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Vogelna ul. 10, 1001 Ljubljana (SI). **MLINSEK, Gregor**
[SI/SI]; Virmase 202, 4220 Skofja Loka (SI).

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(71) Applicants (*for all designated States except US*): **UNIVERZA V LJUBLJANI** [SI/SI]; Fakulteta za farmacijo, Askeceva 7, 1000 Ljubljana (SI). **LEK PHARMACEUTICAL AND CHEMICAL COMPANY D.D.** [SI/SI]; Legal Affairs and Industrial Property, Industrial Property Department, Verovskova 57, 1526 Ljubljana (SI).

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(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **URLEB, Uros** [SI/SI]; Preiceva 29, 1000 Ljubljana (SI). **ZEGA, Anamarija** [SI/SI]; Jozeta Pahorja 4, 6210 Ljubljana (SI). **STEGNAR, Mojca** [SI/SI]; Pod hrasti 60, 1000 Ljubljana (SI). **TRAMPUS, Bakija, Alenka** [SI/SI]; Jakopiceva 2, 1000 Ljubljana (SI). **SOLMAJER, Tomaz** [SI/SI];

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(54) Title: AMIDINOPHENYLALANINE DERIVATIVES AS THROMBIN INHIBITORS

(57) Abstract: The compounds of the formula (formula 1) and pharmaceutically acceptable salts thereof and a process for preparing the same and pharmaceutical compositions containing the same are described wherein the substituents have the meaning as specified in the description. The compounds are used as thrombin inhibitors.



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AMIDINOPHENYLALANINE DERIVATIVES AS THROMBIN INHIBITORS

Technical Field

The invention belongs to the field of pharmaceutical industry and relates to novel azaphenylalanine derivatives, procedures for their preparation and pharmaceutical compositions containing them. Novel azaphenylalanine derivatives have the enzymatic activity (serine proteases) and anticoagulant effect.

Technical Problem

There exists a constant need for new medicinal products with anticoagulant activity that may be administered either orally or parenterally and that are highly selective and of low toxicity. Recently, low molecular weight thrombin inhibitors appear to be of growing importance in this area.

Prior Art

Thrombin as a serine protease is one of key enzymes in the processes of blood coagulation and in the development of thrombosis (Edit, J. F.; Allison, P.; Noble, S.; Ashton, J.; Golino, P.; McNard, J.; Buja, L. M.; Willerson, J. T., *J. Clin. Invest.* 1989, 84, 18.).

The crystalline structure of serine proteases of thrombin and serine is known (Bode, W.; Turk, D.; Karshikov, A. J., *Protein Sci.* 1992, 1, 426).

Currently used anticoagulant agents are toxic and of limited effectiveness.

Low molecular weight thrombin inhibitors should have selective efficacy and can be used in oral administration. There are reversible and irreversible thrombin antagonists (Kimball, S. D., *Curr. Pharm. Design* 1995, 1, 441, Das, J.; Kimball, S. D., *Bioorg. Med. Chem.* 1995, 3, 999, Kimball, S. D., *Blood Coagulation and*

Fibrinolysis 1995, 6, 511, Breznik, M.; Peèar, S., *Farm. Vestn.* 1997, 48, 545, Sanderson, P. E. J.; Naylor-Olsen, A. M.; *Current Med. Chem.* 1998, 5, 289, Rewinkel, J. B. M.; Adang, A. E. P., *Curr. Pharm. Design* 1999,5,1043,Wiley, M. R.; Fisher, M. J.; *Exp. Opin. Ther. Patents* 1997, 7, 1265, Menear, K.; *Current Med. Chem.* 1998, 5, 457.

The majority of reversible thrombin antagonists derive from peptidomimetically modified structure D-Phe-Pro-Arg. The aim of the modification is to provide chemical stability, selectivity and effectiveness.

The active substance used in Japan and the USA is argatroban (Kikumoto, R.; Tamao, Y.; Tezuka, T.; Tonomura, S.; Hara, H., Ninomiya, K.; Hijikata, A.; Okamoto, S., *Biochemistry* 1984, 23, 85).

Amidinophenylalanine derivatives and aminopyridylalanine derivatives have selective antithrombotic activity (Danilewicz, J., et al. WO 95/13274 (1995)).

By replacing amidino-piperidine P1 group with a weakly basic 2-amino-6-methylpyridine structure, a selective and effective thrombin inhibitor that can be used in oral administration has been obtained (Sanderson, P. E. J.; et al., *Bioorg. Med. Chem.* 1998, 8, 817).

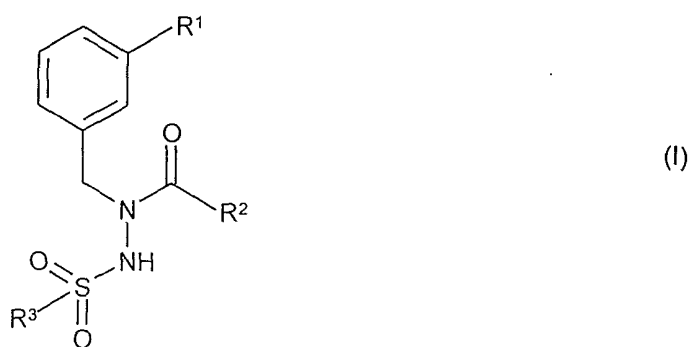
SI 20025 describes thrombin inhibitors which are the derivatives of azaphenylalanine.

Accordingly, the object of the present invention is to overcome the problems encountered in the prior art.

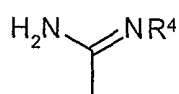
Description of the solution of technical problem including examples

The above problems are solved e.g. by a compound as defined in claim 1 and a process as defined in claim 6 as well as pharmaceutical compositions as defined in claim 8.

The invention relates particularly to novel azaphenylalanine derivatives and analogs thereof of the general formula (I)



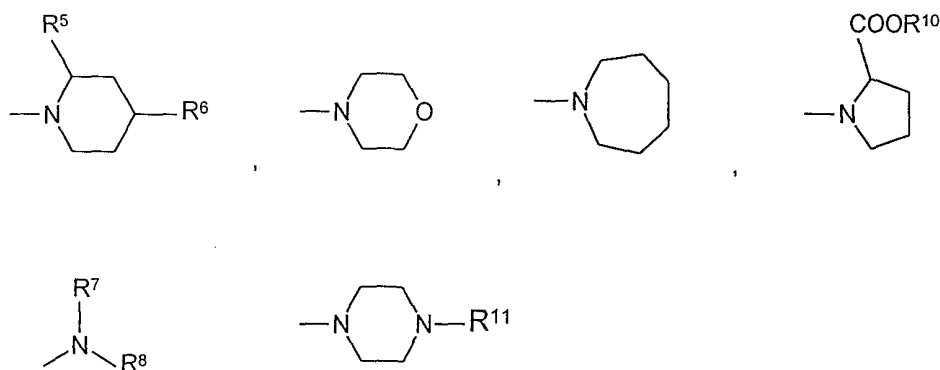
wherein



R¹ represents a residue of the formula

wherein R⁴ = H, alkyl (C₁-C₃), OH, O-alkyl (C₁-C₃), NH₂;

R² represents a residue of the formula



wherein R⁵ = H, alkyl (C₁-C₃), CF₃, COOR⁹,

R⁶ = H, alkyl (C₁-C₃), CF₃, COOR⁹,

R⁷ = alkyl (C₁-C₃), cycloalkyl (C₃-C₆),

R⁸ = alkyl (C₁-C₃), cycloalkyl (C₃-C₆),

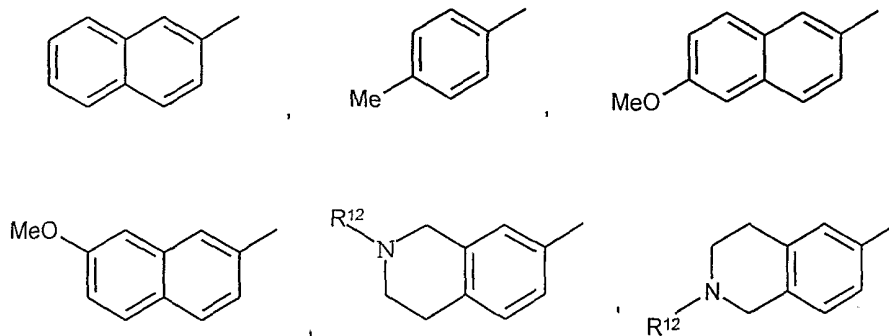
R⁹ = H, alkyl (C₁-C₃),

R¹⁰ = H, alkyl (C₁-C₃), benzyl

R¹¹ = H, SO₂Me, CO₂Me;

R³ represents a residue of the formula

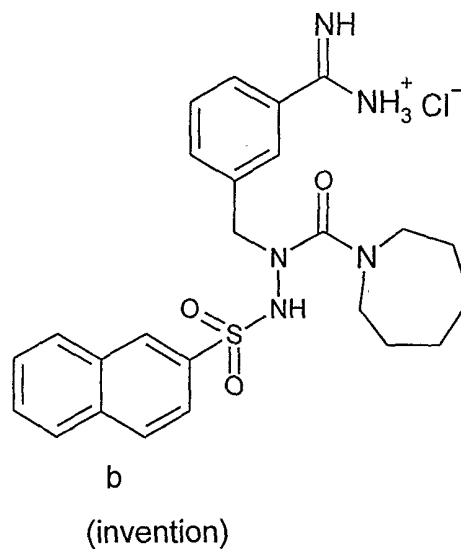
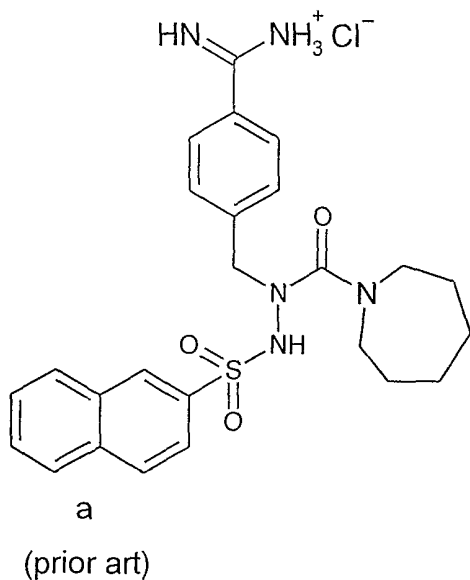
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wherein $R^{12} = \text{H}$, alkyl ($\text{C}_1\text{-C}_3$), acetyl

and pharmaceutically acceptable salts thereof.

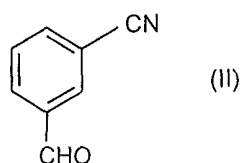
In comparison to the compounds, described in SI 20025, the compounds of the present invention are of greater activity. K_i thrombin (μM) of the compound a, according to the patent SI 20025, is 0.032, K_i thrombin (μM) of the compound b, according to the present invention, is 0.005.



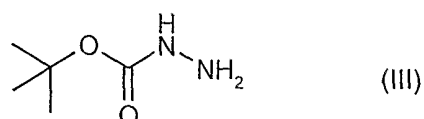
The invention also particularly relates to a process for the preparation of azaphenylalanine derivatives and analogs of the general formula (I).

