When the addition was complete the reaction was allowed to warm to room temperature overnight. The resultant solution was concentrated to half the volume by removing the solvent at reduced pressure, then the DCM was washed twice with an equal volume of 10% sodium bicarbonate solution. The organic layer was dried over magnesium sulphate and the solvent removed under reduced pressure to leave the title compound as a yellow oil (148.0g, 0.59 mol, 94%).

$[alpha]_D$ = +23.0° (c = 2, methanol)

delta$_H$ (250 MHz, $CDCl_3$) 4.18 (1H, t, J= 6.5Hz, $BrC\underline{H}CO_2H$), 1.89 (2H, m, $CHC\underline{H}_2CH$), 1.78 (1H, m, $Me_2C\underline{H}$), 1.49 (9H, s, $(CH_3)_3C$) and 0.94 (6H, 2xd, J= 7Hz, $(CH_3)_2CH$)

delta$_C$ (63.9 MHz, $CDCl_3$) 167.0, 82.0, 46.3, 43.4, 27.6, 26.3, 22.2, and 21.6.

c) Benzyl (2-benzloxycarbonyl-3R-(tert-butoxycarbonyl)-5-methylhexanoate.

Dibenzyl malonate (124.5g, 0.44 mol) was taken up in dry DMF and potassium tert-butoxide (49.2g, 0.44 mol) was added portionwise with stirring and cooling. When a homogeneous solution had formed it was cooled to 0^o then tert-butyl-2R-bromo-5-methylpentanoate (110.0g, 0.44 mol) in DMF (200 ml) was added dropwise over 1h. When addition was complete the reaction was transfered to a cold room at $<5^o$ and left for 4 days. The reaction mixture was partitioned between ethyl acetate and saturated ammonium chloride then the aqueous layer extracted with further ethyl acetate (4x500ml), drying and solvent removal left an oil (228g) heavily contaminated with DMF. This oil was taken into ether (1 litre) and washed with brine (2x1l) then the organic layer dried (magnesium sulphate), solvent removed under reduced pressure to leave the desired material (179g) contaminated with a small amount of dibenzyl malonate.

$[alpha]_D = +22.5^o$ (c = 2, methanol)

delta$_H$ (250 MHz, $CDCl_3$) 7.40 - 7.25 (10H, m, Aromatic H), 5.14 (4H, 2xABq, $C\underline{H}_2Ph$), 3.77 (1H, d, J= 10Hz, $BnO_2CC\underline{H}CO_2Bn$), 3.09 (1H, dt, J= 10,6Hz, $CH_2C\underline{H}CO_2tBu$), 1.50 (3H, m, CH_2 + $C\underline{H}Me_2$)1.41 (9H, s, $C(C\underline{H}_3)_3$) and 0.88 (6H, 2xd, J= 7Hz).

d) [4-Benzyloxy-3-benzyloxycarbonyl-2R-isobutyl-succinyl]-L-phenylalanine-N-methylamide

Benzyl(2-benzyloxycarbonyl-5-methyl-3R-tert-butoxycarbonyl)-hexanoate (281.4g, 0.56 mol) was taken up in 5% water in TFA (410 ml) and allowed to stand at 5° overnight. After this time the TFA was evaporated under reduced pressure then the residue partitioned between DCM (1l) and brine (200ml). Solvent removal left an oil which crystallised on standing (230g).

The crude acid from this reaction was dissolved in DMF (1l), then HOBT (95.3g, 0.64 mol), NMM (64g, 0.64 mol) and phenylalanine-N-methylamide (113.0g, 0.64 mol) were added at room temperature. The mixture was cooled to 0° before dropwise addition of DCC (131.0g, 0.64 mol) in THF (1l). This solution was stirred to room temperature over the weekend. The precipitated DCU was removed by filtration then the solvents were removed from the filtrate under reduced pressure to leave an oil. This oily residue was dissolved in ethyl acetate then washed with 10% citric acid, 10% sodium bicarbonate and saturated brine. The organic layer was dried (magnesium sulphate), filtered then the solvent removed under reduced pressure to give the title compound as an oil (400g). This material was columned on silica using gradient elution (0 - 50% ethyl

acetate in hexane) to remove impurities and separate a small amount of the minor diastereoisomer. The material from the column (195g) was recrystallised from DIPE to give the title compound as a white crystalline solid (140.2g, 0.25 mol, 47%)

m.p. 98 -99°
Analysis calculated for $C_{33}H_{38}N_2O_6$
Requires C 70.95 H 6.86 N 5.01
Found C 70.56 H 6.89 N 5.06

