

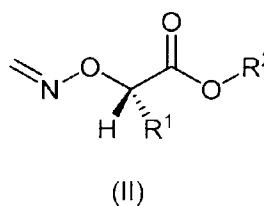
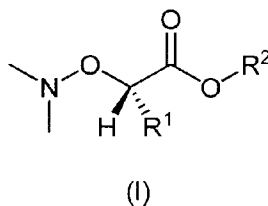
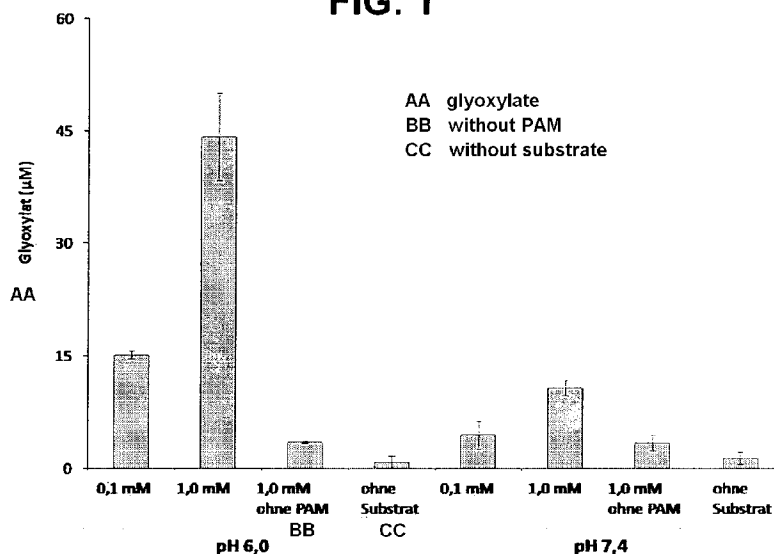


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(54) Title: METHOD FOR IMPROVED BIOACTIVATION OF PHARMACEUTICALS

FIG. 1



(57) **Abrégé/Abstract:**

The invention relates to a prodrug comprising a partial structure having the general formula (I) or (II), where R¹ and R² are hydrogen, alkyl, or aryl radicals.

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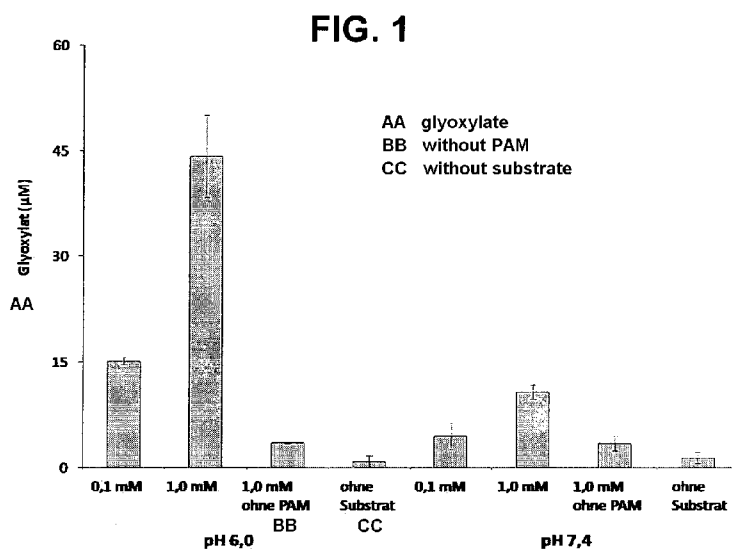
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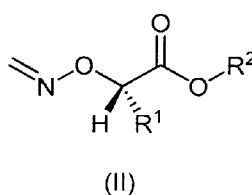
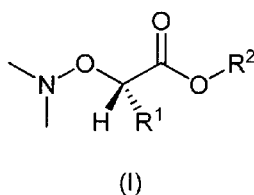
(54) Title: METHOD FOR IMPROVED BIOACTIVATION OF MEDICATIONS

(54) Bezeichnung : VERFAHREN ZUR VERBESSERTEN BIOAKTIVIERUNG VON ARZNEISTOFFEN



(57) **Abstract:** The invention relates to a prodrug comprising a partial structure having the general formula (I) or (II), where R¹ and R² are hydrogen, alkyl, or aryl radicals.

(57) **Zusammenfassung:** Prodrug umfassend eine die allgemeine Formel (I) oder (II) aufweisende Teilstruktur, wobei R¹ und R² Wasserstoff, Alkyl- oder Arylreste sind.



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- mit internationalem Recherchenbericht (Artikel 21 Absatz 3)
- vor Ablauf der für Änderungen der Ansprüche geltenden Frist; Veröffentlichung wird wiederholt, falls Änderungen eingehen (Regel 48 Absatz 2 Buchstabe h)

Method for Improving Bioactivation of Pharmaceuticals

The present invention relates to a method for improving the bioactivity of pharmaceuticals.

The requirement for a therapeutic effect of a pharmaceutical after oral administration is the absorption thereof from the gastrointestinal tract. The most important mechanism of such an effect is passive diffusion. The degree of resorption by way of passive diffusion is dependent, inter alia, on the lipophilicity.

Another problem with the treatment of many diseases by drugs is the necessity to pass the blood-brain barrier. The blood-brain barrier constitutes an effective barrier with respect to the absorption of substances in the brain. It assures selective take-up and prevents substances from penetrating. Moreover, the blood-brain barrier acts not only as a physical but also as an enzymatic barrier. A variety of processes are involved in the penetration of substances into the brain. In comparison with other indications, only few pharmaceuticals are on the market which manifest the effect thereof in the central nervous system (CNS). Of these, the predominant part reaches the CNS by way of diffusion. In this way, diseases such as epilepsy, chronic pain or depression are treated. Other severe functional disorders such as brain tumors or amyotrophic lateral sclerosis, for example, are very difficult to treat this way today.

So as to be able to overcome biomembranes by way of passive diffusion, a substance should be lipophilic, have a molecular weight lower than 500 Da and it should be present in the uncharged state. To specifically absorb small, highly polar molecules such as amino acids or sugar, different transporter systems such as nucleoside transporters, influx and efflux transports for organic anions or cations, glucose transporters, peptide transporters and amino acid transporters, for example, are expressed at the biomembranes with barrier function (gastrointestinal tract, blood-brain barrier).

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For this reason, a variety of prodrug systems are employed to improve the pharmacokinetic properties. A prodrug is a pharmaceutical that is pharmacologically inactive or substantially inactive and is not converted into an active metabolite until it is metabolized in the organism.

N-hydroxyamidines (amidoximes) and *N*-hydroxyguanidines represent known prodrug principles for increasing the oral bioavailability of amidines [Clement, B. Methoden zur Behandlung und Prophylaxe der Pneumocystis carinii Pneumonie (PCP) und anderen Erkrankungen sowie Verbindungen und Formulierungen zum Gebrauch bei besagten Methoden. [DE 4321444] [Methods for the treatment and prophylaxis of Pneumocystis carinii pneumonia (PCP) and other diseases and compounds and formulations for use in said methods] and guanidines. The nitrogen atoms of the amino and imino groups are present in a mesomeric equilibrium in the salts of the amidines and guanidines, and the concepts can be employed for both nitrogen atoms.

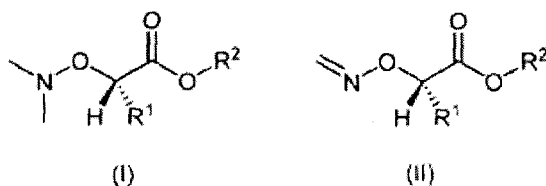
The conversion into an active metabolite takes place via different enzyme systems, depending on the underlying prodrug concept. The enzyme system that occurs practically in all forms of life is cytochrome P450 (CYP450), which catalyzes, inter alia, the following reactions:

N-oxidation, *S*-oxidation, *N*-dealkylation, *O*-dealkylation, *S*-dealkylation, desamination, dehalogenation and hydroxylation of aromatic and aliphatic compounds.

The implication of the diversity of the CYP450 enzyme system is that different substrates and pharmaceuticals compete with the system during the conversion. This results in interactions, reciprocal effects and undesirable mutual influencing. For this reason, CYP450-independent bioactivation is desirable when developing prodrugs.