They may be suitably prepared as follows:

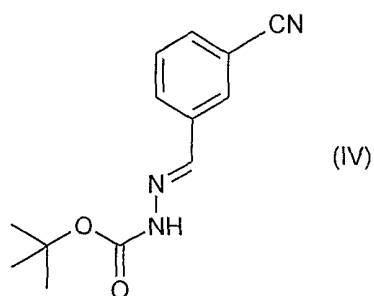
a) 3-cyanobenzaldehyde of formula (II)



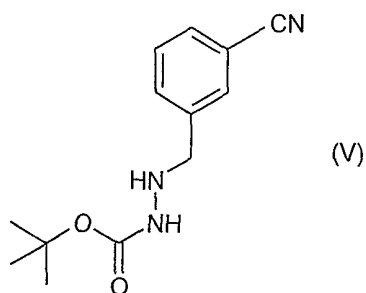
is converted with BOC-carbazate of the formula (III)



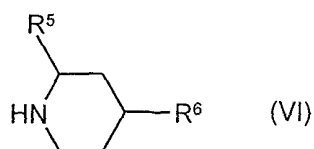
to the compound of the formula (IV),



which is subjected to reduction by catalytic hydrogenation or with NaCNBH_3 and is converted to the compound (V),

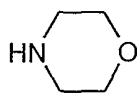


which reacts with triphosgene and amine of the formula (VI),

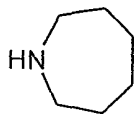


wherein R^5 and R^6 have the same meanings as in the formula (I),

or with triphosgene and amine of the formula (VII or VIII),

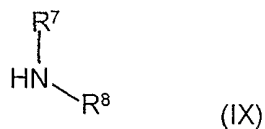


(VII)



(VIII)

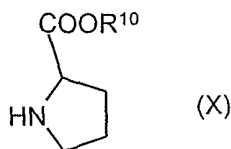
or with triphosgene and amine of the formula (IX),



(IX)

wherein R^7 and R^8 have the same meanings as in the formula (I),

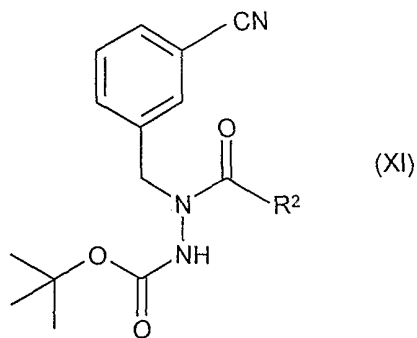
or with triphosgene and amine of the formula (X),



(X)

wherein R^{10} has the same meaning as in the formula (I),

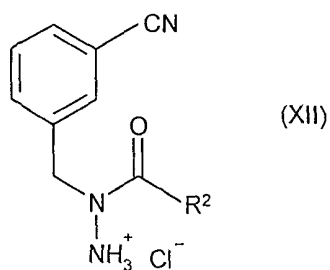
in „one pot“ reaction to the compound (XI)



(XI)

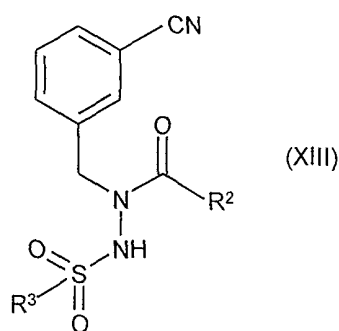
wherein R^2 has the same meaning as in the formula (I).

The protecting BOC group in the compound (XI) is removed with HCl (g) in HOAc at room temperature to obtain the compound (XII),



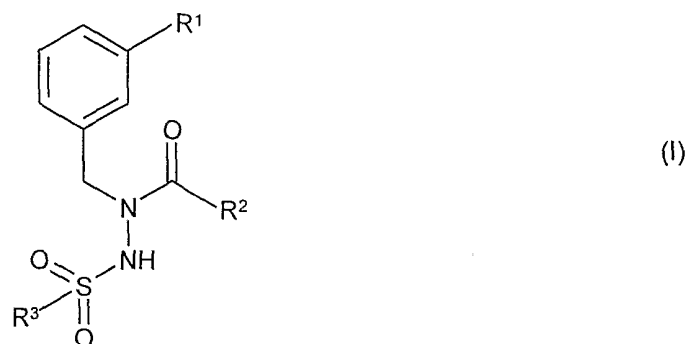
wherein R^2 has the same meanings as in the formula (I)

which reacts with aromatic sulfonylchloride up to the compound (XIII),



wherein R^2 and R^3 have the same meanings as in the formula (I).

The compound (XIII) is converted to the compound of the formula (I)



in a usual manner. This can be achieved e.g. by converting compound (XIII) by addition of a hydroxylamine and an anhydrous alcohol such as e.g.-ethanol or a mineral acid such as e.g. HCl(g) and e.g. ammonium acetate, thus obtaining compound (I).

When the compound (XIII) reacts with hydroxylamine in anhydrous ethanol, R⁴ denotes OH group, after the reaction of the compound (XIII) with HCl (g) and ammonium acetate R⁴ denotes hydrogen.

The starting compounds are prepared, unless otherwise directed, according to the procedures described in the literature; e.g., the compound of the formula IV as described by A. Fässler, et. al., J. Med. Chem. 1996, 39, 3203-3215.

The invention further relates to the use of compounds of the formula I as therapeutically active compounds. The novel compounds are preferably thrombin inhibitors. They inhibit thrombin and formation of fibrin. They are useful in the treatment or prevention of a variety of thrombosis forms: (i) venous thromboembolism due to formation of a thrombus within a vein (*venous thrombosis*) associated with acquired (prolonged bedrest, surgery, injury, malignancy, pregnancy and postpartum states or inherited (deficiency of natural coagulation inhibitors) risk factors, obstruction or occlusion of a lung artery by a detached thrombus (*pulmonary embolism*), (ii) cardiogenic thromboembolism due to formation of a thrombus in the heart associated with cardiac arrhythmia, heart valve defect, prosthetic heart valves or heart disease, embolism of peripheral arteries caused by a detached thrombus, most commonly in the brain (*ischemic stroke*), (iii) arterial thrombosis due to underlying atherosclerotic processes in the arteries which obstructs or occludes an artery and causes myocardial ischemia (*angina pectoris, acute coronary syndrome*) or heart muscle death cell (*myocardial infarction*), obstructs or occludes a peripheral artery (*ischemic peripheral artery disease*) and obstructs or occludes the artery after the procedure on the blood vessel (*reocclusion or restenosis after transluminal coronary angioplasty, reocclusion or restenosis after percutaneous transluminal angioplasty of peripheral arteries*) and (iv) in the number of states (e.g., in complications in pregnancy, in metastazing malignant diseases, after extensive injuries, in bacterial sepsis) when thrombogenic activation causes widespread formation of thrombi within the vascular system (*disseminated intravascular coagulation*).

The compounds of the present invention may be also used as an adjunct therapy in conjunction with thrombolytic therapy in recent myocardial infarction, in combination with aspirin in patients with unstable angina pectoris designed to undergo percutaneous transluminal angioplasty and in the treatment of patients with thrombosis and with heparin-induced thrombocytopenia.

The compounds of the present invention may further be used for the prevention of coagulation of blood which is in contact with nonbiological surfaces (vascular prosthesis, vascular stents, prosthetic heart valves, extracorporeal circulation systems, hemodialysis) and *in vitro* to prevent coagulation in biological samples for testing or storage.

The present invention also relates particularly to pharmaceutical compositions comprising the compounds of the formula I. They can be formulated as injectable or oral formulations. The active drug component may be combined with suitable standard additives with respect to the intended form of administration. The pharmaceutical compositions may be prepared according to the standard procedures. The preparation may be formulated in such a manner as to permit controlled and sustained release of the active ingredient. Dosage, frequency and mode of administration depend on a variety of factors, they also depend on individual active ingredient and its pharmacokinetic parameters and on the patient's therapeutic needs.

IN VITRO ASSAYS FOR DETERMINING PROTEINASE INHIBITION

Coagulation assays

The Anticoagulant effect of thrombin inhibitors was measured by thrombin time (TT), activated partial thromboplastin time (aPTT), and prothrombin time (PT) assays. Inhibitors were added to normal pooled human plasma over a range of concentrations and clotting was recorded in an automated coagulometer (Fibrintimer, Dade/Behring). The concentration of inhibitor that doubled the clotting time was determined for each assay.

1. Thrombin time (TT)

a) Principle of method

In a plasma sample thrombin converts fibrinogen into fibrin and the clot is formed. The time for clot formation was measured. Thrombin time is prolonged due to disorders in fibrin polymerisation or due to the presence of thrombin inhibitors.

b) Reagents

Thrombin (Test Thrombin Reagent, 1.5 IU/mL, Dade/Behring): lyophilized bovine thrombin was dissolved in 5 mL of HEPES (25 mmol/L, pH 7.4). The reagent was warmed to 37°C prior to the assay.

Normal pooled plasma: venous blood from 11 apparently healthy volunteers was collected (1 part 0.11 mol/L sodium citrate solution with 9 parts of blood) and centrifuged immediately at 2000 x g for 30 minutes at 4°C. Plasma was removed, pooled and stored in aliquots at -70°C until use.

Inhibitors: were dissolved in DMSO (10 mM stock solution) and diluted with distilled water to working solutions (the highest concentration 100 µM).

c) Method

Inhibitors (10 μL working solution, concentrations from 2.5 μM to 100 μM) and pooled plasma (90 μL) were incubated at 37°C for 5 minutes and then thrombin (200 μL) was added. Clot formation was measured in a coagulometer in duplicate.

2. Activated partial thromboplastin time (aPTT)

a) Principle of method

Incubation of plasma with the optimal quantity of phospholipids and a surface activator leads to activation of factors of the intrinsic coagulation pathway. The addition of calcium ions triggers the coagulation process. The time of fibrin clot formation was measured. aPTT was used as a screening test for coagulation disorders of the intrinsic coagulation pathway. It is prolonged due to intrinsic coagulation factors deficit or due to the presence of inhibitors.

b) Reagents

Pathromtin SL (Dade/Behring): silicon dioxide particles, vegetable phospholipids, sodium chloride (2.4 g/L), Hepes (14.3 g/L, pH 7.6), sodium azide (<1 g/L). The reagent was used at room temperature (15-25°C).

Calcium chloride solution: 0.025 mol/L, warmed to 37°C.

Normal pooled plasma: venous blood from 11 apparently healthy volunteers was collected (1 part 0.11 mol/L sodium citrate solution with 9 parts of blood) and centrifuged immediately at 2000 x g for 30 minutes at 4°C. Plasma was removed, pooled and stored in aliquots at -70°C until use.

Inhibitors: were dissolved in DMSO (10 mM stock solution) and diluted with distilled water to working solutions (the highest concentration 100 μM).

c) Method

Inhibitor (10 μL working solution, concentrations from 5 μM to 100 μM) and pooled plasma (90 μL) were incubated at 37°C for 5 minutes. Pathromtin (100 μL) was added and the sample was incubated for another 2 minutes at 37°C. The addition of

calcium chloride (100 μ L) triggered the coagulation process and clot formation was detected with a coagulometer in duplicate.

3. Prothrombin time (PT)

a) Principle of method

An optimal amount of thromboplastin and calcium are added to plasma sample and the time of fibrin clot formation was measured. PT is a rapid, sensitive screening test for coagulation disorders of the extrinsic pathway. It is well suited for the induction and monitoring of oral anticoagulant therapy, for diagnosing genetic or acquired deficiencies in coagulation factors and checking the synthesis performance of the liver in hepatic diseases. PT is prolonged due to extrinsic coagulation factors deficit or due to the presence of inhibitors.

b) Reagents

Thromboplastin (Thromborel S, Dade/Behring): was dissolved in 4 mL of distilled water. Reagent was at 37°C prior to use at least 30 minutes.