$delta_H$ (250MHz, $CDCl_3$) 7.42 - 7.13 (15H ,m, Aromatic H), 6.58 (1H, d, J=7.7Hz, CONH), 5.75 (1H, m, CONHMe), 5.20 - 5.05 (4H, m, OCH_2Ph), 4.50 (1H, dt, J= 6.9,7.7Hz, $CHCH_2Ph$), 3.79 (1H, d, J= 9.1Hz, $CH(CO_2Bn)$), 3.15 - 2.91 (2H, m, CH_2Ph), 2.65 (3H, d, J= 4.8Hz, $CONHCH_3$), 1.52 (1H, m, $CHCH_2CH$), 1.32 (1H, m, $CH(CH_3)$), 1.05 (1H, m, $CHCH_2CH$), and 0.74 (6H, 2xd, J= 6.5Hz, $CH(CH_3)_2$)

e) [4-Hydroxy-2R-isobutyl-3-ethenylsuccinyl]-L-phenylalanine-N-methylamide.

[4-Benzyloxy-3-benzyloxycarbonyl-2R-isobutylsuccinyl]-L-phenylalanine-N-methylamide (29.6g, 53mmol) was taken up in ethanol, ammonium formate (16.7g, 265mmol) added followed by 10% palladium on charcoal (6g) as a slurry in isopropyl alcohol. After 30 minutes at room temperature the catalyst was removed by filtration, then washed with ethanol to give a solution of the crude diacid. To this was added piperidine (5.0g) and the mixture stirred at room temperature for 15 minutes before addition of aqueous formaldehyde (40%

solution, 25ml). After 18 hours at room temperature the mixture was refluxed for 1 h. Solvents were removed under reduced pressure and the residue partitioned between ethyl acetate and citric acid. The acid layer was extracted with further portions of ethyl acetate (2x250ml), the combined organic layers were extracted with potassium carbonate (3x200ml). These base extracts were acidified to pH 4 and re-extracted with DCM then the organic layer dried over magnesium sulphate. Solvent removal under reduced pressure gave the desired product as a white solid (9.35g, 27.0mmol, 51%).

m.p. 149-151°C

delta$_H$ (250MHz, $CDCl_3$) 8.37 (2H, d, J= 9.0Hz, CONH), 7.39 (1H, m, CONHMe), 7.27 - 7.06 (5H, m, Aromatic H), 6.40 (1H, s, CH_2CHCO_2H), 5.78 (1H, s, CH_2CHCO_2H), 4.93 (1H, q, J= 7Hz, $CHCH_2Ph$), 3.92 (1H, m, $CH_2CHCONH$), 2.95 (2H, m, CH_2Ph), 2.71 (3H, d, J= 4.1Hz, $NHCH_3$), 1.68 (1H, m), 1.45 (2H, m), and 0.86 (6H, 2xd, J= 5.8Hz, $CH(CH_3)_2$).

delta$_C$ (63.9Hz, $CDCl_3$) 173.3, 172.8, 169.6, 139.1, 136.3, 129.2, 128.3, 127.0, 126.6, 54.4, 43.5, 41.4, 39.1, 26.2, 25.7, 22.5 and 22.4

f) [4-Hydroxy-2R-isobutyl-3S-(phenylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide

[4-Hydroxy-2R-isobuty-3-ethenylsuccinyl]-L-phenyl-alanine-N-methylamide (15.0g, 44mmol) was dissolved in thiophenol

(150ml) and the mixture stirred in the dark under nitrogen at 60° for 2 days. Ether was added to the cooled reaction mixture and the precipitated product collected by filtration. The solid was washed with large volumes of ether and dried under vacuum to give the title compound (13.1g, 28.7mmol, 65%).

m.p. 199-201°C
Analysis calculated for $C_{25}H_{32}N_2O_4S$
Requires C 65.76 H 7.06 N 6.14 S 7.02
Found C 65.69 H 7.06 N 6.07 S 7.05

$delta_H$ (250MHz, D_6-DMSO) 8.40 (1H, d, J= 9Hz, CONH), 7.82 (1H, m, CONHMe), 7.35 - 7.10 (7H, m, Aromatic H), 7.04 (3H, m, Aromatic H), 4.62 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J= 14,5Hz, $CHCH_2Ph$), 2.89 (1H, dd, J= 14,9Hz, $CHCH_2Ph$), 2.62 (3H, d, J= 4.5Hz, $CONHCH_3$), 2.41 (3H, m, 2xCH + CH_2SPh), 2.23 (1H, d, J= 12Hz, CH_2SPh), 1.43 (1H, m, $CHCH_2CH$), 1.30 (1H, bm, $CH(CH_3)_2$), 0.90 (1H, m, $CHCH_2CH$) and 0.78 (6H, 2xd, J= 6.5Hz, $CH(CH_3)_2$.

g) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthio-methyl) succinyl]-L-phenylalanine-N-methylamide