It is therefore the object of the invention to provide a prodrug system which employs a path of bioactivation that is independent of the cytochrome P450 (CYP450) enzyme. This object is achieved by the subject matter described in the claims. The dependent claims provide advantageous embodiments of the invention.

According to the invention, the object is achieved in one aspect by a prodrug comprising a partial structure having the general formula (I) or (II)



where R^1 and R^2 are hydrogen, alkyl radicals or aryl radicals.

In a preferred embodiment of the invention, the term "partial structure", as it is used herein, denotes that the structural element indicated in the respective formula is part of the formula of substance, preferably of a prodrug. For example, the compound O-carboxymethyl benzamidoxime (1) constitutes a corresponding prodrug of the pharmaceutical benzamidine, wherein the partial structure is a partial structure of formula (II), and R^1 and R^2 are hydrogen atoms, respectively. This partial structure is a substituent on a benzene ring and together with the same constitutes the pharmaceutical benzamidine.

In a preferred embodiment of the invention, the term "prodrug", as it is used herein, denotes a substance that as such is inactive or pharmacologically substantially inactive, which is not converted into a pharmaceutical that is pharmacologically active until it is metabolized in the organism. The prodrug can, but does not have to, exhibit improved oral bioavailability than the actual active pharmaceutical. As an alternative, it is possible to use a prodrug because, in comparison with the pharmaceutical, it exhibits improved solubility, bioactivation, blood-brain barrier crossing, physical-chemical stability, lower toxicity and/or a tolerable or more pleasant flavor. For example, erythromycin A 2'-ethyl succinate is not administered as a prodrug to children due to the bitter taste, and not perhaps because of inadequate resorption or solubility of erythromycin A (Bhadra et al. (2005), J. Med. Chem.).

In a further preferred embodiment of the invention, the original prodrug is not metabolized from the prodrug into the pharmaceutical in a one-step reaction, but rather by way of a plurality of reaction steps, wherein each of the metabolites obtained from a reaction step can exhibit one or more of the same and/or different more advantageous properties compared to the original prodrug. To this end, not all of the metabolites may exhibit advantageous properties over the prodrug. For example, a first metabolization product of the prodrug can exhibit increased pharmacological activity compared to the prodrug, a second metabolization product derived from the first metabolization product can likewise exhibit increased pharmacological activity compared to the prodrug, and a third metabolization product derived from the second metabolization product can exhibit increased blood-brain barrier crossing and physical-chemical stability compared to the prodrug.

In a preferred embodiment of the invention, the term "physical-chemical structure", as it is used herein, denotes the capacity of a substance, for example a prodrug or a pharmaceutical, to be stored and/or used in the form of a relevant aqueous solution, for example dissolved in water, a buffer or a physiological salt solution, without chemical decomposition, for example hydrolysis. In a further preferred embodiment of the invention, the term, as it is used herein, denotes that the substance can be synthesized in stable and synthetic form. In a further preferred embodiment of the invention, the term, as it is used herein, denotes that, during the synthesis of the substance, isolated relevant synthesis precursors are more stable than analogous products, precursors or intermediate products of other substances produced according to an analogous or identical synthesis strategy, so that subsequent synthesis products or synthesis intermediate products can be produced in a more stable form, or can be produced at all.

In one embodiment, the object is achieved by a prodrug, characterized in that the partial structure which the prodrug comprises is part of a hydroxylamine, an *N*-oxide, a nitron, a diazeniumdiolate (NONOat) or a similar N-O-containing nitric oxide donor, a hydroxamic acid, a hydroxyurea, an oxime, an amidoxime (*N*-hydroxyamidine), an *N*-

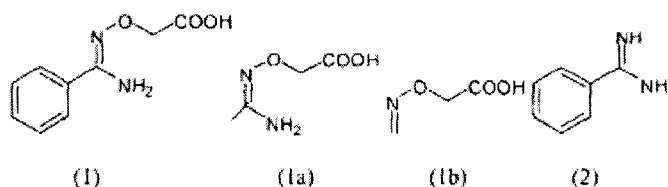
hydroxyamidinohydrazone or an *N*-hydroxyguanidine.

In the case of the prodrug carboxymethyl benzamidoxime (1) of the pharmaceutical benzamidine, for example, the partial structure is a partial structure of the formula (II), R^1 and R^2 are hydrogen atoms, respectively, and the partial structure that the prodrug comprises is part of an amidoxime (N-hydroxyamidine).

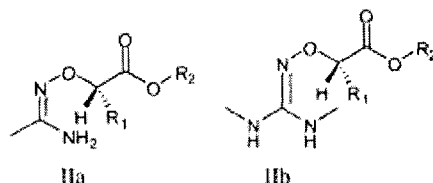
In one embodiment, the object is achieved by a prodrug, characterized in that the prodrug is metabolized into a pharmaceutical, which is a pharmaceutical for treating diseases associated with nitric oxide deficiency.

In one embodiment, the object is achieved by a prodrug, characterized in that the prodrug or the corresponding pharmaceutical is selected from the group consisting of protease inhibitors, DNA- and RNA-intercalating compounds, inhibitors of viral enzymes, and N-methyl-D-aspartate receptor antagonists.

In a preferred embodiment of the present invention, the term "higher-level partial structure", as it is used herein, shall be understood such that this higher-level partial structure comprises a partial structure of formula (I) or (II) on the one hand, and is part of the overall structure of the substance in question on the other hand. For example, in the case of the carboxymethyl benzamidoxime (1) prodrug of the pharmaceutical benzamidine (2), the higher-level partial structure, which here is denoted by (1a), comprises the partial structure of formula (IIa), where R^1 and R^2 are hydrogen, and the partial structure, which here is denoted by (1b), is the partial structure of formula (II), where R^1 and R^2 are likewise hydrogen.

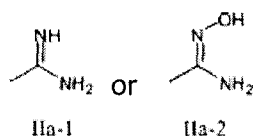


In one embodiment, the object is achieved by a prodrug, characterized in that the partial structure has the general formula IIa or IIb

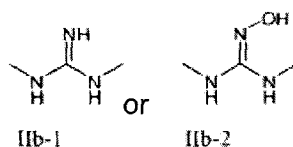


For example, in the case of the carboxymethyl benzamidoxime (1) prodrug of the pharmaceutical benzamidine (2), the higher-level partial structure comprises the partial structure of formula (IIa), where R^1 and R^2 are hydrogen, the partial structure is the partial structure of formula (II), where R^1 and R^2 are likewise hydrogen, and the pharmaceutical has the structure (IIa-1) in the prodrug rather than the partial structure of formula (IIa).

In one embodiment, the object is achieved by a prodrug, characterized in that the prodrug is a prodrug of a pharmaceutical, wherein the partial structure of the general formula IIa, after metabolization, comprises a structure having the formula

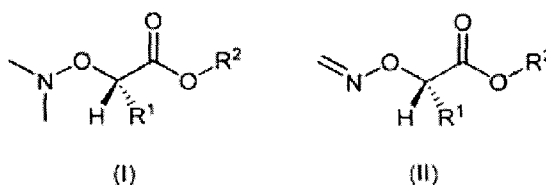


and the partial structure of general formula IIb, after metabolization, comprises a structure having the formula



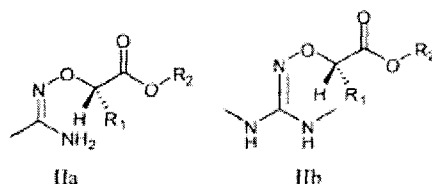
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In a further aspect of the invention, the object is achieved by the use of a partial structure forming the general formula (I) or (II)



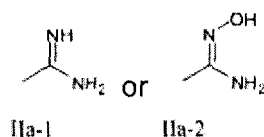
as part of the overall structure of a prodrug which is prodrug or a pharmaceutical, where R^1 and R^2 are hydrogen, alkyl radicals or aryl radicals.

In one embodiment, the object is achieved by the use of a prodrug, wherein the partial structure has the general formula (II), and is part of a higher-level partial structure IIa or IIb



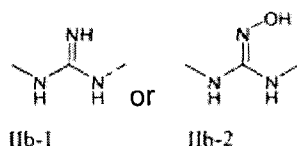
in the place of an amidine or guanidine group of a pharmaceutical to improve solubility, oral bioavailability, blood-brain barrier crossing, the flavor and/or the physical-chemical stability.