Normal pooled plasma: venous blood from 11 apparently healthy volunteers was collected (1 part 0.11 mol/L sodium citrate solution with 9 parts of blood) and centrifuged immediately at 2000 x g for 30 minutes at 4°C. Plasma was removed, pooled and stored in aliquots at -70°C until use.

Inhibitors: were dissolved in DMSO (10 mM stock solution) and diluted with distilled water to working solutions (the highest concentration 100 μ M).

c) Method

Inhibitor (10 μ L working solution, concentrations from 5 μ M to 100 μ M) and pooled plasma (90 μ L) were incubated at 37°C for 5 minutes and then Thromborel S (200 μ L) was added. Clot formation was measured with a coagulometer in duplicate.

Enzyme assay

The enzyme inhibitory effect of the compounds can be identified by determination of inhibition constant K_i . It denotes the degree of dissociation of the enzyme-inhibitor complex. Low dissociation constant means high potency of inhibitor. K_i can be determined during reaction of the enzyme with a specific chromogenic substrate which under hydrolysis by the enzyme develops color. Reaction in time is recorded by spectrophotometry and K_i is calculated from kinetic parameters (V_{max} , K_m , reaction rate).

1. Determination of thrombin K_i

a) Principle of method

The ability of a compound to act as an inhibitor of human thrombin catalytic activity was assessed by determination of K_i .

b) Reagents

Buffer: HBSA, pH 7.5 (10 mM HEPES, 150 mM NaCl, 0.1% w/v bovine serum albumin)

Substrate (S-2238: H-D-Phe-Pip-Arg-pNA HCl, 25 mg; Chromogenix): dissolved in distilled water to 1mM concentration. (K_m is 2.6 μ M)

Human thrombin (308 NIH; Sigma): dissolved in saline to give a stock solution of 20 NIH/mL.

Inhibitors: were dissolved in DMSO (10 mM stock solution) and diluted with distilled water to working solutions (the highest final concentration of DMSO was 3%).

c) Method

A mixture of 50 μ L of buffer, 50 μ L of inhibitor in water (final concentrations from 10 to 100 μ M) and 50 μ L of thrombin (0.5 NIH U/mL f.c.) was incubated for 15 minutes at room temperature. The reaction was started with 50 μ L of S-2238 (20 μ M or 40 μ M f.c.) and the absorbance of each sample at 405 nm (at 25°C) was measured in triplicate every 10 seconds for a period of 15 minutes using a microtiter plate reader (Tecan Sunrise).

Thrombin activity was determined from the change in absorbance in the linear part of the velocity graph. K_i was calculated according to Cheng and Prusoff (Biochem Pharmacol, 1973) where K_i is $IC_{50}/(1+S/K_m)$. The K_m for the substrate was determined under the test conditions with at least 6 substrate concentrations varying around K_m and calculated with the non-linear regression programme Curve expert.

2. Determination of trypsin K_i

a) Principle

The ability of a compound to act as an inhibitor of trypsin catalytic activity was assessed by determination of K_i .

b) Reagents

Buffer: HBSA, pH 7.5 (10 mM Hepes, 150 mM NaCl, 0.1% w/v bovine serum albumin)

Substrate (S-2222: N-benzoyl-Ile-Glu-Gly-Arg-pNA HCl, 25 mg; Chromogenix): dissolved in distilled water to 2mM concentration. (K_m is 21 μ M)

Trypsin (6000 E/mg prot.; Sigma): dissolved in distilled water to give a stock solution of 300 E/mL.

Inhibitors: were dissolved in DMSO (10 mM stock solution) and diluted with distilled water to working solution (the highest final concentration of DMSO was 10%).

c) Method

50 μ L of buffer, 50 μ L of test compound in water (final concentrations from 50 to 300 μ M) and 50 μ L of trypsin (3 mE/mL f.c.) were incubated for 15 minutes at room temperature. The reaction was started with 50 μ L of S-2222 (50 μ M or 100 μ M f.c.) and the absorbance of each sample at 405 nm (at 25°C) was measured in triplicates every 10 seconds for a period of 15 minutes using a microtiter plate reader (Tecan Sunrise).

Trypsin activity was determined from the change in absorbance in the linear part of velocity graph. K_i was calculated according to Cheng and Prusoff (Biochem Pharmacol, 1973) where K_i is $IC_{50}/(1+S/K_m)$. The K_m for the substrate was

determined under test conditions with at least 6 substrate concentrations varying around K_m and calculated with the non-linear regression program Curve expert.

3. Selectivity to thrombin

By the use of other serine protease like trypsin with the appropriate chromogenic substrate, selectivity of inhibitors with respect to thrombin was determined. Selectivity of an inhibitor is expressed as a ratio of K_i for trypsin to K_i for thrombin.

In vivo ASSAYS

1. Stasis-induced thrombosis

Principle of method

Using a combination of thrombogenic challenge (0.045 ng/kg of tissue factor) and stasis, compounds were tested for their ability to affect thrombus formation in a venous thrombosis model in the rat.

Method

Thrombus formation by a combination of stasis and hypercoagulability was induced as described by Vogel et al. (Thromb. Res., 1989, 54, 399-410). Male sprague-Dawley rats (250-300 g) were anaesthetized with sodium pentobarbitone (30 mg/kg, i.p.). The abdomen of the animals was surgically opened and after careful dissection, the vena cava was exposed and dissected free from surrounding tissue. Saline, or the various compounds were administered i.v. 5 min before thrombosis induction. Two loose sutures were prepared 0.7 cm apart on the inferior vena cava and all collateral veins were ligated. Human tissue factor (0.045 ng/kg, i.v.) was injected into the dorsal vein of the penis. Ten seconds after the end of the injection, stasis were established by tightening the two, firstly the proximal and then the distal. The abdominal cavity was provisionally closed and stasis was maintained for 20 minutes. The cavity was then reopened, the ligated segment was opened longitudinally and the thrombus formed was removed, rinsed, blotted on filter paper, dried overnight at

60°C and weighed. Under these experimental conditions, control thrombus weight was 5.5 ± 0.1 mg (n=15).

2. Bleeding time

Principle of method

The hemorrhagic risk associated to a treatment with selected compounds were determined using an experimental model of bleeding: transection of the tail of rats.

Method

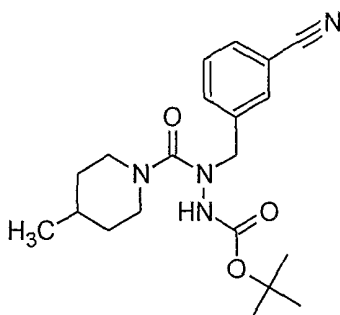
Bleeding time was determined by transection of the tail, 2 mm from the tip, of phenobarbital-anaesthetised rats (30 mg/kg i.p.). The compounds were injected i.v. at the indicated doses 5 min before tail transection. Blood was carefully blotted every 15 s on a filter paper. Haemostasis was considered to be achieved when no more blood stain was observed over 1 min.

Examples

The invention is further described by means of the following examples, but not in any limitative case:

EXAMPLE 1

tert-Butyl 2-(3-cyanobenzyl)-2-[(4-methyl-1-piperidiny)carbonyl]-1-hydrazinecarboxylate



Triphosgene (2.296 g) was dissolved in 15 ml of anhydrous dichloromethane and cooled to -5°C . While stirring under argon atmosphere, a solution of *N*-1-(*tert*-butoxycarbonyl)-*N*-2-[(3-cyanophenyl)methyl]hydrazine (3.833 g) and *N,N*-diisopropylethylamine (4.040 ml) in 25 ml of dichloromethane was added dropwise. After addition, the reaction mixture was stirred for another 5 minutes, and a mixture of 4-methyl piperidine (1.836 ml) and *N,N*-diisopropylethylamine (4.040 ml) in 25 ml dichloromethane was then added at once and stirred at room temperature for another 20 minutes. The solvent was evaporated in vacuo and the residue was dissolved in 50 ml of ethyl acetate, washed with 25 ml of 5% citric acid, with 25 ml of 5% solution of NaHCO_3 and with 25 ml of brine. The organic phase was dried over MgSO_4 , filtered and the filtrate evaporated under reduced pressure.

The resulting mixture was suspended in 20 ml of diethylether and 5 ml of hexane was added. The precipitate was filtered by suction.

Yield: 1.756 g (30.4 %)

Melting range: $147-150^{\circ}\text{C}$

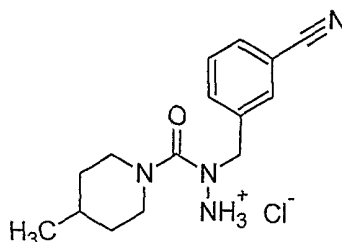
IR (KBr, cm^{-1}): 3275.0, 2922.7, 2229.3, 1722.3, 1632.3, 1497.3, 1445.5, 1367.6, 1252.9, 1157.0, 979.2, 805.1, 736.2, 573.7

NMR (DMSO- d_6) : δ (ppm): 0.86 (d, $J = 6,4$ Hz, 3H, CH₃), 0.90-1.05 (m, 2H, Pip-H^{3,5}), 1.35 (s, 9H, Boc), 1.45-1.60 (m, 4H, Pip-H^{3',5'}), 2.60-2.75 (m, 2H, Pip-H^{2,6}), 3.70-3.85(m, 2H, Pip-H^{2',6'}), 4.52 (br s, 2H, CH₂), 7.58-7.66 (m, 1H, Bn-H⁵), 7.79-7.85 (m, 1H, Bn-H⁶), 7.93-8.05 (m, 2H, Bn-H^{2,4}), 11.05 (s, 1H, NH).

Molecular weight of C₂₀ H₂₇ N₄ O₃: calculated: 372; found: 373 (MH ⁺)

EXAMPLE 2

2-(3-Cyanobenzyl)-2-[(4-methyl-1-piperidiny)carbonyl]hydrazinium chloride



tert-butyl 2-(3-cyanobenzyl)-2-[(4-methyl-1-piperidiny)carbonyl]hydrazine

carboxylate (1.173 mg) was dissolved in 30 ml of glacial acetic acid and HCl gas was bubbled through the solution for 20 minutes. The progress of the reaction was monitored by TLC. Glacial acetic acid was evaporated on a rotavapor, and diethylether was poured over the oily product. The liquid phase was removed, the resulting precipitate is dried at 50°C and desiccated over NaOH for 24 hours.

Yield: 0.875 g (%) of the mixture

Melting range: 170-173°C

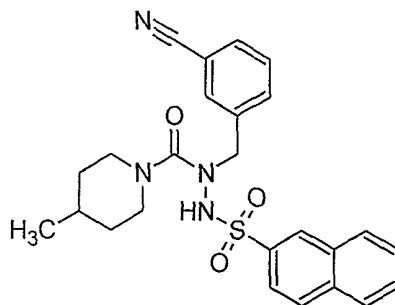
IR (KBr, cm⁻¹): 3417.8, 2934.6, 2664.1, 2227.3, 1711.6, 1641.3, 1433.7, 1402.3, 1249.6, 1132.0, 1017.8

NMR (DMSO- d_6): δ (ppm): 0.92 (d, $J = 6,4$ Hz, 3H, CH₃), 0.99-1.16 (m, 2H, Pip-H^{3,5}), 1.52-1.71 (m, 4H, Pip-H^{3',5'}), 2.90-3.05 (m, 2H, Pip-H^{2,6}), 3.80-3.95(m, 2H, Pip-H^{2',6'}), 4.62 (s, 2H, CH₂), 7.59-7.69 (m, 1H, Bn-H^{4,5}), 7.79-7.88 (m, 1H, Bn-H^{2,6}).