[4-Hydroxy-2R-isobutyl-3S-(phenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide (16.8g, 37 mmol) and HOBT (6.6g, 44 mmol) were dissolved in DCM / DMF (4:1) and the mixture cooled to 0° before adding WSCDI (8.5g, 44 mmol) and NMM (4.5g, 44 mmol). The mixture was stirred at 0° for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (3.8g, 55 mmol) and NMM (5.6g, 55 mmol) were dissolved in DMF then this mixture added dropwise to the cooled

solution of the activated ester. After 1h the reaction was poured into ether / water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water then dried under vacuum at 50°. This material was recrystallised from methanol / water (1:1) to remove a trace of the minor diastereomer (9.03g, 19.2 mmol, 52%).

m.p. 227-229°C

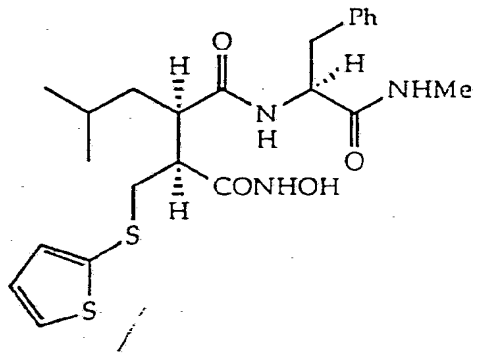
$[\alpha]_D$ = -88° (c = 10 , methanol)

δ_H (250MHz, D_6-DMSO) 8.84 (1H, d, J= 1.5Hz, NHOH), 8.35 (1H, d, J= 8.7Hz, CONH), 7.87 (1H, m, CONHMe), 7.29 - 6.92 (11H, m, Aromatic H + NHOH), 4.60 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J= 13.5,4.3, $CHCH_2Ph$), 2.77 (1H, dd, J= 13.5,10, $CHCH_2Ph$), 2.60 (3H, d,J= 4.6Hz), 2.53 (1H, m), 2.41 (1H, m), 2.20 (1H, dd, J= 13.4,2.2Hz, CH_2SPh), 2.09 (1H, dd, J=13.4,2.4Hz, CH_2SPh), 1.38 (2H, m, $CHMe_2$ + $CHCH_2CH$), 0.88 (1H, m, $CHCH_2CH$), 0.82 (3H, d, J= 6.4Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J+ 6.4Hz, $CH(CH_3)_2$).

δ_C (63.9MHz, D_6-DMSO) 172.9, 171.6, 166.3, 138.1, 136.7, 129.1, 128.9, 128.0, 127.3, 126.4, 125.2, 54.2, 46.4, 46.0, 37.7, 32.4, 25.6, 25.2, 24.2, and 21.7.

Example 2

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



a) [4-N-Hydroxy-2R-isobutyl-3S-(thiophenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide

The title compound was prepared from [4-Hydroxy-2R-isobutyl-3-ethenylsuccinyl]-L-phenylalanine-N-methylamide (400mg, 1.16mmol) by the method described in example 1f, substituting thiophenethiol in the place of thiophenol to give a material (320mg, 0.73mmol, 63%) with the following characteristics.

m.p. 184-186°C

delta$_H$ (250MHz, D_6-DMSO) 8.29 (1H, d, J= 8.1Hz, CONH), 7.84 (1H, m, CONHMe), 7.57 (1H, d, J= 5.1Hz, Thiophene H), 5H, m, Aromatic H), 7.00 (2H, m, Thiophene H), 4.50 (1H, m, $CHCH_2Ph$), 2.91 (1H, m, $CHCH_2Ph$), 2.75 (1H, m, $CHCH_2Ph$), 2.56 (3H, d, J= 4.0Hz, $CONHCH_3$), 2.34 (3H, m), 1.99 (1H, d, J= 9.3Hz,

$C\underline{H}_2SHet$), 1.42 (1H, m, $CHC\underline{H}_2CH$), 1.29 (1H, bm, $C\underline{H}(CH_3)_2$), 0.87 (1H, m, $CHC\underline{H}_2CH$), 0.79 (3H, d, J= 6.4Hz, $CH(C\underline{H}_3)_2$), and 0.72 (3H, d, J= 6.4Hz, $CH(C\underline{H}_3)_2$).

b) [4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiophenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide

Prepared by the method described in example 1g to give material with the following characteristics

m.p. 236-238°C

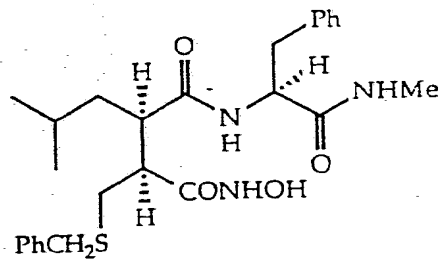
Analysis calculated for $C_{23}H_{30}N_2O_4S_2$
Requires C 57.84 H 6.54 N 8.80
Found C 57.64 H 6.48 N 8.85