In one embodiment, the object is achieved by the use of a prodrug, wherein the prodrug is a prodrug of a pharmaceutical that has the same structure as the prodrug, except that instead of the higher-level partial structure IIa it comprises one of the partial structures IIa-1 or IIa-2



or instead of the higher-level partial structure IIb it comprises one of the partial structures IIb-1 or IIb-2

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In one embodiment, the object is achieved by the use of a prodrug for activating the pharmaceutical by peptidylglycine α -amidating monooxygenase (PAM).

In a preferred embodiment of the invention, the expression "activating the pharmaceutical by peptidylglycine α -amidating monooxygenase (PAM)", "activating a prodrug by way of the PAM activation path", bioactivation or the like, as it is used herein, denotes that the prodrug is recognized by PAM as a substrate and metabolized. In a preferred embodiment of the invention, the expression "introducing a pharmaceutical into the PAM activation path, comprising the production of a prodrug of the pharmaceutical", as it is used herein, denotes that a corresponding prodrug form is produced of a pharmaceutical to be introduced into the PAM activation path, this prodrug form being recognized by PAM and metabolized. In a preferred embodiment, the affinity of the prodrug for PAM, as compared with the pharmaceutical, is 1-1000 times, 2-100 times, 3-50 times, 4-40 times, 5-20 times or even 6-15 times greater, as a person skilled in the art will be able to determine using the K_M values.

In one embodiment, the object is achieved by the use of a prodrug, characterized in that the partial structure is part of a hydroxylamine, an *N*-oxide, a nitron, a diazeniumdiolate (NONOat) or a similar N-O-containing nitric oxide donor, a hydroxamic acid, a hydroxyurea, an oxime, an amidoxime (*N*-hydroxyamidine), an *N*-hydroxyamidinohydrazone or an *N*-hydroxyguanidine.

In a further aspect of the invention, the object is achieved by a method for introducing a pharmaceutical comprising a free amidine or guanidine function into the PAM activation path, comprising the production of a prodrug of the pharmaceutical.

In a further aspect of the invention, the object is achieved by a method for treating a patient, comprising the administration of a prodrug to the patient.

In a further aspect of the invention, the object is achieved by the use of a prodrug for producing a pharmaceutical.

In a preferred embodiment of the invention, the pharmaceutical is a pharmaceutical, or the prodrug is a prodrug, for combating viral infections such as influenza, for combating HIV infections, for the prophylaxis and treatment of visceral and cutaneous leishmaniasis, for the prophylaxis of *Pneumocystis carinii* pneumonia (PCP), for treating trypanosomiasis (African sleeping sickness), for treating malaria, for treating babesiosis, for inhibiting blood coagulation, for example for the primary prevention of venous thromboembolic events, for the prophylaxis of stroke in patients with atrial fibrillation, for lowering blood pressure, for inhibiting the growth of malignant tumors, for neuroprotection, for combating viral infections such as influenza, for the (diuretic) elimination of water from the body, for example with cardiac insufficiency, pulmonary edema, poisoning, renal insufficiency or cirrhosis of the liver, for treating allergies, for treating asthma, for treating inflammatory diseases, for example rheumatism or pancreatitis, or for the prophylaxis of ischemia (insufficient blood supply).

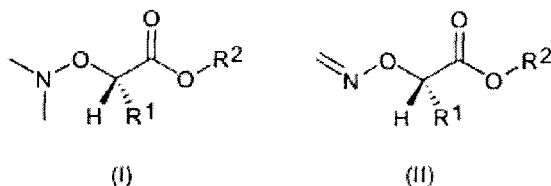
In a further aspect of the invention, the object is achieved by the use of a prodrug according to any one of claims 7 to 11 and claim 14, or by a method according to claim 13, wherein the use or the method is a use or a method for treating diseases associated with nitric oxide deficiency.

In one embodiment, the object is achieved by the use of a prodrug, characterized in that the pharmaceutical or the prodrug is selected from the group consisting of protease

inhibitors, DNA- and RNA-intercalating compounds, inhibitors of viral enzymes, and N-methyl-D-aspartate receptor antagonists.

In one embodiment, the object is achieved by the use of a prodrug, wherein the use is a use for the prophylaxis and/or treatment of visceral and/or cutaneous leishmaniasis, trypanosomiasis, phase 2 of trypanosomiasis or pneumonia caused by *Pneumocystis carinii*, for inhibiting the growth of malignant tumors, for inhibiting blood coagulation, for lowering blood pressure, for neuroprotection, or for combating viral infections, including influenza and HIV infections.

In a further aspect of the invention, the object is achieved by a pharmaceutical comprising a partial structure having the general formula (I) or (II)



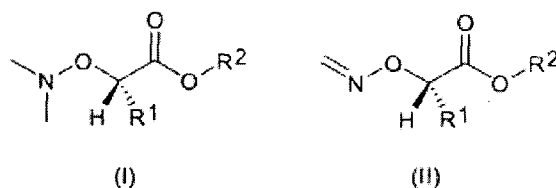
where R^1 and R^2 are hydrogen, alkyl radicals or aryl radicals.

In one embodiment, the object is achieved by a pharmaceutical comprising a partial structure having the general formula (I) or (II), characterized in that the partial structure is part of a hydroxylamine, an *N*-oxide, a nitron, a diazeniumdiolate (NONOat) or a similar N-O-containing nitric oxide donor, a hydroxamic acid, a hydroxyurea, an oxime, an amidoxime (*N*-hydroxyamidine), an *N*-hydroxyamidinohydrazone or an *N*-hydroxyguanidine.

In one embodiment, the object is achieved by a pharmaceutical according to any one of the preceding claims, characterized in that the pharmaceutical is designed to treat diseases associated with nitric oxide deficiency.

In one embodiment, the object is achieved by a pharmaceutical, characterized in that the pharmaceutical is selected from the group consisting of protease inhibitors, DNA- and RNA-intercalating compounds, inhibitors of viral enzymes, and N-methyl-D-aspartate receptor antagonists.

In a further aspect of the invention, the object is achieved by the use of an O-carboxyalkylated N-O-containing functionality for producing a pharmaceutical comprising a partial structure forming the general formula (I) or (II)



where R^1 and R^2 are hydrogen, alkyl radicals or aryl radicals, for improving the solubility, bioavailability, blood-brain barrier crossing, bioactivation and/or the physical-chemical stability of the pharmaceutical.

In one embodiment, the object is achieved by the use of a pharmaceutical comprising an O-carboxyalkylated N-O-containing functionality for activating the pharmaceutical by peptidylglycine α -amidating monooxygenase (PAM).

In one embodiment, the object is achieved by the use of a pharmaceutical, characterized in that the partial structure is part of a hydroxylamine, an N-oxide, a nitron, a diazeniumdiolate (NONOat) or a similar N-O-containing nitric oxide donor, a hydroxamic acid, a hydroxyurea, an oxime, an amidoxime (N-hydroxyamidine), an N-hydroxyamidinohydrazone or an N-hydroxyguanidine.

In one embodiment, the object is achieved by the use of a pharmaceutical, characterized in that the pharmaceutical is designed to treat diseases associated with nitric oxide deficiency.

In one embodiment, the object is achieved by the use of a pharmaceutical, characterized in that the pharmaceutical is selected from the group consisting of protease inhibitors, DNA- and RNA-intercalating compounds, inhibitors of viral enzymes, and N-methyl-D-aspartate receptor antagonists.

In one embodiment, the object is achieved by the use of a pharmaceutical, characterized in that the pharmaceutical is designed for the prophylaxis and/or treatment of visceral and/or cutaneous leishmaniasis, trypanosomiasis, phase 2 of trypanosomiasis or pneumonia caused by *Pneumocystis carinii*, to inhibit the growth of malignant tumors, to inhibit blood coagulation, to lower blood pressure, for neuroprotection, or to combat viral infections, including influenza and HIV infections.

In a further aspect of the invention, pharmaceutical compounds, pharmaceutical compositions and pharmaceutical products are provided, which comprise the compounds according to the invention and/or the salts thereof. The pharmaceutical compositions preferably contain carriers and/or adjuvants and ideally they are pharmaceutically compatible. A person skilled in the art is generally familiar with such carriers and adjuvants. The compounds according to the invention are also provided for use in medicine.