Molecular weight of C₁₄H₁₉ClN₄O: calculated: 294.78; found: 295(MH ⁺)

EXAMPLE 3

N'-(3-Cyanobenzyl)-*N'*-[(4-methyl-1-piperidiny]carbonyl]-2-naphthalenesulfonohydrazide



2-(3-cyanobenzyl)-2-[(4-methyl-1-piperidiny]carbonyl]hydrazine (0.870 g) was dissolved in 50 ml of dichloromethane, cooled to -5°C and while stirring, 1.190 ml of triethylamine and 0.640 g of naphthalen-2-sulfonylchloride were added. The reaction mixture was stirred at -5°C for 30 minutes and then at room temperature for another 24 hours. The solvent was evaporated under reduced pressure and 100 ml of ethyl acetate was added to the residue. In the separatory funnel it was washed twice with 50 ml of water, with 50 ml of 10% of citric acid and with 50 ml of brine. The organic phase was dried over Na_2SO_4 , filtered and the filtrate was evaporated in vacuo. The resulting product was suspended in diethylether and the precipitate was filtered by suction. The precipitate was then recrystallized from ethanol and the crystals were filtered by suction.

Yield: 0.464 g (35.6%)

Melting range: $92-95^{\circ}\text{C}$

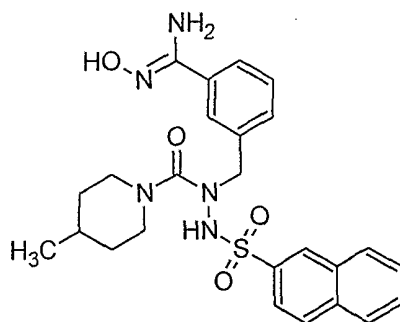
IR (KBr, cm^{-1}): 3191.7, 2931.6, 2221.0, 1680.5, 1433.5, 1333.1, 1168.8, 857.9, 749.4, 692.2

^1H NMR (DMSO-d_6): δ (ppm): 0.10-0.35 (m, 2H, Pip- $\text{H}^{3,3',5,5'}$), 0.47 (d, 3H, $J = 6.0$ Hz, CH_3), 1.22-1.32 (m, 3H, Pip- $\text{H}^{3,3',4,4',5,5'}$), 2.30-2.45 (m, 1H, Pip- $\text{H}^{2,2'}$), 2.65-2.80 (m, 1H, Pip- $\text{H}^{6,6'}$), 3.45-3.65 (m, 2H, Pip- $\text{H}^{2,2',6,6'}$), 4.38 (br d, 2H, CH_2), 7.47-7.55 (m, 2H, Bn- $\text{H}^{5,6}$), 7.58-7.63 (m, 1H, Bn- H^2), 7.63-7.80 (m, 4H, Bn- H^4 in Nf- $\text{H}_{3,6,7}$), 8.02-8.18 (m, 3H, Nf- $\text{H}^{4,5,8}$), 8.43 (s, 1H, Nf- H^1), 9.60 (s, 1H, NH). Two sets of signals

Molecular weight of $C_{25}H_{26}N_4O_3S$: calculated: 462.6; found: 463 (MH^+)

EXAMPLE 4

N'-Hydroxy-3-[[1-[(4-methyl-1-piperidiny)carbonyl]-2-(2-naphthylsulfonyl)hydrazino]methyl]benzenecarboximidamide



N'-(3-cyanobenzyl)-*N'*-[(4-methyl-1-piperidiny)carbonyl]-2-naphthalene sulfonohydrazide (0.223 g) was suspended in 20 ml of anhydrous ethanol under argon atmosphere and 0.032 g of hydroxylamine was added. The reaction mixture was heated at reflux temperature for 24 hours and evaporated under reduced pressure. The resulting product was purified by column chromatography (eluent: chloroform/methanol = 9/1) to yield white crystals.

Yield: 0.215 g (38%)

Melting range: 169-173°C

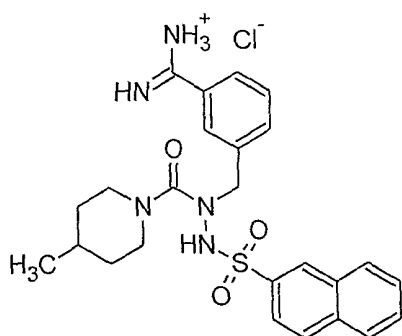
IR (KBr, cm^{-1}): 3365.8, 3194.6, 2943.8, 2864.7, 1652.6, 1447.5, 1332.2, 1169.3, 1076.1, 756.2, 700.3, 553.2

1H -NMR (DMSO- d_6): δ (ppm): 0.01-0.36 (m, 2H, Pip- $H^{3,5}$), 0.44 (d, 3H, $J = 6.4$ Hz, CH_3), 1.15-1.27 (m, 3H, Pip- $H^{3,4,5}$), 2.27-2.42 (m, 1H, Pip- H^2), 2.56-2.75 (m, 1H, Pip- H^6), 3.45-3.63 (m, 2H, Pip- $H^{2,6}$), 4.35 (br d, 2H, CH_2), 7.14 (d, 1H, $J = 7.9$, Bn- H^4), 7.25-7.40 (m, 1H, Bn- H^5), 7.48-7.59 (m, 2H, Bn- $H^{2,6}$), 7.63-7.81 (m, 3H, Nf- $H^{3,6,7}$), 8.02-8.19 (m, 3H, Nf- $H^{4,5,8}$), 8.46 (s, 1H, Nf- H^1), 9.40 (s, 1H, NH), 9.78 (s, 1H, OH).

Molecular weight of $C_{25}H_{30}N_5O_4S$: calculated: 495.6; found: 496 (MH^+)

EXAMPLE 5

Imino(3-[[1-[(4-methyl-1-piperidyl)carbonyl]-2-(2-naphthylsulfonyl)hydrazino]methyl]phenyl)methanaminium chloride



N'-(3-cyanobenzyl)-*N'*-[(4-methyl-1-piperidyl)carbonyl]-2-naphthalene sulfonohydrazide (0.200 g) was suspended in 10 ml of ethanol, cooled in an ice bath and HCl was bubbled into the suspension for 30 minutes. The mixture was stirred at room temperature for 4 hours and then the solvent was evaporated under reduced pressure. The solid residue was washed with isopropyl ether and filtered by suction. The resulting precipitate was dissolved in 10 ml ethanol and ammonium acetate (0.092 g) was added. The resulting mixture was stirred at room temperature for 20 hours and then HCl was passed through the solution for 30 minutes. After 24 hours the white precipitate was formed which was filtered by suction.

Yield: 50.4 mg (52%)

Melting range: 148-152°C

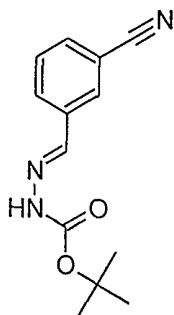
IR (KBr, cm^{-1}): 3396.1, 3101.7, 2945.8, 1669.1, 1435.8, 1335.9, 1165.9, 1072.4, 749.4, 668.4

$^1\text{H-NMR}$ (DMSO- d_6): δ (ppm): 0.01-0.40 (m, 2H, Pip- $\text{H}^{3,5}$), 0.47 (d, 3H, $J = 6.4$ Hz, CH_3), 1.15-1.29 (m, 3H, Pip- $\text{H}^{3,4,5}$), 2.27-2.42 (m, 1H, Pip- H^2), 2.56-2.75 (m, 1H, Pip- H^6), 3.45-3.60 (m, 2H, Pip- $\text{H}^{2,6}$), 4.41 (br d, 2H, CH_2), 7.14 (d, 1H, $J = 7.9$, Bn- H^4), 7.25-7.40 (m, 1H, Bn- H^5), 7.48-7.59 (m, 2H, Bn- $\text{H}^{2,6}$), 7.63-7.81 (m, 3H, Nf- $\text{H}^{3,6,7}$), 8.02-8.19 (m, 3H, Nf- $\text{H}^{4,5,8}$), 8.46 (s, 1H, Nf- H^1), 9.40 (s, 1H, NH).

Molecular weight of $C_{24}H_{27}N_5O_3S$ (free base): calculated: 465.57; found: 466 (MH^+)

EXAMPLE 6

tert-Butyl 2-(3-cyanobenzylidene)-1-hydrazinecarboxylate



3-Cyanobenzaldehyde (40.0 mmol) suspended in EtOH (100 mL) was added to a stirred solution of *tert*-butylcarbazate (40.0 mmol) in EtOH. The mixture was heated at reflux temperature and after 4 h the EtOH was partially evaporated in vacuo. Water (100 mL) was added and the precipitated product was collected by filtration and washed with diethylether to give *tert*-butyl 2-(3-cyanobenzylidene)-1-hydrazinecarboxylate as a white solid.

Yield: 77%.

MP: 115-158 °C.

IR (KBr): 3297, 2977, 2232, 1703, 1530, 1477, 1376, 1248, 1160, 1061, 941, 865 cm^{-1} .

MS (FAB): $MH^+ = 246$.

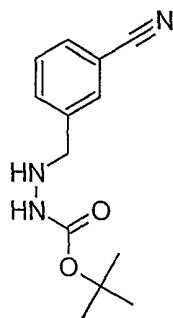
1H NMR (300 MHz $CDCl_3$): 1.55 (s, 9H, Boc-H), 7.55 (m, 2H, Bn-H), 7.93 (m, 3H, Bn-H in CH), 8.24 (s, 1H, NH).

Elemental analysis: calculated for $C_{13}H_{15}N_3O_2$: C, 63.66; H, 6.16; N, 17.13, found C, 63.38; H, 6.18; N, 17.09.

EXAMPLE 7

tert-Butyl 2-(3-cyanobenzyl)-1-hydrazinecarboxylate

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tert-Butyl 2-(3-cyanobenzylidene)-1-hydrazinecarboxylate (60.0 mmol) was dissolved in MeOH (250 mL) and 10% Pd/C (10 w/w %) was added. The mixture was hydrogenated for 6 h. The catalyst was filtered off and the filtrate was evaporated in vacuo to yield oily *tert*-butyl 2-(3-cyanobenzyl)-1-hydrazinecarboxylate which crystallized overnight.

Yield: 95 %.

MP: 75-80 °C.

IR (KBr): 3368, 2980, 2226, 1679, 1482, 1366, 1286, 1166, 793 cm⁻¹

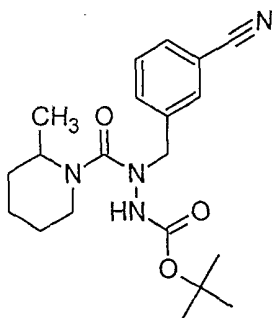
MS (FAB): MH⁺ = 248.

¹H NMR (300 MHz CDCl₃): 1.47 (s, 9H, Boc-H), 4.04 (br s, 2H, CH₂), 4.28 (br s, 1H, NH), 6.09 (br s, 1H, NH), 7.12-7.41(m, 4H, Bn-H).

Elemental analysis: calculated for C₁₃H₁₇N₃O₂ · ¼ H₂O: C, 62.01; H, 7.00; N, 16.69; found C, 61.90; H, 7.22; N, 16.69.