$delta_H$ (250MHz, D_6-DMSO) 8.80 (1H, s, CONHO$\underline{H}$), 8.08 (1H, d, J=8Hz, CON$\underline{H}$), 7.52 (1H, m, CON$\underline{H}$Me), 7.32 (1H, dd, J= 4.6,2.9Hz, Thiophene H), 7.17 - 6.95 (5H, m, Aromatic H), 6.89 (2H, m, Thiophene H), 4.46 (1H, m, $CHC\underline{H}_2Ph$), 2.89 (1H, dd, J=13.6,4.4Hz, $CHC\underline{H}_2Ph$), 2.72 (1H, dd, J= 13.6,10.5Hz, $CHC\underline{H}_2Ph$), 2.54 (3H, d, J= 4.3Hz, $CONHC\underline{H}_3$), 2.46 (1H, d, J= 12.1Hz, $C\underline{H}_2S$), 2.35 (1H, bt, J= 10.2Hz), 2.14 (1H, bt, J= 10.2Hz), 1.98 (1H, dd, J=12.7,2.5Hz, $CHC\underline{H}_2Ph$), 1.35 (1H, bt, J= 11.4Hz, $CHC\underline{H}_2CH$), 1.22 (1H, bm, $CH(C\underline{H}_3)_2$), 0.86 (1H, bt, J=12.6Hz, $CHC\underline{H}_2CH$), 0.74 (3H, d, J= 6.3Hz, $CH(C\underline{H}_3)_2$), and 0.68 (3H, d, J= 6.4Hz, $CH(C\underline{H}_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.5, 171.6, 166.1, 138.0, 133.8, 132.7, 129.4, 129.2, 128.1, 127.8, 126.5, 54.2, 46.2, 46.0, 38.5, 37.6, 25.8, 25.2, 24.2, and 21.7.

Example 3

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



Prepared by the method described in example 1g to give material with the following characteristics

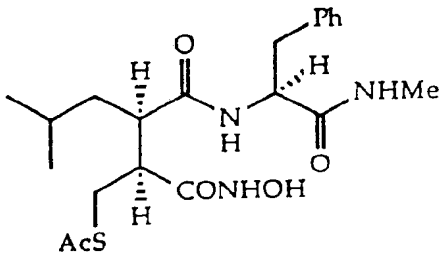
m.p. °

Analysis calculated for $C_{27}H_{37}N_3O_5S.0.5H_2O$
Requires C 61.81 H 7.30 N 8.00
Found C 61.85 H 7.15 N 7.45

$delta_H$ (250MHz, D_6-DMSO) 8.40 (1H, s, CONHOH), 8.22 (1H, m, NHMe), 7.20 (5H, m, Aromatic H), 6.58 (4H, m), 4.10 (1H, m, $CHCH_2Ph$), 3.22 (3H, s, OCH_3), 3.04 - 2.45 (4H, m, $2xCH_2Ar$), 2.42 (3H, d, J= 6Hz, $NHCH_3$), 2.32 - 2.08 (4H, m), 0.78 (2H, m, $CHCH_2CH$), and 0.40 - 0.18 (7H, m, $(CH_3)_2CH$).

Example 4

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



Prepared by the method described in example 1g to give material with the following characteristics

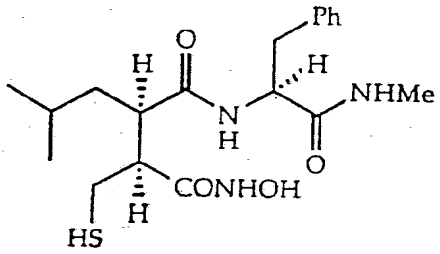
m.p. 226-227°C

Analysis calculated for $C_{21}H_{31}N_3O_5S.H_2O$
Requires C 55.37 H 7.30 N 9.22
Found C 55.57 H 6.99 N 9.53

$delta_H$ (250MHz, D_6-DMSO) 8.84 (1H, s, NHOH), 8.36 (1H, d, J= 8Hz, CONH), 7.80 (1H, d, J= 6Hz, NHMe), 7.20 (%h, m, Aromatic H), 4.58 (1H, m, $CHCH_2Ph$), 3.16 - 2.62 (2H, m, $CHCH_2Ph$), 2.54 (3H, d, J= 4Hz, $NHCH_3$), 2.22 (3H, s, CH_3COS), 2.36 - 2.10 (4H, m, $CHCHCH_2S$), 1.36 (2H, m, $CHCH_2CH$), and 0.98 - 0.66 (7H, m, $CH(CH_3)_2$).