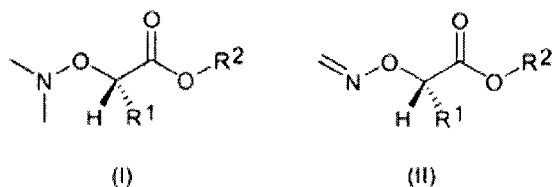
It is sufficient if the pharmaceutical comprises at least one or more active amidine, N-hydroxyamidine (amidoxime), guanidine or N-hydroxyguanidine functions in the proposed form. The pharmaceutical can thus contain, for example, a plurality of amidoxime functions (for example two, as with pentoxime ester) or N-hydroxyguanidine functions, wherein then at least one of these groups is modified in the aforementioned manner. Similarly, mixtures of pharmaceuticals can also be employed, of which at least one is modified according to the invention.

The compounds according to the invention can be administered once, as a bolus administration, every day, weekly or monthly. The manner of the administration can likewise be easily determined. In general, the possible forms of administration include

oral, rectal, parenteral such as intravenous, intramuscular, subcutaneous, transdermal administration, intrapulmonary administration and administration as an aerosol, intravesical instillation, intraperitoneal or intracardiac injection, uptake via mucous membranes or intravaginal application, for example by means of suppositories. The oral form of administration can be a liquid, semi-solid or solid formulation, in particular in the form of tablet, sugar-coated tablet, pellet or microcapsule. To this end, the active ingredient, or the active ingredient mixture, is received in a suitable non-toxic solvent, such as water, monohydric alcohols, in particular ethanols, multihydric alcohols, in particular glycerin and/or propanediol, polyglycols, in particular polyethylene glycols, and/or Miglyol, glycerinformal, dimethyl isosorbide, natural or synthetic oils, for those embodiments in which liquid formulations are used. The conventional base products, such as bentonite, Veegum, guar meal and/or cellulose derivatives, in particular methyl cellulose and/or carboxymethyl cellulose, and polymers made of vinyl alcohols and/or vinyl pyrrolidones, alginates, pectins, polyacrylate, solid and/or liquid polyethylene glycols, paraffins, fatty alcohols, vaseline and/or waxes, fatty acids and/or fatty acid esters are used to produce semi-solid or solid preparations.

Moreover, the known extenders, such as colloidal silicic acid, talcum, lactose, starch powder, sugar, gelatin, metal oxides and/or metal salts may be present in solid formulations. Further additives such as stabilizers, emulsifiers, dispersing agents and preservatives are an obvious choice.

Surprisingly, it has been found that O-carboxyalkylated N-O-containing functionalities of the general formula (I) or (II), which are bound to a pharmaceutical molecule via bonds at the nitrogen (N),



where (I) and (II) are, for example, part of a hydroxyl amine, an *N*-oxide, a nitron, a diazeniumdiolate (NONOat) or similar N-O-containing nitric acid donor, a hydroxamic acid, an oxime, an amidoxime (*N*-hydroxyamidine), an *N*-hydroxyamidinohydrazone or an *N*-hydroxyguanidine, and R^1 (which must be *pro-R* configured) and R^2 are hydrogen, alkyl radicals or aryl radicals, utilize a bioactivation path that is independent of cytochrome P450 (CYP450) enzymes. This constitutes an unexpected result because it is known that CYP450 enzymes generally catalyze oxidative O-dealkylations, which in the case of the prodrug principle proposed here would also be necessary to release the actual pharmaceutical.

The proposed etherification of N-O-containing functionalities with carboxyalkyl radicals produces the special advantage that an enzyme different from the CYP450 enzyme can be utilized for bioactivation: peptidylglycine α -amidating monooxygenase (PAM). This prevents, for example, side effects and the aforementioned interactions with other simultaneously administered pharmaceuticals.

In higher organisms (vertebrates), peptidylglycine α -amidating monooxygenase (PAM) constitutes a bifunctional enzyme, which is composed of a monooxygenase domain (PHM, peptidylglycine α -hydroxylating monooxygenase, EC 1.14.17.3) and a lyase domain (PAL, peptidyl- α -hydroxyglycine α -amidating lyase, EC 4.3.2.5). On an overall basis, PAM is subject to a strongly tissue-specific and development-dependent regulation by splicing and expression. Within the meaning of a post-translational modification, PAM is able to activate diverse physiologically occurring peptide

hormones, neurotransmitters and growth factors (for example, substance P, neuropeptide Y, oxytocin, vasopressin, calcitonin). In the process, the peptides are C-terminally amidated by separating a terminal glycine by means of oxidative *N*-dealkylation in a monooxygenase reaction.

A particular advantage of the etherification of the N-O-containing functionalities with carboxyalkyl radicals, as proposed according to the invention, is the improved solubility resulting from the insertion of a carboxylic acid that is negatively charged under physiological conditions (pH 6-8).

An additional advantage is that the etherification of the N-O-containing functionalities proposed according to the invention – using (alkoxycarbonyl)alkyl ethers or (aryloxy carbonyl)alkyl ethers – increases the lipophilicity so much that passive diffusion is made possible, whereby the bioavailability and/or blood-brain barrier crossing is improved.

The possibility of using a comparatively small radical – in the simplest case, a carboxymethyl radical – as the prodrug group, so that the size of the pharmaceutical molecule increases only moderately, is likewise advantageous.

Wand *et al.* [*Metabolism* 1985, 34, 11, 1044] analyzed PAM activities in different human tissues and detected the highest activity in tissues of the CNS (in particular in the pituitary gland). In contrast, no activity was found in the classic foreign matter-metabolizing organs, the liver and the kidneys. Activities for which the planned prodrug concept could also be utilized were likewise detected in plasma, the heart and lungs.

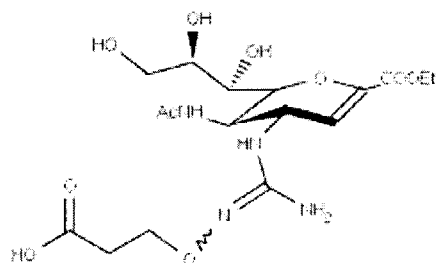
In particular the high activities of this enzyme in the CNS can be utilized to transport O-carboxyalkylated prodrugs through the blood-brain barrier, so as to then convert them. However, bioactivation in the cardiovascular system after peroral application and absorption from the gastrointestinal tract is also possible.

The prodrug system according to the invention can be applied to different pharmaceuticals which have an amidine or guanidine function. The following pharmaceuticals are particularly preferred:

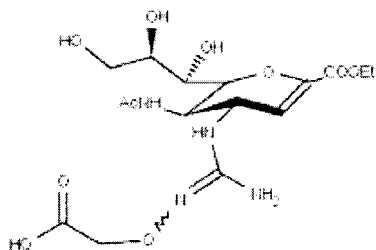
pentamidine, dabigatran, BSF 411693 (Abbott), idazoxan hydrochloride, irbesartan, linogiride, lofexidine hydrochloride, tetrahydrozoline hydrochloride, tolazoline, xylometazoline hydrochloride, pentamidine isethionate, taribavirin, thiamine (Vitamin B1), bosentan, dibromopropamide isethionate, hydroxystilbamidine isethionate, sibrifiban, orbofiban, xemilofiban, argatroban, ximelagatran, melagatran, 2-piperidinic acid, orbofiban acetate, epinastine (Relestat), RO 43-8857, AB1 (Chlorambucil, analogues), AMG-126737, AY-0068, B-623, BABIM, BIBT-986 (Boehringer Ingelheim), CI-1031 (company: Biosciences), CJ-1332 (company: Curacyte), CJ-463 (company: Curacyte), CJ-672 (company: Curacyte), CT50728 (Portolla Pharmaceuticals), CVS-3983, DX-9065a, Lamifiban (Roche), LB-30870 (company: LG LifeSciences Ltd), LY-178550 (company: Lilly), PHA-927F and analogues, RO-44-3888 (Roche), sepimostat, FUT-187 (Torii), viramidine (Ribapharm), WX-FX4 (Willex), YM-60828 (Yamanouchi Pharmaceutical Co. Ltd), ZK-807191 (Berlex Biosciences), NAPAP (SR 25477), BIIL 315 (Boehringer Ingelheim), BIIL 260 (Boehringer Ingelheim), BIIL 284/260 (Boehringer Ingelheim), tanogitran, moxilubant, stilbamidine, panamidine, fradafiban, diminazene, roxifiban, furamidine, PD0313052, PHA 927F, PHA 798, fidexaban, otamixaban, thromstop (Thrombstop), zanamivir, amiloride hydrochloride, anagrelide hydrochloride, proguanil, cimetidine, clonidine hydrochloride, guanoxan, peramivir, romifidine, tirapazamine, tizanidine, tolondine nitrate, metformin, diminazene, debrisoquine, sulfamethazine, eptifibatide, famotidine, Bayer pharmaceutical, streptomycin, nafamostat, FUT-175, inogatran, guanethidine (Thilodigon), 3DP-10017, APC-366, CVS-1123, diphenyl phosphonate derivative, E-64, FOY-305, MBGB, MIBG, RWJ-422521, Synthalin, WX-293, WX-340, BMS-189090, JTV-803 (Japan Tobacco), napsagatran, ismelin, Tan 1057A, Hydikal, Phenformin (Retardo), netropsin (Sinanomycin), BIIB 722 (sabiporide), guanadrel, deoxyspergualin, BMS 262084, Siamformet (Orabet), PPACK (Pebac), MERGETPA (Plummer's carboxypeptidase inhibitor), peramivir, famotidine, zaltidine.