EXAMPLE 8

tert-Butyl 2-(3-cyanobenzyl)-2-[(2-methyl-1-piperidiny)carbonyl]-1-hydrazinecarboxylate



The product was prepared from *tert*-butyl 2-(3-cyanobenzyl)-1-hydrazinecarboxylate and 2-methylpiperidine using the procedure described in EXAMPLE 1.

Yield: 42 %.

MP: oil

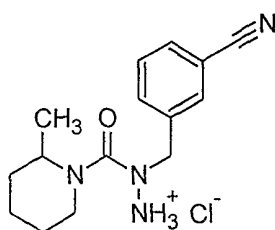
IR (KBr): 3271, 2941, 2230, 1725, 1644, 1428 cm^{-1} .

MS (FAB): $\text{MH}^+ = 373$.

^1H NMR (300 MHz, CDCl_3): 1.22 (d, $J = 6,8$ Hz, 3H, CH_3), 1.45 (s, 9H, Boc), 1.45-1.79 (m, 5H, Pip-H), 2.97-3.10 (m, 1H, Pip-H), 3.44-3.54 (m, 1H, Pip-H), 3.63-3.76 (m, 1H, Pip-H), 4.18-4.32 (m, 1H, Pip-H), 4.48 (s, 2H, CH_2), 6.30 (s, 1H, NH), 7.46 (t, $J = 7.9$ Hz, 1H, Bn-H), 7.56-7.67 (m, 3H, Bn-H).

EXAMPLE 9

2-(3-Cyanobenzyl)-2-[(2-methyl-1-piperidiny)carbonyl]hydrazinium chloride



The product was prepared from *tert*-butyl 2-(3-cyanobenzyl)-2-[(2-methyl-1-piperidiny)carbonyl]-1-hydrazinecarboxylate using the procedure described in EXAMPLE 2.

Yield: 91 %.

MP: 135-137 $^{\circ}\text{C}$.

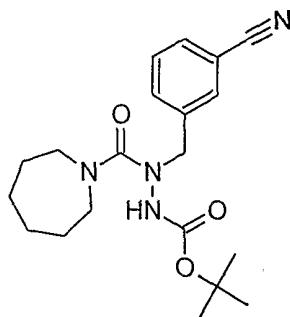
IR (KBr): 3429, 2949, 2229, 2708, 2230, 1694, 1526, 1420 cm^{-1} .

MS (FAB): $\text{MH}^+ = 273$.

^1H NMR (300 MHz, CDCl_3): 1.23 (d, $J = 6,8$ Hz, 3H, CH_3), 1.40-1.51 (m, 1H, Pip-H), 1.52-1.79 (m, 5H, Pip-H), 2.98-3.10 (m, 1H, Pip-H), 3.60-3.79 (m, 1H, Pip-H), 4.25-4.38 (m, 1H, Pip-H), 4.64 (s, 2H, CH_2), 7.53 (t, $J = 7.9$ Hz, 1H, Bn-H), 7.61-7.76 (m, 3H, Bn-H), 9.60 (br s, 2H, NH_2).

EXAMPLE 10

tert-Butyl 2-(1-azepanylcarbonyl)-2-(3-cyanobenzyl)-1-hydrazinecarboxylate



The product was prepared from *tert*-butyl 2-(3-cyanobenzyl)-1-hydrazinecarboxylate and azepane using the procedure described in EXAMPLE 1.

Yield: 63 %.

MP: oil.

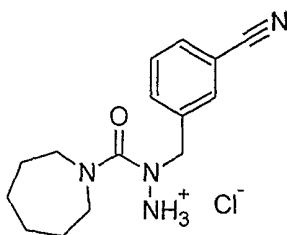
IR (KBr): 3287, 2925, 2223, 1726, 1634 cm^{-1} .

MS (FAB): $\text{MH}^+ = 373$.

^1H NMR (300 MHz, CDCl_3): 1.45 (s, 9H, Boc), 1.53-1.62 (m, 4H, Pip- $\text{H}^{4,5}$), 1.69-1.80 (m, 4H, Pip- $\text{H}^{3,6}$), 3.39-3.46 (m, 4H, Pip- $\text{H}^{2,7}$), 4.50 (s, 2H, CH_2), 6.25 (br s, 1H, NH), 7.45 (t, $J = 7.9$ Hz, 1H, Bn-H), 7.56-7.69 (m, 3H, Bn-H).

EXAMPLE 11

2-(1-Azepanylcarbonyl)-2-(3-cyanobenzyl)hydrazinium chloride



The product was prepared from *tert*-butyl 2-(1-azepanylcarbonyl)-2-(3-cyanobenzyl)-1-hydrazinecarboxylate using the procedure described in EXAMPLE 2.

Yield: 66 %.

MP: oil.

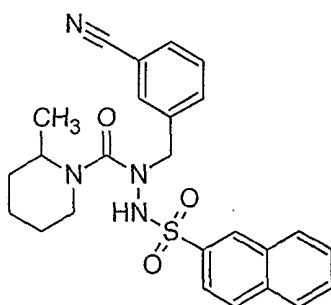
IR (KBr): 2926, 2702, 2230 1703, 1521 cm^{-1} .

MS (FAB): $MH^+ = 273$.

1H NMR (300 MHz, $CDCl_3$): 1.53-1.64 (m, 4H, Pip- $H^{4,5}$), 1.65-1.81 (m, 4H, Pip- $H^{3,6}$), 3.38-3.46 (m, 4H, Pip- $H^{2,7}$), 4.70 (s, 2H, CH_2), 7.48- 7.64 (m, 2H, Bn-H), 7.69-7.80 (m, 2H, Bn-H), 9.60 (br s, 2H, NH_2).

EXAMPLE 12

N'-(3-cyanobenzyl)-*N'*-[(2-methyl-1-piperidiny)carbonyl]-2-naphthalenesulfonohydrazide



The product was prepared from 2-(3-cyanobenzyl)-2-[(2-methyl-1-piperidiny)carbonyl]hydrazinium chloride and naphthalene-2-sulfonyl chloride using the procedure described in EXAMPLE 3.

Yield: 14 %.

MP: 116-119 °C.

IR (KBr): 3231, 2943, 2227, 1653 cm^{-1} .

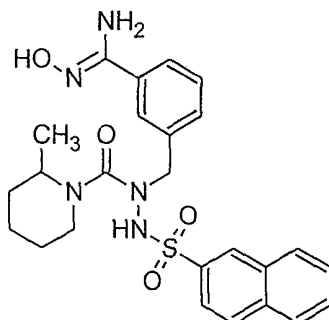
MS (FAB): $MH^+ = 273$.

1H NMR (300 MHz $CDCl_3$): 0.35-0.55 (m, 2H, Pip-H), 0.75-0.90 (m, 2H, Pip-H), 1.03-1.45 (m, 6H, Pip-H in CH_3), 2.54-2.70 in 2.75-2.95 (m, 1H, Pip-H) 3.50-3.75 in 3.75-4.00 (m, 1H, Pip-H), 4.31 (br d, 2H, CH_2), 7.35-7.70 (m, 7H, Bn-H in Nph-H), 7.75-7.85(m, 1H, Nph-H), 7.86-8.00 (m, 3H, Nph-H), 8.42 (s, 1H, Nph-H), (two sets of signals).

Elemental analysis: calculated for $C_{25}H_{26}N_4O_3S$: C, 64.91; H, 5.67; N, 12.11; found: C, 65.07; H, 5.58; N, 11.93.

EXAMPLE 13

N'-hydroxy-3-[[1-[(2-methyl-1-piperidiny)carbonyl]-2-(2-naphthylsulfonyl)hydrazino]methyl]benzenecarboximidamide



The product was prepared from *N'*-(3-cyanobenzyl)-*N'*-[(2-methyl-1-piperidiny)carbonyl]-2-naphthalenesulfonohydrazide using the procedure described in EXAMPLE 4.

Yield: 48 %.

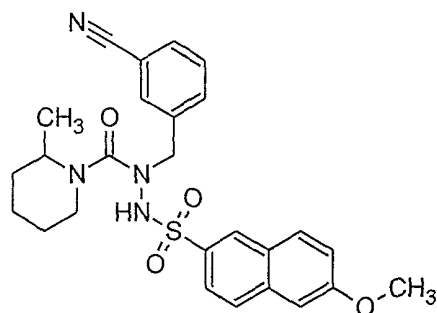
MP: 101-103 °C.

IR (KBr): = 3370, 2932, 1651 cm^{-1} .

MS (FAB): MH^+ = 496.

EXAMPLE 14

N'-(3-Cyanobenzyl)-6-methoxy-*N'*-[(2-methyl-1-piperidiny)carbonyl]-2-naphthalenesulfonohydrazide



The product was prepared from 2-(3-cyanobenzyl)-2-[(2-methyl-1-piperidiny)carbonyl]hydrazinium chloride and 6-methoxy-2-naphthalenesulfonyl chloride using the procedure described in EXAMPLE 3.

Yield: 26 %.

MP: 169-173 °C.

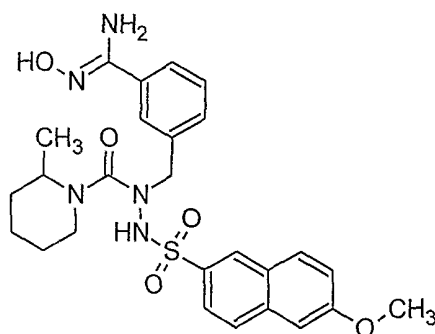
IR (KBr): 3172, 2934, 2229, 1671, 1623 cm^{-1} .

MS (FAB): $MH^+ = 493273$.

1H NMR (300 MHz, $CDCl_3$): 0.47-0.60 (m, 2H, Pip-H), 0.79-0.92 (m, 2H, Pip-H), 1.12-1.50 (m, 6H, Pip-H in CH_3), 2.50-2.75 in 2.80-3.15 (m, 1H, Pip-H), 3.55-3.76 in 3.80-3.95 (m, 1H, Pip-H), 3.95 (s, 3H, OCH_3); 2.55-2.74 (m, 1H, Pip-H), 3.45-3.65 (m, 2H, Pip-H), 3.84 (s, 3H, CH_3), 4.32 (br d, 2H, CH_2), 7.28-7.33 (m, 1H, Nph-H), 7.44-7.60 (m, 5H, Bn-H and Nph-H), 7.75-7.88 (m, 3H, Nph-H), 8.34 (d, 1H, $J = 1.88$ Hz, Nph-H).

EXAMPLE 15

N'-Hydroxy-3-({2-[(6-methoxy-2-naphthyl)sulfonyl]-1-[(2-methyl-1-piperidiny)carbonyl]hydrazino}methyl)benzenecarboximidamide



The product was prepared from *N'*-(3-Cyanobenzyl)-6-methoxy-*N'*-[(2-methyl-1-piperidiny)carbonyl]-2-naphthalenesulfonohydrazide using the procedure described in EXAMPLE 4.

Yield: 45 %.

MP: 172-175 °C.

IR (KBr): 3368, 2937, 1647, 1160 cm^{-1} .

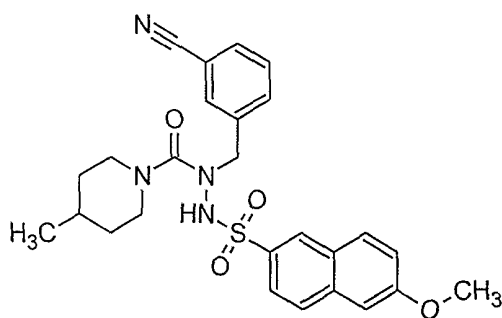
MS (FAB): $MH^+ = 526$.