Example 5

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(thiolmethyl) succinyl]-L-phenylalanine-N-methylamide



[4-(N-Hydroxyamino)-2R-isobutyl-3S-(acetylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (30mg, 0.06mmol) was stirred in methanol (3ml) with methylamine (1ml methanolic solution) at room temperature. After 30 minutes the crystalline product (20mg, 0.05mmol, 74%) was filtered off and dried.

m.p. 234°C

Analysis calculated for $C_{19}H_{39}N_3O_4S.1.5H_2O$

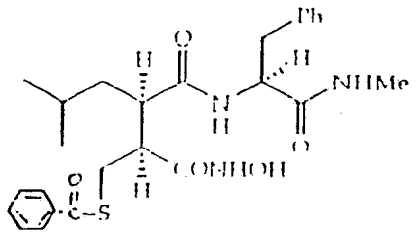
Requires C 54.10 H 7.63 N 9.94 S 7.60

Found C 54.28 H 7.16 N 10.43 S 7.80

$delta_H$ (250MHz, D_6-DMSO) 8.28 (1H, d, J= 9Hz, NHOH), 7.80 (1H, m, NHMe), 7.22 (5H, m, Aromatic H), 4.60 (1H, m, $CHCH_2Ph$), 3.08 - 2.56 (2H, m, $CHCH_2Ph$), 2.50 (3H, d, J= 4Hz, $NHCH_3$), 2.40 - 2.02 (4H, m, $CHCHCH_2SH$), 1.44 - 1.22 (2H, m, $CHCH_2CH$) and 0.98 - 0.72 (7H, m, $CH(CH_3)_2$).

Example 6

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(benzoylthiomethyl)-succinyl]-L-phenylalanine-N-methylamide



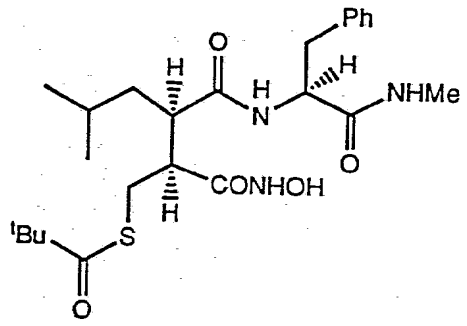
The title compound was prepared by the method described in Example 1g to give material with the following characteristics

m.p. 227 - 228°
Analysis calculated for $C_{21}H_{31}N_3O_5S$
Requires C 62.50 H 6.66 N 8.41
Found C 62.32 H 6.67 N 8.40

$delta_H$ (250 MHz, $CDCl_3$:D_6DMSO (1:1)) 8.82 (1H, s, NHOH), 8.25 (1H, d, J=8.4Hz, NHOH), 7.87 (2H, dd, J=8.5, 1.1Hz), 7.60 (2H, m, Ar-H and CONH), 7.50 (2H, t, J=8.2Hz), 7.28 (2H, d, J=8.4Hz), 7.16 (2H, t, J=7.2Hz), 7.04 (1H, t, J=8.5Hz), 4.65 (1H, m, $CHCH_2Ph$), 3.06 (1H, dd, J=14.1, 5.0Hz, $CHCH_2Ph$), 2.90 (1H, dd, J=13.9, 10Hz, $CHCH_2Ph$), 2.73 (2H, m SCH_2Ph), 2.65 (3H, d, J=4.7Hz, NHMe), 2.33 (1H, dt, J=11.0, 4.7Hz), 1.51 (1H, t, J=7Hz, CH_2CHMe_2), 1.24 (1H, m, $CHMe_2$), 0.97 (1H, t, J=7Hz, CH_2CHMe_2), 0.84 (3H, d, J=6.5Hz, $CHMe_2$) and 0.79 (3H, d, J=6.5Hz, $CHMe_2$).

Example 7

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(pivaloylthiomethyl) succinyl]-L-phenylalanine-N-methylamide



[4-Hydroxy-2R-isobutyl-3S-(pivaloylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,8g, 1.7 mmol) and HOBT (0.31g, 2.1 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.4g, 2.1mmol) and NMM (0.21g, 2.1mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.18g, 2.6mmol) and NMM (0.26g, 2.6mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.38g, 0.7mmol, 45%).

m.p. 225°C

[alpha]$_D$ = -3.5° (c=2, methanol)