The annex contains a table with the chemical formulas, the CAS registry numbers and the indications of the pharmaceuticals.

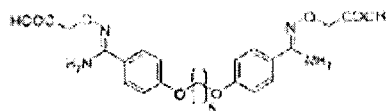
Hereinafter, 4 prodrugs according to the invention are shown by way of example:



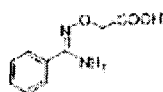
Carboxyethoxy prodrug of zanamivir



Carboxymethoxy prodrug of zanamivir



Bis(carboxymethoxy) prodrug of pentamidine



Carboxymethyl benzamidoxime

The surprising discovery that non-peptidic O-carboxyalkylated N-O-containing functionalities are accepted as substrates of PAM is demonstrated in the exemplary embodiments based on amidoxime- and N-hydroxyguanidine-based model compounds.

O-carboxymethyl benzamidoxime (1) was tested for the PAM substrate properties thereof as a model compound of amidoximes. O-carboxymethyl benzamidoxime is a possible prodrug of the pharmaceutical benzamidoxime. The PAM-catalyzed bioactivation of O-carboxymethyl benzamidoxime (1) into benzamidoxime (2) occurs with glyoxalic acid being released at the same time.

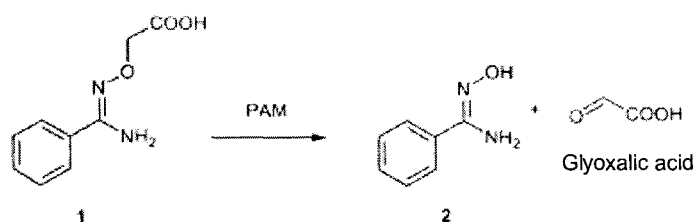


FIG. 1 shows the results of the colorimetric determination of the glyoxalate formation. The determined glyoxalate concentrations are mean values $\pm$ standard deviations from two incubations, each of which was measured twice. The formation of glyoxalate as the cleavage product of the PAM catalysis of 1 was verified in a concentration-dependent manner. The incubations at the pH optimum of PAM (pH 6.0) resulted in considerably higher conversions in comparison with the incubation at pH 7.4. In the colorimetric assay, a 5-point calibration of glyoxalate was carried out simultaneously with the testing of 1. The calibration was linear in the determined concentration range ($r^2 = 1.000$).

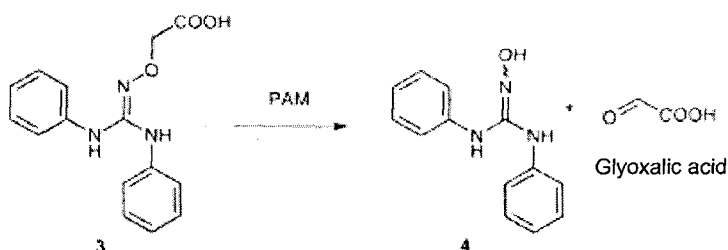
Since, according to these results, *O*-carboxymethyl benzamidoxime (1) was accepted as a substrate by PAM, the reaction was characterized in more detail by determining the K_M and V_{max} values.

For this purpose, an HPLC analysis was developed. The calibration line for benzamidoxime was linear in the determined concentration range ($r^2 = 1.000$) and the recovery rate was 130.6% ($r^2 = 0.999$). Two independent experiments ($n = 2$) yielded a K_M value of $307 \pm 80 \mu\text{M}$ and V_{max} value of $393 \pm 40 \text{ nmol min}^{-1} \text{ mg}^{-1}$ PAM. FIG. 2 is a representative illustration of such a determination.

For the CYP450 substrate studies, the aforementioned HPLC analysis was modified so that additionally the detection of the conceivable metabolite benzamidine is possible as a product from the *N*-reduction of benzamidoxime (2). At pH 6.0 and pH 7.4, neither benzamidoxime (a possible prodrug of benzamidine) nor benzamidine were detected in any of the CYP450 enzyme sources.

Based on the benzamidoxime model compound 1, the *O*-carboxymethyl function is removed only from PAM, but not from cytochrome P450 within the meaning of a monooxygenase reaction.

N-carboxymethoxy-*N'*,*N''*-diphenyl guanidine (3) was tested for the PAM substrate properties thereof as a model compound of hydroxyguanidines.



The PAM-catalyzed bioactivation of *N*-carboxymethoxy-*N,N'*-diphenyl guanidine (3) into *N,N'*-diphenyl-*N''*-hydroxy guanidine (4) takes place with glyoxalic acid being released at the same time.

The results from the colorimetric assay using 3 were comparable to those of the amidoxime model compound 1. For determining the K_M and V_{max} values, an HPLC analysis was developed which is able to separate the prodrug 3 and hydroxy guanidine 4 within 15 minutes on an RP column. The calibration line for *N,N'*-diphenyl-*N''*-hydroxy guanidine (4) was linear in the determined concentration range ($r^2 = 0.999$) and the recovery rate was 111.7% ($r^2 = 0.999$). Two independent experiments ($n = 2$) yielded a K_M value of $37 \pm 5 \mu\text{M}$ and V_{max} value of $373 \pm 53 \mu\text{mol min}^{-1} \text{mg}^{-1}$ PAM. FIG. 3 is a representative illustration of such a determination.

From the determined K_M value, an affinity for PAM that is approximately 8 times greater in comparison with the amidoxime prodrug 1 can be derived, while the conversion rate is comparable.

For the CYP450 substrate studies, the HPLC analysis developed for the PAM substrate studies was modified so that additionally the detection of the conceivable metabolite *N,N'*-diphenyl guanidine is possible as a product from the *N*-reduction of hydroxy guanidine 4. At pH 6.0 and pH 7.4, neither 4 nor *N,N'*-diphenyl guanidine were detected in any of the CYP450 enzyme sources that were used after an incubation time of 180 minutes.

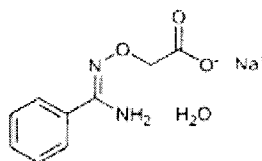
Analogously to *O*-carboxymethyl benzamidoxime (1), the *O*-carboxymethyl function is removed only from PAM, but not from cytochrome P450 within the meaning of a monooxygenase reaction, based on the hydroxyguanidine model compound 3.

Materials and Methods

Sodium salt of *O*-carboxymethyl benzamidoxime monohydrate (1)

Modified instruction according to Koch [Ber. Dtsch. Chem. Ges. 1889, 22, 3161]:

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A solution of 681 mg benzamidoxime (5.0 mmol), 1.04 g bromoacetic acid (7.5 mmol) and 500 mg sodium hydroxide pellets (12.5 mmol) in 5 ml ethanol is boiled for 5 hours under reflux. Thereafter, the solvent is removed under vacuum until a deposit starts to form. The deposit is allowed to fully precipitate, filtered off and dried. The product is recrystallized from ethanol (96%)/water (95:5).

Yield: 937 mg white fine felt-like crystals (80%)

Melting pt.: 226°C (dec.)

¹H-NMR (DMSO-*d*₆):

δ/ppm = 4.13 (s, 2H, O-CH₂), 6.09 (br s, 2H, NH₂), 7.37 (m, 3H, 3',4',5'-CH), 7.67 (m, 2H, 2',6'-CH).