1H NMR (300 MHz, $CDCl_3$): 0.35-0.50 (m, 2H, Pip-H), 0.79-1.00 (m, 2H, Pip-H), 1.12-1.50 (m, 6H, Pip-H in CH_3), 2.40-2.75 in 2.80-3.00 (m, 1H, Pip-H), 3.55-3.76 in 3.80-3.95 (m, 1H, Pip-H), 3.95 (s, 3H, OCH_3); 4.35 (br d, 2H, CH_2), 5.04 (s, 2H, NH_2), 7.15-7.65 (m, 6H, Nph-H in Bn-H), 7.74-7.87 (m, 3H, Nph-H), 8.34 (s, 1H, Nph-H).

Elemental analysis: calculated for $C_{26}H_{31}N_5O_5S \cdot EtOH$: C, 58.83; H, 6.52; N, 12.25; found: C, 59.41; H, 5.96; N, 12.09.

EXAMPLE 16

N'-(3-Cyanobenzyl)-6-methoxy-*N'*-[(4-methyl-1-piperidiny)carbonyl]-2-naphthalenesulfonohydrazide



The product was prepared from 2-(3-cyanobenzyl)-2-[(4-methyl-1-piperidiny)carbonyl]hydrazinium chloride using the procedure described in EXAMPLE 3.

Yield: 50 %.

MP: 169-173 °C.

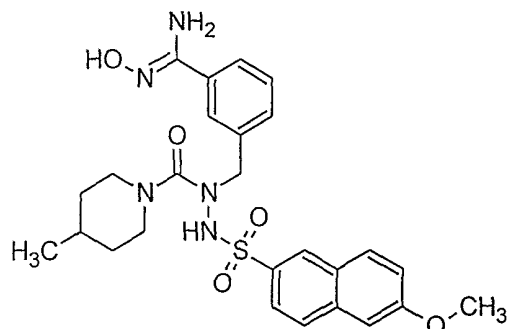
IR (KBr): 3168, 2932, 2232, 1671 cm^{-1} .

MS (FAB): $\text{MH}^+ = 493$.

^1H NMR (300MHz, DMSO-d_6): 0.15-0.40 (m, 2H, Pip-H), 0.53 (d, 3H, $J = 6.0$ Hz, CH_3), 1.25-1.39 (m, 3H, Pip-H), 2.25-2.38 (m, 1H, Pip-H), 2.55-2.74 (m, 1H, Pip-H), 3.45-3.65 (m, 2H, Pip-H), 3.84 (s, 3H, CH_3), 4.32 (br d, 2H, CH_2), 7.28-7.33 (m, 1H, Nph-H), 7.44-7.54 (m, 3H, Bn-H and Nph-H), 7.60 (d, 1H, $J = 1.88$ Hz, Bn-H), 7.60- 7.76 (m, 2H, Bn-H and Nph-H), 7.94 (d, 1H, $J = 8.66$ Hz, Nph-H), 8.05 (d, 1H, $J = 8.66$ Hz, Nph-H), 8.34 (d, 1H, $J = 1.88$ Hz, Nph-H 1), 9.46 (s, 1H, NH).

EXAMPLE 17

N'-Hydroxy-3-({2-[(6-methoxy-2-naphthyl)sulfonyl]-1-[(4-methyl-1-piperidiny)carbonyl]hydrazino}methyl)benzenecarboximidamide



The product was prepared from *N'*-(3-cyanobenzyl)-6-methoxy-*N'*-[(4-methyl-1-piperidiny)carbonyl]-2-naphthalenesulfonohydrazide using the procedure described in EXAMPLE 4.

Yield: 36 %.

MP: 154-156 °C.

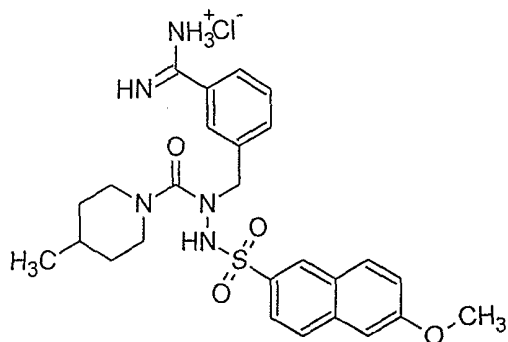
IR (KBr): 3473, 3365, 2950, 1646 cm^{-1} .

MS (FAB): $\text{MH}^+ = 526$

^1H NMR (300 MHz, CDCl_3): 0.30 (br d, 3H, Pip-H), 0.58 (d, 3H, $J = 6.0$ Hz, CH_3), 1.26 (br d, 3H, Pip-H), 2.45 (br s, 1H, Pip-H), 2.63 (br d, 1H, Pip-H), 3.74 (br d, 2H, Pip-H), 3.97 (s, 3H, OCH_3), 4.34 (br d, 2H, CH_2), 4.94 (s, 2H, NH_2) 7.17-7.35 (m, 4H, Nph-H and Bn-H), 7.49-7.55 (m, 2H, Bn-H), 7.77-7.89 (m, 4H, Bn-H and Nph-H), 8.35 (m, 1H, Bn-H). 8.65 (br s, 1H, NH), 9.02 (s, 1H, OH).

EXAMPLE 18

Imino[3-({2-[(6-methoxy-2-naphthyl)sulfonyl]-1-[(4-methyl-1-piperidiny)carbonyl]hydrazino}methyl)phenyl]methanaminium chloride



The product was prepared from *N'*-(3-cyanobenzyl)-6-methoxy-*N'*-[(4-methyl-1-piperidinyl)carbonyl]-2-naphthalenesulfonohydrazide using the procedure described in EXAMPLE 5.

Yield: 26 %.

MP: 151-154 °C.

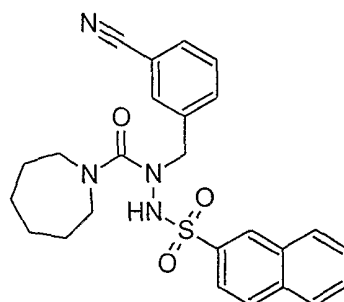
IR (KBr): 3054, 1677, 1624 cm^{-1} .

MS (FAB): $\text{MH}^+ = 510$.

^1H NMR (300 MHz, CDCl_3): 0.28 (br d, 3H, Pip-H), 0.54 (d, 3H, $J = 6.0$ Hz, CH_3), 1.27 (br d, 3H, Pip-H), 2.45 (br s, 1H, Pip-H), 2.64 (br s, 1H, Pip-H), 3.66 (br s, 2H, Pip-H), 3.95 (s, 3H, OCH_3), 4.32 (m, 2H, CH_2), 7.16-7.28 (m, 2H, Nph-H), 7.36-7.40 (m, 2H, Bn- $\text{H}^{2,6}$), 7.73-7.87 (m, 4H, Bn- H^5 and Nph-H), 7.95 (m, 1H, Bn- H^4), 8.78 (br s, 2H, NH_2), 8.16 (br s, 2H, NH_2).

EXAMPLE 19

N'-(1-Azepanylcabonyl)-*N'*-(3-cyanobenzyl)-2-naphthalenesulfonohydrazide



The product was prepared from *N'*-(1-azepanylcabonyl)-*N'*-(3-cyanobenzyl)-2-naphthalenesulfonohydrazide and naphthalene-2-sulfonyl chloride using the procedure described in EXAMPLE 3.

Yield: 43 %.

MP: 128-130 °C.

IR (KBr): 3190, 2942, 2223, 1674 cm^{-1} .

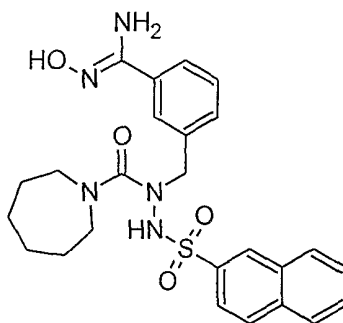
MS (FAB): $\text{MH}^+ = 463$.

^1H NMR (300 MHz, DMSO-d_6): 0.63-1.40 (m, 8H, Pip- $\text{H}^{3,4,5,6}$), 2.90-3.20 (m, 4H, Pip- $\text{H}^{2,7}$), 4.35 (br d, 2H, CH_2), 7.45-5.55 (m, 2H, Nph-H in Bn-H), 7.50-7-75 (m, 5H, Nph-H in Bn-H), 8.02- 8.15 (m, 3H, Nph-H), 8.45 (s, 1H, Nph-H), 9.56 (s, 1H, NH).

Elemental analysis: calculated for $C_{25}H_{26}N_4O_3S$: C, 64.91; H, 5.67; N, 12.11; found: C, 64.65; H, 5.78; N, 12.10.

EXAMPLE 20

3-[[1-(1-Azepanylcabonyl)-2-(2-naphthylsulfonyl)hydrazino]methyl]-*N'*-hydroxybenzenecarboximidamide



The product was prepared from *N'*-(1-azepanylcabonyl)-*N'*-(3-cyanobenzyl)-2-naphthalenesulfonylhydrazide using the procedure described in EXAMPLE 4.

Yield: 79%.

MP: 152-155 °C.

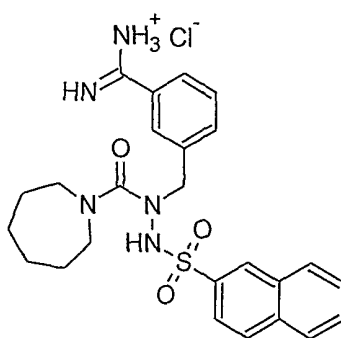
IR (KBr): 3365, 2924, 1646 cm^{-1} .

MS (FAB): $MH^+ = 496$.

1H NMR (300 MHz, DMSO- d_6): 0.65-1.40 (m, 8H, Pip- $H^{3,4,5,6}$), 2.90-3.20 (m, 4H, Pip- $H^{2,7}$), 4.34 (br d, 2H, CH_2), 5.87 (br s, 2H, NH_2), 7.45-5.55 (m, 2H, Nph-H in Bn-H), 7.50-7.75 (m, 5H, Nph-H in Bn-H), 8.02- 8.15 (m, 3H, Nph-H), 8.45 (s, 1H, Nph-H), 9.35 (br d, 1H, NH), 9.67 (br s, 1H, OH).

EXAMPLE 21

(3-[[1-(1-Azepanylcabonyl)-2-(2-naphthylsulfonyl)hydrazino]methyl]phenyl)(imino)methanaminium chloride



The product was prepared from *N'*-(1-azepanylcarbonyl)-*N'*-(3-cyanobenzyl)-2-naphthalenesulfonylhydrazide using the procedure described in EXAMPLE 5.

Yield: 36 %.

MP: 151-154 °C.

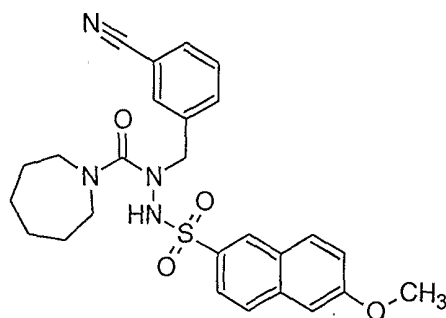
IR (KBr): 3374, 3054, 2926, 1654, 1420, 1160 cm^{-1} .

MS (FAB): $\text{MH}^+ = 480$.