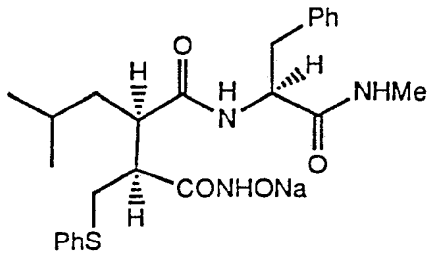
Analysis calculated for $C_{24}H_{39}N_3O_5S.0.5\ H_2O$
Requires: C58.99 H7.84 N8.60
Found: C58.96 H7.63 N8.55

delta$_H$ (250MHz, D_6-DMSO) 8.81 (1H, s, J = 1.5Hz, NHOH), 8.30 (1H, d, J=8Hz, CONH), 7.78 (1H, d, J=6Hz, CONHMe), 7.27-7.03 (5H, m, aromatic H), 4.54 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J = 12,5Hz, $CHCH_2Ph$), 2.79 (1H, dd, J = 13,10Hz, $CHCH_2Ph$) 2.56 (3H, d, J = 4.5Hz, $NHCH_3$), 2.44 (2H, m), 2.20 (1H, dd, J = 13,3Hz, CH_2S), 2.07 (1H, dt), 1.36 (2H, m), 1.13 (9H, s, $C(CH_3)_3$), 0.87 (1H, m, $CH_2CH(CH_3)_2$), 0.79 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

delta$_C$ (63.9MHz, D_6-DMSO) 172.55, 171.59, 168.24, 138.03, 129.18, 128.00, 126.24, 54.21, 46.48, 45.84, 45.55, 37.61, 28.30, 27.13, 25.64, 25.25, 24.24, and 21.63.

Example 8

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide sodium salt



[4-(N-Hydroxyamino)-2R-isobutyl-3S-(phenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,2g, 0.4 mmol) was dissolved in 20ml of methanol and 1eq of 0.1N NaOH(aq) added. The solvent was removed in vacuo and the residue dissolved in water and freeze-dried (0.21g,0.4 mmol,100%).

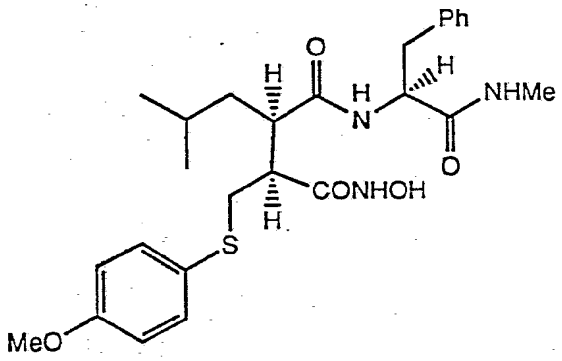
m.p. 184°C

$[\alpha]_D$ = -7.7° (c=2, methanol)

δ_H (250MHz, D_6-DMSO) 8.62 (1H, s, J = 1.5Hz, NHOH), 8.28 (1H, d, J = 8Hz, CONH), 7.26 - 7.04 (10H, m, aromatic H), 4.43 (1H, m, $CHCH_2Ph$), 3.00 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.84 (1H, dd, J = 14,10Hz, $CHCH_2Ph$), 2.55 (3H, d, J = 4.5Hz, $NHCH_3$), 2.46 (3H, m), 2.21 (1H, m), 1.39 (1H, m), 1.14 (1H, m), 1.00 (1H,m), and 0.70 (6H, d , J = 5.7Hz)

Example 9

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)



succinyl]-L-phenylalanine-N-methylamide[4-Hydroxy-2R-isobutyl-3S-(4-methoxyphenylthiomethyl)succinyl]-L-phenylalanine-N-methylamide (0,5g, 1 mmol) and HOBT (0.18g, 1.2 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.23g, 1.2mmol) and NMM (0.12g, 1.2mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.1g, 1.5mmol) and NMM (0.15g, 1.5mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.36g, 0.7mmol, 72%).

m.p. 225°C

$[\alpha]_D$ = +8° (c=0.5, methanol)

Analysis calculated for $C_{26}H_{35}N_3O_5S$
Requires: C62.25 H7.04 N8.38
Found: C62.43 H7.09 N8.37

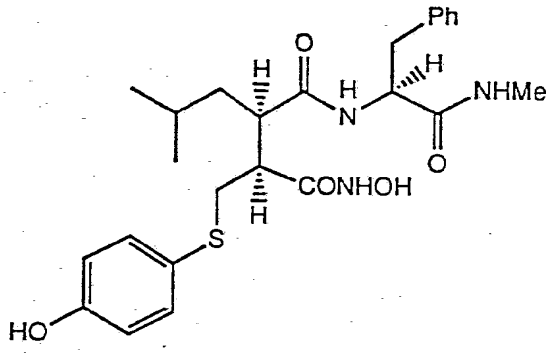
δ_H (250MHz, D_6-DMSO) 8.83 (1H, s, J = 1.5Hz, NHOH), 8.28 (1H, d, J = 8Hz, CONH), 7.83 (1H, d, J = 6Hz, CONHMe), 7.28 - 6.86 (9H, m, aromatic H), 4.52 (1H, m, CHCH$_2$Ph), 3.73 (3H, s, OCH3), 2.91 (1H, dd, J = 14,4Hz, CHCH$_2$Ph), 2.75 (1H, dd, J = 14,10Hz, CHCH$_2$Ph), 2.57 (3H, d, J = 4.5Hz, NHCH$_3$), 2.50 - 2.34 (2H,m), 2.16 -

1.99 (2H, m, $CH_2CH(CH3)_2$) 1.36 (2H, m), 0.88 (1H, m, $C\underline{H}_2CH(CH_3)_2$), 0.80 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$), and 0.73 (3H, d, J = 6Hz, $CH(C\underline{H}_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.79, 171.62, 168.39, 138.14, 131.34, 129.19, 128.00, 126.44, 114.59, 55.32, 54.20, 38.68, 25.63, 25.17, 24.26, and 21.70.