¹³C-NMR (CDCl₃):

δ/ppm = 73.6 (O-CH₂), 125.7, 128.0, 129.0 (ArCH), 132.8 (ArC), 151.4 (C=N), 173.2 (CO).

MS (ESI):

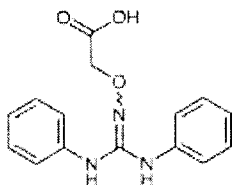
m/z = 217 [M + Na]⁺, 195 [M + H]⁺, 119 [M - C₄H₂ - C₂H₂ + H]⁺, 105 [C₆H₅N₂]⁺.

C₉H₉N₂NaO₃·1.0 H₂O (234.18)

Calculated C 46.16 H 4.73 N 11.96

Found C 46.43 H 4.44 N 11.65

***N*-carboxymethoxy-*N'*,*N''*-diphenyl guanidine (3)**



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546 mg aminooxyacetic acid semichloride (5 mmol) and 697 μ l triethylamine (5 mmol) are stirred for 30 minutes in 10 ml dry DMF. The precipitate is filtered off and 970 mg *N,N'*-diphenyl carbodiimide (5 mmol) is added to the filtrate. The batch is stirred for four hours at room temperature, solvent-extracted with ethyl acetate, and the product is recrystallized from ethanol.

Yield: 285 mg of a white solid (20%)

Melting pt.: 176°C

DC: R_f = 0.29 (dichloromethane/methanol, 9:1)

¹H-NMR (DMSO-*d*₆):

δ /ppm = 4.37 (s, 2H, O-CH₂), 6.75-6.87 (m, 2H, ArH), 7.03-7.20 (m, 8H, ArH), 8.02, 8.21 (2x br s, 1H, NH), 12.05 (br s, 1H, COOH).

¹³C-NMR (DMSO-*d*₆):

δ /ppm = 70.0 (O-CH₂), 116.7, 118.7, 119.8 121.0, 128.5 (ArCH), 140.7 (ArC), 142.3 (ArC), 147.5 (C=N), 171.8 (CO).

MS (ESI):

m/z = 308 [M + Na]⁺, 286 [M + H]⁺, 210 [M - C₂H₄O₃]⁺.

MS (EI):

m/z (%) = 209 (38), 208 (37), 119 (20), 118 (38), 93 (100), 91 (47), 77 (43), 66 (31), 51 (30).

C₁₅H₁₅N₃O₃·0.3 H₂O (290.71)

Calculated C 61.97 H 5.41 N 14.45

Found C 62.18 H 5.72 N 14.57

HPLC system

Waters Breeze HPLC system with Waters 1525 pumps, Waters 2487 absorption detector, Waters 717 Plus autosampler and Breeze recording and evaluation software (Version 3.30), Gynkotek STH 585 column oven.

HPLC columns:

Synergi Max-RP 80 A (250 x 4.6 mm, 4 μ m) with C-18 precolumn (4 x 3 mm) (Phenomenex);

LiChroCART, LiChrospher 100, RP-8 (125 x 4 mm, 5 μ m) with LiChrospher 60 precolumn, RP-select B (4 x 4 mm, 5 μ m) (Merck);

LiChroCART, LiChrospher RP-select B (250 x 4.6 mm, 5 μ m) with LiChrospher 60 precolumn, RP-select B (4 x 4 mm, 5 μ m) (Merck).

Additional devices and materials:

Cary 50 UV-Vis photometer (Varian); 96-well plates (Greiner); GFL-1083 shaking water bath (Gesellschaft für Labortechnik, Burgwedel); microliter centrifuge (Hettich GmbH); InoLab pH Level 1 pH measuring device (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim) with a LiQ Plast pH electrode (Hamilton); VF2 vortexer (Janke und Kunkel GmbH & Co. KG, Staufen); 1.5 ml reaction vessels (Sarstedt AG & Co., Nümbrecht).

Enzyme sources:

The recombinant peptidylglycine α -amidating monooxygenase (PAM, rat, EC 1.14.17.3) that was used was provided by Unigene Laboratories, Inc. (New Jersey, USA) (specific activity = $5.8 \cdot 10^6$ U/mg protein); bovine liver catalase (EC 1.11.1.6), specific activity = 12600 U/mg solid (Aldrich).

The cytochrome P450 enzyme sources that were used were obtained in the Clement von Grünwald working group according to the following instructions:

Porcine liver microsomes and 9000 g supernatant:

The pork livers were procured from a local butcher (Bordesholm) and the organs were transported directly after slaughter in an ice-cooled 20 mM phosphate buffer (1 mM Na₂ EDTA, pH 7.4). For further processing, the liver lobes were first perfused with 50 mM phosphate buffer (1 mM Na₂ EDTA, pH 7.4) and washed. The tissue was cut into pieces and run through a commercially available meat grinder. The suspension was diluted an equal volume of phosphate buffer and homogenized using a flow homogenizer. The microsomes and 9000 g supernatant were further obtained by differential ultracentrifugation. For storage, the resulting preparations were aliquotted and frozen at -80°C.

Human liver microsomes and 9000 g supernatant:

To obtain human microsomes, human liver tissue from cancer patients of the surgical department of the University Clinic of Christian-Albrecht University was obtained who had to undergo hemihepatectomy.

The liver tissue pieces were flash-frozen in a saccharose-containing phosphate buffer (10 mM K₂HPO₄, 10 mM KH₂PO₄, 250 mM saccharose, 1 mM Na₂ EDTA, pH 7.4, 4°C). As soon as a sufficient quantity of organ parts (> 3) was available, the corresponding pieces were thawed and pooled so as to compensate for differences due to interindividual variations. The tissue pieces were cut into smaller parts at 4°C, washed several times with buffer solution (without EDTA), and processed into a suspension using a homogenizer. The microsomes and 9000 g supernatant were further obtained from this suspension by differential ultracentrifugation. For storage, the resulting preparations were aliquotted and frozen at -80°C.

PAM assay: incubation conditions

A typical incubation batch of 300 µl (total volume) contained 25000 U/ml peptidylglycine α-amidating monooxygenase (PAM, company: Unigene Laboratories), 250 U/ml catalase, 1 µM copper(II) (employed as acetate/monohydrate), 2 mM sodium ascorbate, 5 mM potassium iodide and the respective substrate in 0.1 mM or 1 mM concentration, in buffers having different pH values. The buffer system used was 30 mM MES for the incubation at pH 6.0 and 50 mM HEPES for the incubation at pH 7.4. The pH value was adjusted in each case with diluted sodium hydroxide. The incubation was carried out at 37°C in the shaking water bath for 60 minutes, 100 µl was withdrawn, and the reaction was stopped with 50 µl 10% TFA_(aq)/acetonitrile (2:3). The remaining batch was incubated for another 180 minutes at 37°C and stopped with 100 µl 10% TFA_(aq)/acetonitrile (2:3).

The stopped samples were shaken for 5 minutes (vortexer) and frozen at -80°C. To analyze the samples, they were thawed, shaken for 5 minutes, and the precipitated protein was centrifuged at 10000 rpm. The supernatant was used for the colorimetric

glyoxylate determination and/or HPLC measurement.

For the K_M and V_{max} determination, 100 μ l batches were treated at pH 6.0 under the aforementioned conditions, however with the difference that the incubation time was 30 minutes.

Colorimetric determination of glyoxylate

200 μ l of the incubation batch that was freed of protein was mixed with 20 μ l of a phenylhydrazine solution (20 mg in 2 ml *aqua bidest.*) and shaken for 5 minutes in the shaking water bath at 37°C. Thereafter, the mixture was cooled for 15 minutes to 0°C, 100 μ l ice-cold 6 N HCl was added and allowed to sit at 0°C for an additional 5 minutes. Then, 20 μ l of a potassium hexacyanoferrate(III) solution (100 mg in 2 ml *aqua bidest.*) was added. The batch was allowed to rest for 15 minutes at room temperature and 200 μ l was withdrawn for the measurement using a Plate Reader (Cary 50 UV-Vis photometer, 520 nm).

Calibration:

For a 5-point calibration, glyoxalic acid in concentrations of 2, 5, 10, 50 and 100 μ M in a 2:1 mixture of assay buffer (pH 6.0):10% TFA_(aq)/acetonitrile (2:3) was measured as described above. This calibration took place simultaneously for each assay of a test compound that was carried.