^1H NMR (300 DMSO- d_6): 0.60-1.45 (m, 8H, Pip- $\text{H}^{3,4,5,6}$), 2.90-3.20 (m, 4H, Pip- $\text{H}^{2,7}$), 4.40 (br d, 2H, CH_2), 7.44 (d, $J = 8,3$ Hz, 2H, Bn- $\text{H}^{2,6}$), 7.62-7.80 (m, 5H, Bn- $\text{H}^{3,5}$ in Nph-H), 8.02-8.18 (m, 3H, Nph-H), 8.45 (s, 1H, Nph-H), 9.09 (s, 2H, NH_2), 9.35 (s, 1H, NH)

EXAMPLE 22

N'-(1-Azepanylcarbonyl)-*N'*-(3-cyanobenzyl)-6-methoxy-2-naphthalenesulfonylhydrazide



The product was prepared from 2-(3-cyanobenzyl)-2-[(4-methyl-1-piperidiny]carbonyl]hydrazinium chloride using the procedure described in EXAMPLE 3.

Yield: 15 %.

MP: 128-130 °C.

IR (KBr): 3169, 2939, 2229, 1670 cm^{-1} .

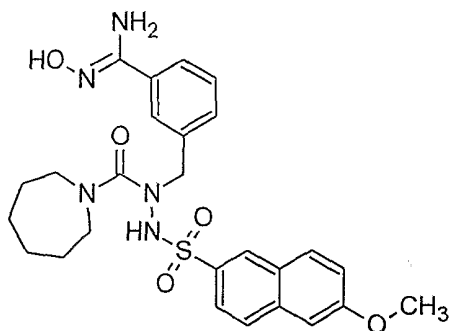
MS (FAB): $\text{MH}^+ = 493$.

^1H NMR (300 MHz, DMSO-d_6): 0.65-1.50 (m, 8H, Pip- $\text{H}^{3,4,5,6}$), 2.85-3.20 (m, 4H, Pip- $\text{H}^{2,7}$), 3.91 (s, 3H, OCH_3) 4.35 (br d, 2H, CH_2), 7.28-7.32 (m, 1H, Bn-H), 7.42-7.52 (m, 3H, Bn-H in Nph-H), 7.60 (br s, 1H, Nph-H), 7.66-7.71 (m, 2H, Nph-H), 7.92 (d, 1H, $J = 8.7$, Nph-H), 8.04 (d, 1H, $J = 8.7$, Nph-H), 8.33 (d, 1H, $J = 1.5$, Nph-H), 9.44 (s, 1H, NH).

Elemental analysis: calculated for $\text{C}_{26}\text{H}_{28}\text{N}_4\text{O}_4\text{S}$: C, 63.14; H, 6.11; N, 11.33; found: C, 63.26; H, 5.87; N, 11.06.

EXAMPLE 23

3-({1-(1-Azepanylcabonyl)-2-[(6-methoxy-2-naphthyl)sulfonyl]hydrazino}methyl)-*N'*-hydroxybenzenecarboximidamide



The product was prepared from *N'*-(1-azepanylcabonyl)-*N'*-(3-cyanobenzyl)-6-methoxy-2-naphthalenesulfonohydrazide using the procedure described in EXAMPLE 4.

Yield: 24 %.

MP: 173-175 °C.

IR (KBr): 3459, 3368, 2932, 1648, cm^{-1} .

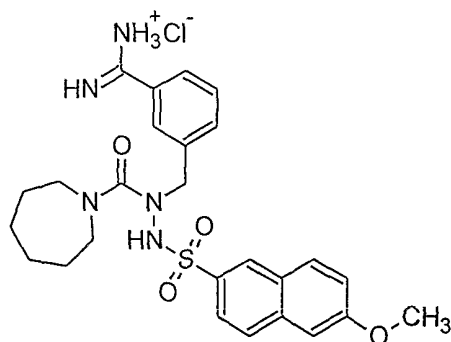
MS (FAB): $\text{MH}^+ = 526$.

^1H NMR (300 MHz, CDCl_3): 0.65-1.50 (m, 8H, Pip- $\text{H}^{3,4,5,6}$), 2.85-3.20 (m, 4H, Pip- $\text{H}^{2,7}$), 3.97 (s, 3H, OCH_3) 4.35 (s, 2H, CH_2), 4.95, (s, 1H, NH) 7.28-7.32 (m, 1H, Bn-H), 7.42-7.52 (m, 3H, Bn-H in Nph-H), 7.60 (br s, 1H, Nph-H), 7.66-7.71 (m, 2H, Nph-H), 7.92 (d, 1H, $J = 8.7$ Hz, Nph-H), 8.04 (d, 1H, $J = 8.7$ Hz, Nph-H), 8.33 (d, 1H, $J = 1.5$ Hz, Nph-H), 8.10 (br s, 1H, NH), 8.35 (s, 1H, OH).

Elemental analysis: calculated for C₂₆H₃₁N₅O₅S: C, 59.18; H, 6.30; N, 13.27;
found: C, 59.68; H, 6.16; N, 12.88.

EXAMPLE 24

[3-({1-(1-Azepanylcabonyl)-2-[(6-methoxy-2-naphthyl)sulfonyl]hydrazino}methyl)phenyl](imino)methanaminium chloride



The product was prepared from *N'*-(1-azepanylcabonyl)-*N'*-(3-cyanobenzyl)-6-methoxy-2-naphthalenesulfonylhydrazide using the procedure described in EXAMPLE 5.

Yield: 21 %.

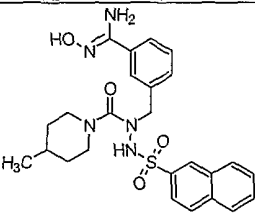
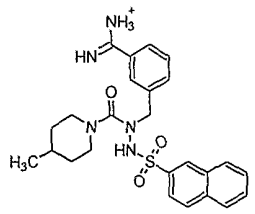
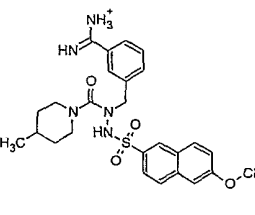
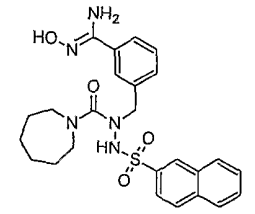
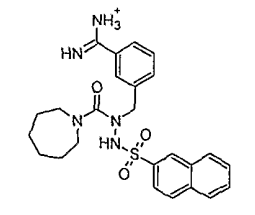
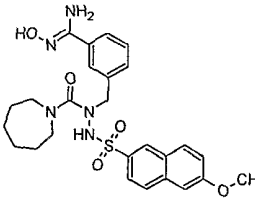
MP: 151-154 °C

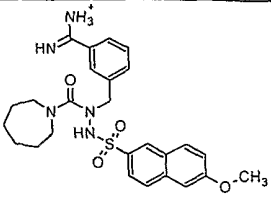
IR (KBr): 3057, 2929, 1677, 1623, 1474, 1157 cm⁻¹.

MS (FAB): MH⁺ = 510.

¹H NMR (300 MHz CDCl₃): 0.65-1.40 (m, 8H, Pip-H^{3,4,5,6}), 2.95-3.20 (m, 4H, Pip-H^{2,7}), 3.97 (s, 3H, OCH₃) 4.35 (br s, 2H, CH₂), 7.44 (d, *J* = 8,3 Hz, 2H, Bn-H^{2,6}), 7.62-7.80 (m, 5H, Bn-H^{3,5} in Nph-H), 8.02-8.18 (m, 3H, Nph-H), 8.45 (s, 1H, Nph-H), 9.03 (s, 2H, NH₂), 9.35 (s, 2H, NH₂).

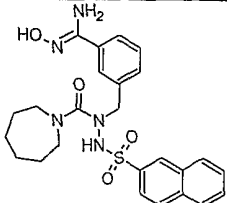
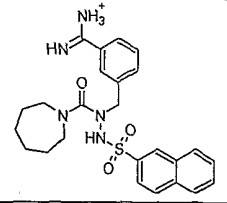
Results of biological *in vitro* testing for selected compounds

	Ki thrombin (μM)	Ki trypsin (μM)	Ki trypsin/ Ki thrombin	aPTT (μM)	PT (μM)	TT (μM)
	0.469	>68.3	146	-	-	-
	0.004	0.112	28	-	-	-
	0.011	-	-	2.1	2.7	2.0
	0.594	32.236	54	-	-	-
	0.005	0.143	29	-	-	-
	0.61	17.945	29	-	-	-

	0.009	0.053	5.84	-	-
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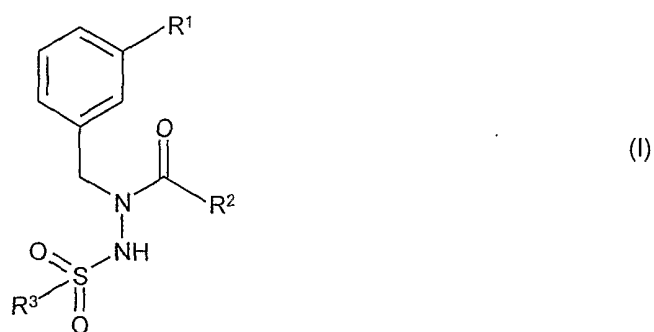
EXAMPLE 27

Results of biological *in vivo* testing for selected compounds

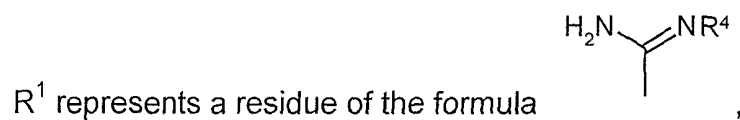
	Stasis-induced thrombosis (ED ₅₀ ± SD – mg/kg)	Bleeding (fold increase at 1 mg/kg)
	290 ± 11	3.3
	70 ± 5	9.4

CLAIMS

1. The compounds of the general formula I

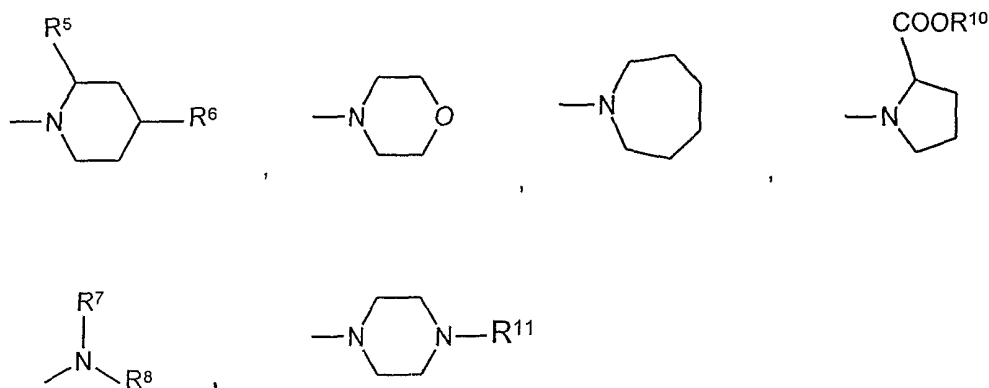


wherein



wherein $R^4 = H, \text{ alkyl (C}_1\text{-C}_3\text{), OH, O-alkyl (C}_1\text{-C}_3\text{), NH}_2$;

R^2 represents a residue of the formula



wherein $R^5 = H, \text{ alkyl (C}_1\text{-C}_3\text{), CF}_3, \text{ COOR}^9$,

$R^6 = H, \text{ alkyl (C}_1\text{-C}_3\text{), CF}_3, \text{ COOR}^9$,

$R^7 = \text{ alkyl (C}_1\text{-C}_3\text{), cycloalkyl (C}_3\text{-C}_6\text{),}$