Example 10

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-hydroxyphenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide



[4-Hydroxy-2R-isobutyl-3S-(4-hydroxyphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,4g, 0.8 mmol) and HOBT (0.15g, 1.0 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (0.20g, 1.0mmol) and NMM (0.1g, 1.0mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (0.09g, 1.3mmol) and NMM (0.13g,1.3mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1)

whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.13g, 0.2mmol, 31%).

m.p. 216°C

$[\alpha]_D$ = -65° (c=0.5, methanol)

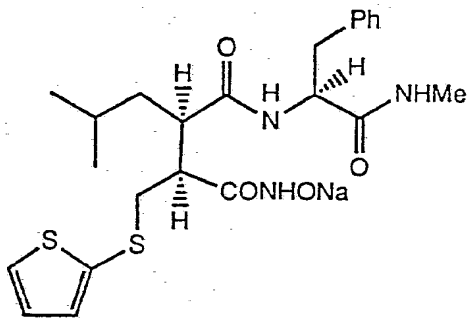
Analysis calculated for $C_{25}H_{33}N_3O_5S$
Requires: C61.58 H6.82 N8.62
Found: C61.43 H6.81 N8.08

δ_H (250MHz, D_6-DMSO) 8.82 (1H, s, J = 1.5Hz, NHOH), 8.26 (1H, d, J = 8Hz, CONH), 7.81 (1H, d, J = 6Hz, CONHMe), 7.27 - 6.64 (9H, m, aromatic H), 4.49 (1H, m, $CHCH_2Ph$), 2.90 (1H, dd, J=14,4Hz, $CHCH_2Ph$), 2.74 (1H, dd, J=14,10Hz, $CHCH_2Ph$), 2.57 (3H, d, J = 4.5Hz, $NHCH_3$), 2.54 - 2.29 (2H, m), 2.14 - 1.98 (2H, m, $CH_2CH(CH3)_2$), 1.35 (2H, m), 0.88 (1H, m, $CH_2CH(CH_3)_2$), 0.80 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.73 (3H, d, J = 6Hz, $CH(CH_3)_2$).

δ_C (63.9MHz, D_6-DMSO) 172.81, 171.66, 168.46, 156.50, 133.02, 132.17, 129.17, 128.02, 126.44, 124.17, 116.00, 54.20, 46.35, 46.13, 37.59, 35.40, 25.62, 25.16, 24.27, and 21.69.

Example 11

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt



[4-Hydroxyamino)-2R-isobutyl-3S-(2-thiophenethiomethyl) succinyl]-L-phenylalanine-N-methylamide (0,2g, 0.4 mmol) was dissolved in 20ml of methanol and 1eq of 0.1N-NaOH(aq) added. The solvent was removed in vacuo and the residue dissolved in water and freeze-dried (0.21g,0.4 mmol,100%).

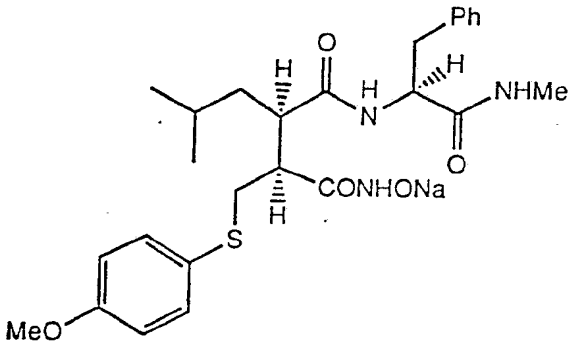
m.p. 170°C

$[\alpha]_D$ = -67° (c=1, methanol)

δ_H (250MHz, d_6-DMSO), 7.51 (1H, d), 7.19 - 6.97 (8H, m, aromatic H), 4.32 (1H, m, $C\underline{H}CH_2Ph$), 3.00 (1H, dd, J = 14,4Hz, $CHC\underline{H}_2Ph$), 2.84 (1H, dd, J = 14,10Hz, $CHC\underline{H}_2Ph$) 2.53 (3H, d, J = 4.5Hz, $NHC\underline{H}_3$), 2.46 2.19 (3H, m), 1.37 (1H, m), 1.09 (1H, m), 0.93 (1H, m), and 0.67 (6H, m)

Example 12

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenyl-thiomethyl)succinyl]-L-phenylalanine-N-methylamide sodium salt



[4-Hydroxyamino)-2R-isobutyl-3S-(4-methoxyphenylthio-methyl)succinyl]-L-phenylalanine-N-methylamide (0,1g, 0.2 mmol) was dissolved in 20ml of methanol and 1eq of 0.1N NaOH(aq) added. The solvent was removed in vacuo and the residue dissolved in water and freeze-dried (0.1g,0.2 mmol,100%).