HPLC analysis for separating O-carboxymethyl benzamidoxime (1) and benzamidoxime (2)

Column:	Synergi Max-RP 80 A (250 x 4.6 mm, 4 μ m)
Column temperature:	20°C
Mobile phase:	79% (v/v) 10 mM octyl sulfonate, pH 2.5 (H ₃ PO ₄) 21% (v/v) acetonitrile
Flow rate:	1.0 mL/min
Run time:	20 min.
Detection:	Absorption measurement at 229 nm

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Injection volume: 20 µL

Retention times:

O-carboxymethyl benzamidoxime (1) 8.9 min + 0.2 min

Benzamidoxime (2) 14.4 min + 0.2 min

Calibration and recovery

For the calibration, benzamidoxime was dissolved in eight concentrations of 0.1-500 µM, dissolved in assay buffer (30 mM MES, 1 µM copper(II) acetate, 2 mM sodium ascorbate, 5 mM potassium iodide, pH 6.0), and measured using the aforementioned HPLC method.

For determining the recovery, the same concentrations were produced in assay buffer (end volume = 100 µl). In addition, *O*-carboxymethyl benzamidoxime (0.5 mM) and 250 U/ml catalase were added, followed by 50 µl 10% TFA_(aq)/acetonitrile (2:3). The samples were shaken using the vortexer and frozen at -80°C. To measure the samples, they were thawed, shaken 5 minutes using the vortexer, and centrifuged for 5 minutes at 10000 rpm.

HPLC analysis for separating *N*-carboxymethoxy-*N'*,*N''*-diphenyl guanidine (3) and *N*-hydroxy-*N'*,*N''*-diphenyl guanidine (4)

Column: LiChrospher RP-select B (250 x 4.6 mm, 5 µm)

Column temperature: 20°C

Mobile phase: 70% (v/v) 40 mM ammonium acetate, pH 5.2
30% (v/v) acetonitrile

Flow rate: 1.0 ml/min

Run time: 15 min.

Detection: Absorption measurement at 229 nm

Injection volume: 20 µl

Retention times:

N-carboxymethoxy-*N'*,*N''*-diphenyl guanidine (3) 5.2 min + 0.1 min*N*-hydroxy-*N'*,*N''*-diphenyl guanidine (4) 9.0 min + 0.2 min

Calibration and recovery

For the calibration, *N*-hydroxy-*N'*,*N''*-diphenyl guanidine (4) was dissolved in eight concentrations of 0.1-500 μ M, dissolved in assay buffer (30 mM MES, 1 μ M copper(II) acetate, 2 mM sodium ascorbate, 5 mM potassium iodide, pH 6.0), and measured using the aforementioned HPLC method.

For determining the recovery, the same concentrations were produced in assay buffer (end volume = 100 μ l). In addition, *N*-carboxymethoxy-*N'*,*N''*-diphenyl guanidine (3) (0.5 mM) and 250 U/ml catalase were added, followed by 50 μ l 10% TFA_(aq)/acetonitrile (2:3). The samples were shaken using the vortexer and frozen at -80°C. To measure the samples, they were thawed, shaken 5 minutes using the vortexer, and centrifuged for 5 minutes at 10000 rpm.

CYP450 assay: incubation conditions

A typical incubation bath of 500 μ l (total volume) contained 0.3 mg protein (pork or human liver enzyme source), 0.1 mM (or 1 mM) test compound in 100 mM phosphate buffer (pH 6.0 or pH 7.4) and 1 mM NADH (or NADPH). The incubation was started after a 5-minute pre-incubation of the enzyme and test compound in buffer, adding NADH (or NADPH), and the product was shaken for 60 minutes or 180 minutes at 37°C in the shaking water bath. The batches were stopped by adding the same volume of acetonitrile, shaken using the vortexer, and frozen at -80°C.

To analyze the samples, they were thawed, shaken 5 minutes using the vortexer, and the protein was separated by means of 5-minute centrifugation at 10000 rpm. The supernatant was used for the HPLC analysis.

HPLC analysis for separating *O*-carboxymethyl benzamidoxime (1), benzamidoxime (2) and benzamidine

Column:	Synergi Max-RP 80 A (250 x 4.6 mm, 4 μ m)
Column temperature:	20°C
Mobile phase:	82.5 % (v/v) 10 mM octyl sulfonate, pH 2.5 (H ₃ PO ₄) 17.5 % (v/v) acetonitrile

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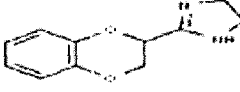
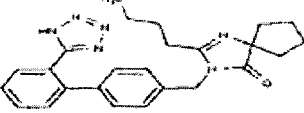
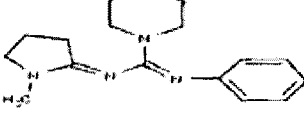
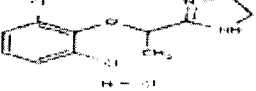
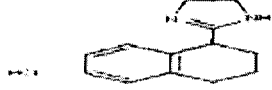
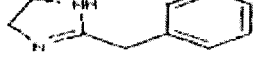
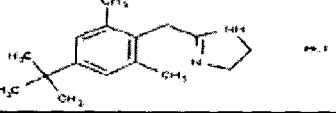
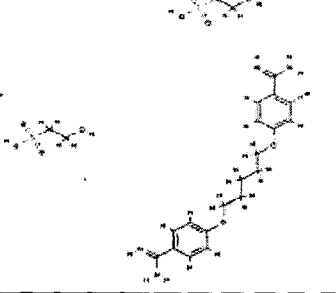
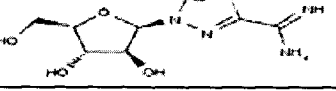
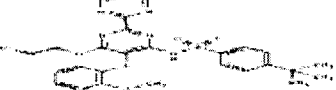
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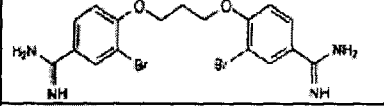
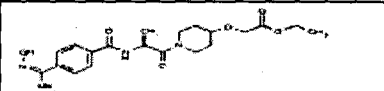
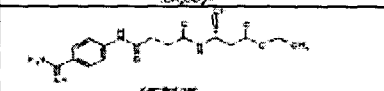
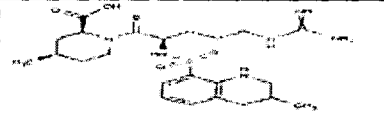
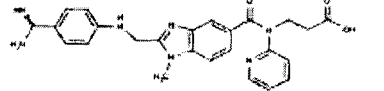
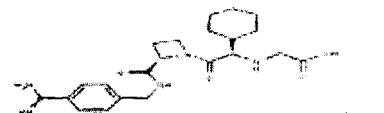
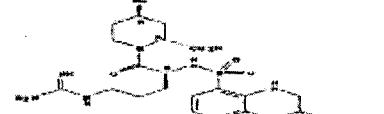
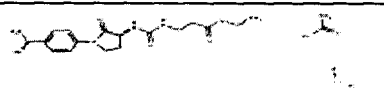
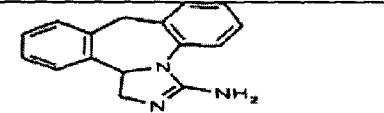
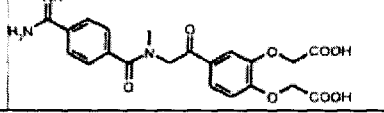
Flow rate: 1.0 ml/min
 Run time: 35 min.
 Detection: Absorption measurement at 229 nm
 Injection volume: 20 µL
 Retention times:
 O-carboxymethyl benzamidoxime (1) 13.6 min + 0.1 min
 Benzamidoxime (2) 22.8 min + 0.3 min
 Benzamidine 26.0 min + 0.3 min