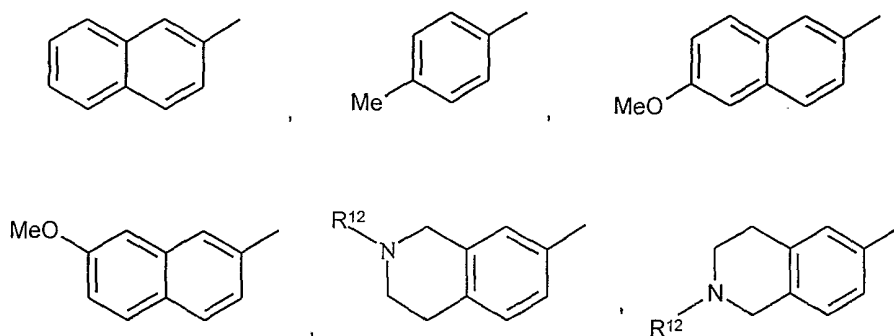
$R^8 = \text{ alkyl (C}_1\text{-C}_3\text{), cycloalkyl (C}_3\text{-C}_6\text{),}$

$R^9 = \text{H, alkyl (C}_1\text{-C}_3\text{)},$

$R^{10} = \text{H, alkyl (C}_1\text{-C}_3\text{), benzyl,}$

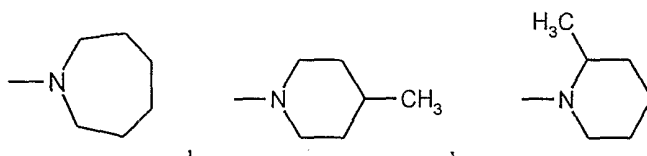
$R^{11} = \text{H, SO}_2\text{Me, CO}_2\text{Me}$

R^3 represents a residue of the formula

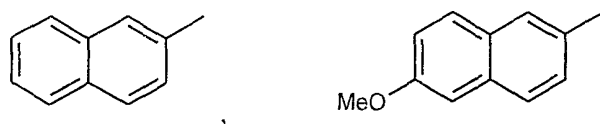


wherein $R^{12} = \text{H, alkyl (C}_1\text{-C}_3\text{), acetyl}$
and pharmaceutically acceptable salts thereof.

2. A compound as claimed in claim 1, of formula I wherein R^2 represents a group selected from:



3. A compound as claimed in preceding claim wherein R^3 represents a group selected from:



4. A compound as claimed in claim 1 wherein said compound is selected from the group consisting of:

N'-hydroxy-3-[[1-[(2-methyl-1-piperidiny)carbonyl]-2-(2-naphthylsulfonyl)hydrazino]methyl]benzenecarboximidamide

N'-Hydroxy-3-({2-[(6-methoxy-2-naphthyl)sulfonyl]-1-[(2-methyl-1-piperidiny)carbonyl]hydrazino)methyl}benzenecarboximidamide

Imino[3-({2-[(6-methoxy-2-naphthyl)sulfonyl]-1-[(2-methyl-1-piperidiny)carbonyl] hydrazino)methyl}phenyl]methanaminium chloride

N'-Hydroxy-3-[[1-[(4-methyl-1-piperidiny)carbonyl]-2-(2-naphthylsulfonyl)hydrazino]methyl]benzenecarboximidamide

Imino(3-[[1-[(4-methyl-1-piperidiny)carbonyl]-2-(2-naphthylsulfonyl)hydrazino]methyl}phenyl)methanaminium chloride

N'-Hydroxy-3-({2-[(6-methoxy-2-naphthyl)sulfonyl]-1-[(4-methyl-1-piperidiny)carbonyl]hydrazino)methyl}benzenecarboximidamide

Imino[3-({2-[(6-methoxy-2-naphthyl)sulfonyl]-1-[(4-methyl-1-piperidiny)carbonyl]hydrazino)methyl}phenyl]methanaminium chloride

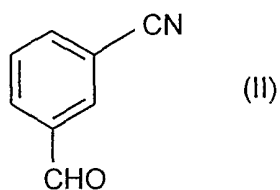
3-[[1-(1-Azepanylcarbonyl)-2-(2-naphthylsulfonyl)hydrazino]methyl]-*N'*-hydroxy benzenecarboximidamide

(3-[[1-(1-Azepanylcarbonyl)-2-(2-naphthylsulfonyl)hydrazino]methyl}phenyl)(imino)methanaminium chloride

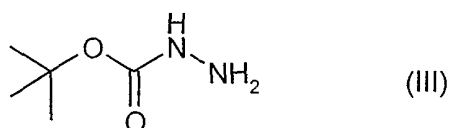
3-({1-(1-Azepanylcarbonyl)-2-[(6-methoxy-2-naphthyl)sulfonyl]hydrazino)methyl}-*N'*-hydroxybenzenecarboximidamide

[3-({1-(1-Azepanylcarbonyl)-2-[(6-methoxy-2-naphthyl)sulfonyl]hydrazino)methyl}phenyl](imino)methanaminium chloride.

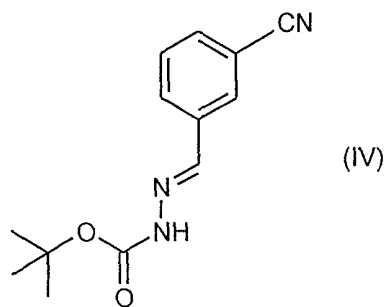
5. Compounds of the formula I according to claim 1 for use in therapy.
6. A process for preparing compounds of the formula I according to claim 1, characterized in that 3-cyanobenzaldehyde of the formula (II)



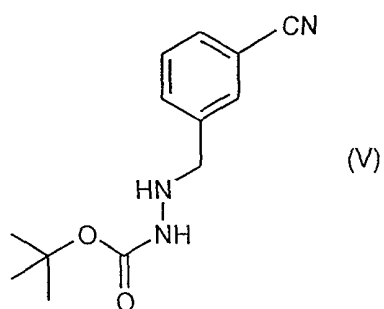
is converted with BOC-carbazate of the formula (III)



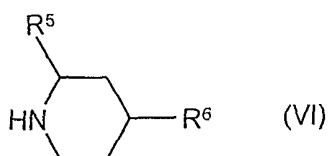
to the compound of the formula (IV),



which is subjected to reduction by catalytic hydrogenation or with NaCNBH_3 is converted to the compound (V),

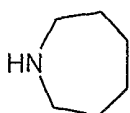
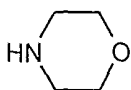


which reacts with triphosgene and amine of the formula (VI),

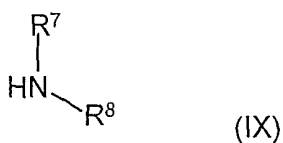


wherein R^5 and R^6 have the same meanings as in the formula (I),

or with triphosgene and amine of the formula (VII or VIII),

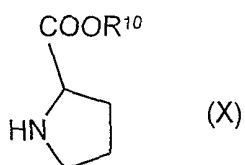


or with triphosgene and amine of the formula (IX),



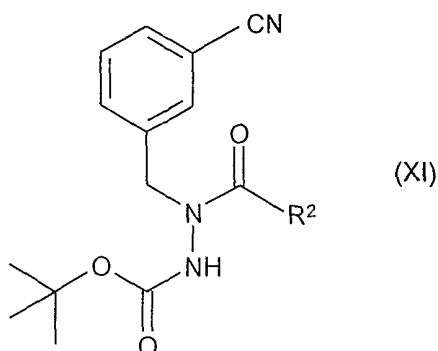
wherein R^7 and R^8 have the same meanings as in the formula (I),

or with triphosgene and amine of the formula (X),



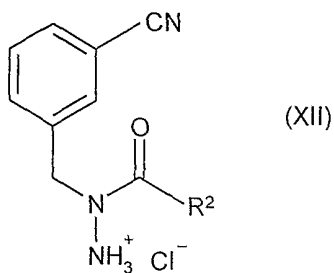
wherein R^{10} has the same meaning as in the formula (I),

in „one pot“ up to the compound (XI),



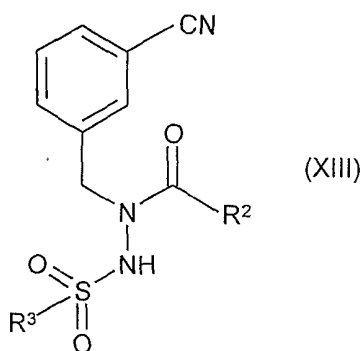
wherein R^2 has the same meaning as in formula (I);

in which the protecting BOC group is removed with HCl (g) in HOAc at room temperature to obtain the compound (XII),



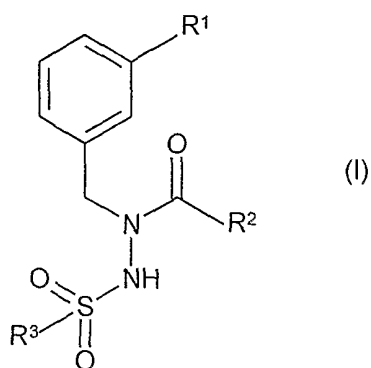
wherein R^2 has the same meaning as in the formula (I),

which reacts with aromatic sulfonylchloride up to the compound (XIII)



wherein R^2 and R^3 have the same meanings as in the formula (I);

which are then converted to the compound of the formula (I).



7. The use of compounds of the formula I of claim 1, in the manufacture of medicaments which inhibit thrombin and formation of fibrin and thrombin.
8. Pharmaceutical compositions comprising a therapeutically effective amount of the compound of the formula I of claim 1 and pharmaceutically acceptable auxiliary substances.
9. Pharmaceutical compositions according to claim 8, for inhibiting thrombin in man and other mammals.
10. Pharmaceutical compositions according to claim 8, for inhibiting formation of fibrin and thrombin in the blood of man and other mammals.
11. A method for inhibiting thrombin in the blood of man and other mammals comprising administering a compound of the formula I of claim 1
12. A method for inhibiting formation of fibrin and thrombin in man and other mammals comprising administering a compound of the formula I of claim 1.

INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/IB 01/02600

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 7 C07D295/20 C07D207/08 C07D211/18 C07D211/34 C07C311/49		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07D C07C		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) CHEM ABS Data, EPO-Internal, BEILSTEIN Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, Y	ZEGA, A. ET AL: "Design and structure-activity relationship of thrombin inhibitors with an azaphenylalanine scaffold: potency and selectivity enhancements via P2 optimization" BIOORGANIC & MEDICINAL CHEMISTRY (2001), 9(10), 2745-2756, no. 9, - 15 June 2001 (2001-06-15) pages 2745-2756, XP002196794 figure 5; tables 2-4 ---	1-12
Y	WO 95 13274 A (PFIZER LTD ; PFIZER RES & DEV (IE); PFIZER (US); DANILEWICZ JOHN CH) 18 May 1995 (1995-05-18) cited in the application claims; examples --- -/--	1-12
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
° Special categories of cited documents :		
A document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family		
Date of the actual completion of the international search	Date of mailing of the international search report	
8 May 2002	21/05/2002	
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Menegaki, F	

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	SANGSOO KIM ET AL: "Rational design of selective thrombin inhibitors" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, 1997, pages 769-774, XP002196796 figures 2,3	1-12

INTERNATIONAL SEARCH REPORT

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International Application No

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