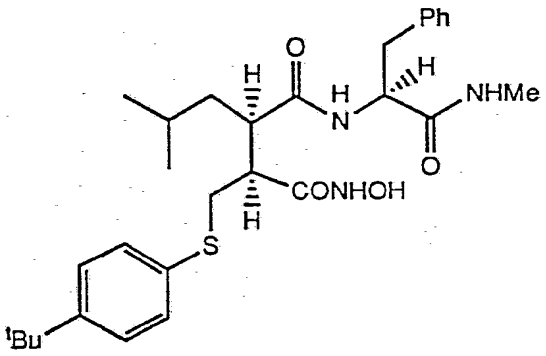
m.p. 174°C

$[\alpha]_D$ = -58° (c=1, methanol)

δ_H (250MHz, D_6-DMSO 7.26 - 7.04 (10H, m, aromatic H), 4.31 (1H, m, $C\underline{H}CH_2Ph$), 3.73 (3H, s, $OC\underline{H}_3$), 3.25 - 2.72 (2H, m, $CHCH_2Ph$), 2.50 (3H, s, $NHC\underline{H}_3$), 2.36 (1H, m), 2.15 (1H, m), 1.37 (1H, m), 0.95 (1H, m), and 0.69 (6H, d, $CHCH_2(C\underline{H}_3)_2$).

Example 13

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(4-tertbutylphenyl-thiomethyl) succinyl]-L-phenylalanine-N-methylamide



[4-Hydroxy-2R-isobutyl-3S-(4-tertbutylphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide (5.0g, 10 mmol) and HOBT (1.76g, 12 mmol) were dissolved in 1:1 DCM/DMF and the mixture cooled to 0°C before adding WSDCI (2.3g, 12mmol) and NMM (1.2g, 12mmol). The mixture was stirred at 0°C for 1h to ensure complete formation of the activated ester. Hydroxylamine hydrochloride (1.0g, 15mmol) and NMM (1.2g, 15mmol) were dissolved in DMF then this mixture was added dropwise to the cooled solution of the activated ester. After 1h the reaction was poured into ether/water (1:1) whereupon the desired product precipitated as white crystals. These were collected by filtration, further washed with ether and water, then dried under vacuum at 50°C. This material was repeatedly recrystallised from methanol/water (1:1) to remove a trace of the minor diastereomer (0.7g, 1.3mmol, 14%).

M.p. 188.5 -190°C

Analysis calculated for $C_{29}H_{41}N_3O_4S$
Requires: C66.00 H7.83 N7.96
Found: C65.80 H7.81 N7.76

$delta_H$ (250MHz, D_6-DMSO) 8.83 (1H, s, NHOH), 8.33 (1H, d, J = 8Hz, CONH), 7.86 (1H, d, J = 6Hz, CONHMe), 7.28 - 6.90 (9H, m, aromatic H), 4.60 (1H, m, $CHCH_2Ph$), 2.94 (1H, dd, J = 14,4Hz, $CHCH_2Ph$), 2.77 (1H, dd, J = 14,10Hz, $CHCH_2Ph$), 2.58 (3H, d, J = 4.5Hz, $NHCH_3$), 2.55 - 2.37 (2H, m), 2.22 - 2.08 (2H, m, $CH_2CH(CH3)_2$), 1.37 (2H, m), 1.26 (9H, s, $C(CH_3)_3$), 0.88 (1H, m, $CH_2CH(CH_3)_2$), 0.81 (3H, d, J = 6Hz, $CH(CH_3)_2$), and 0.74 (3H, d, J = 6Hz, $CH(CH_3)_2$).

$delta_C$ (63.9MHz, D_6-DMSO) 172.88, 171.59, 168.34, 147.87, 138.10, 133.09, 129.13, 127.95, 127.45, 126.36, 125.70, 54.19, 54.20, 46.38, 46.06, 37.70, 34.20, 32.79 31.24, 25.64, 25.19, 24.25, and 21.72.

Example 14

[4-(N-Hydroxyamino)-2R-isobutyl-3S-(2,4-dimethylphenylthiomethyl) succinyl]-L-phenylalanine-N-methylamide