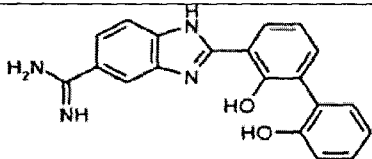
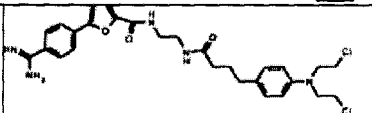
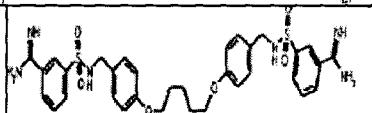
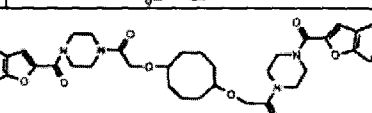
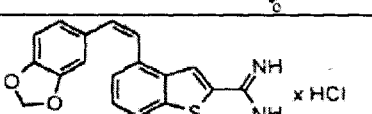
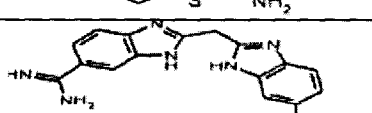
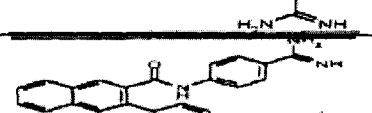
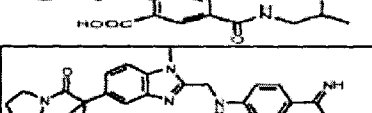
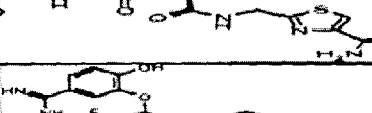
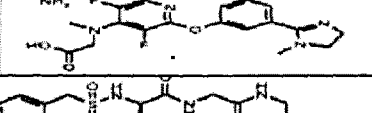
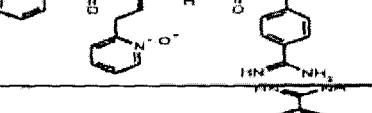
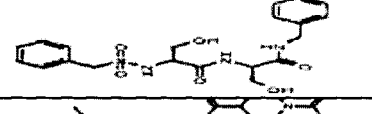
HPLC analysis for separating *N*-carboxymethoxy-*N'*,*N''*-diphenyl guanidine (3), *N*-hydroxy-*N'*,*N''*-diphenyl guanidine (4) and *N'*,*N''*-diphenyl guanidine

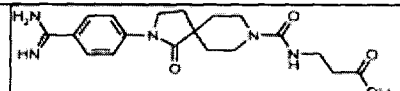
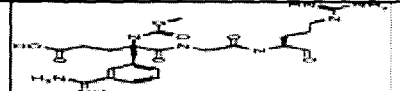
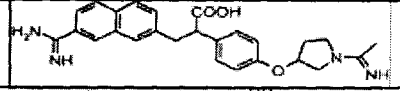
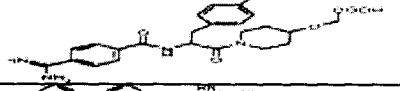
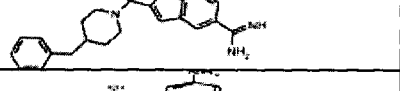
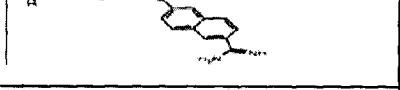
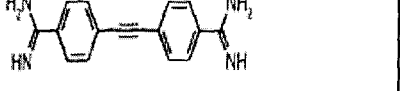
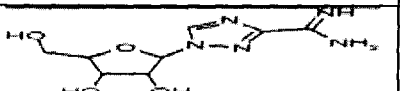
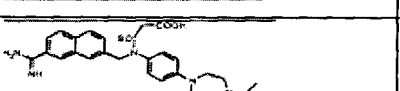
Column: LiChrospher RP-select B (250 x 4.6 mm, 5 µm)
 Column temperature: 20°C
 Mobile phase: 80% 20 mM ammonium acetate, pH 4.3
 20% acetonitrile
 Flow rate: 1.25 ml/min
 Run time: 15 min.
 Detection: Absorption measurement at 205 nm
 Injection volume: 30 µL
 Retention times:
N,*N'*-diphenyl guanidine 6.7 min + 0.2 min
N-carboxymethoxy-*N'*,*N''*-diphenyl guanidine (3) 7.8 min + 0.2 min
N-hydroxy-*N'*,*N''*-diphenyl guanidine (4) 10.7 min + 0.3 min

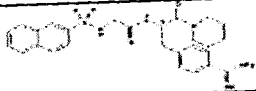
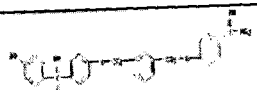
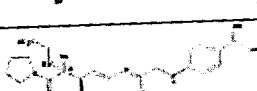
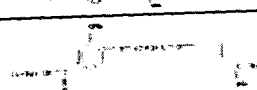
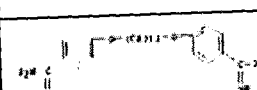
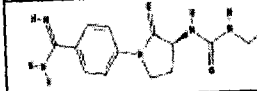
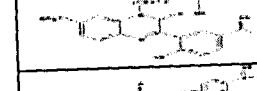
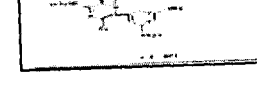
Hereinafter a table is provided of the pharmaceuticals to which the prodrug system according to the invention can preferably be applied:

Structure	Substance name	CAS	Action/indication
	idazoxan hydrochloride	79944-58-4	selective α_2 adrenergic receptor antagonist and antagonist of the imidazoline receptor; initially tested as anti-depressant, but now being examined for schizophrenia
	irbesartan	138402-11-6	angiotensin II receptor antagonist, like most sartans against high blood pressure
	linogiride	75358-37-1	against hyperglycemia
	lofexidine hydrochloride	21498-08-8	α_2 adrenergic receptor antagonist, previously as a blood pressure reducing agent, today primarily used against heroin and opiate withdrawal symptoms
	tetrahydrozoline hydrochloride	522-48-5	in eye drops and nasal sprays, alpha antagonist
	tolazoline	59-98-3	non-selective, competitive α_2 adrenergic receptor antagonist; has vessel-expanding effect, usually used in veterinary medicine as a wake-up agent
	xylometazoline hydrochloride	1218-35-5	nasal spray against the cold, etc.
	pentamidine isethionate	140-64-7	anti-infectious antiprotozoal agent effective in trypanosomiasis, leishmaniasis and some fungal infections
	taribavirin	119567-79-2	polymerase inhibitor against hepatitis C
	thiamine (Vitamin B1)	59-43-8	Vitamin B1 deficiency
	bosentan	147536-97-8	endothelin receptor antagonist for treating pulmonary arterial hypertension

	dibromopropamidine isethionate	496-00-4	antiseptic, eye drops
	hydroxystilbamidine isothionate	495-99-8	treating various fungal infections
	sibrafiban	172927-65-0	GP1Ib/IIIA inhibitor
	orbofiban	163250-90-6	antiplatelet drug
	xemilofiban	149820-74-8	glycoprotein IIb/IIIa antagonist as coagulation inhibitor
	argatroban	74863-84-6 141396-28-3	anticoagulants
	dabigatran	211941-51-1	anticoagulants
	ximelagatran / melagatran	159776-70-2	thrombin inhibitor
	2-piperidine carboxylic acid	74863-84-6	thrombin inhibitor
	orbofiban acetate	165800-05-5	glycoprotein IIb/IIIa receptor antagonist
	epinastine (Relestat)	127786-29-2	antihistamine
	RO 43-8857	1322224-71- 6	GP1Ib/IIIA antagonist, anticoagulant

			FVIIa inhibitor, anticoagulant, preclinical
	AB ₁ (Chlorambucil analogues)	305-03-3	leukemia, lymphomas, breast cancer, preclinical
	AMG-126737	224054-76-6	tryptase inhibitor, preclinical
	AY-0068		tryptase inhibitor, preclinical
	B-623		urokinase inhibitor, malignant diseases, preclinical
	BABIM	74733-75-8	tryptase inhibitor, allergies, asthma, preclinical
		1670-14-0	factor VIIa inhibitor, anticoagulant, preclinical
	BIBT-986 (Tanogitrin) (Boehringer Ingelheim)	637328-69-9	dual FXa and thrombin inhibitors, anticoagulant
	BSF 411693 (Abbott)		thrombin inhibitor, anticoagulant, preclinical
	CI-1031 (Biosciences)	605-69-6	FXa inhibitor, anticoagulant, Phase II
	CJ-1332 (Curacyte)		FXa inhibitor, anticoagulant, preclinical
	CJ-463 (Curacyte)		urokinase inhibitor, malignant diseases, preclinical
	CJ-672 (Curacyte)		matriptase inhibitor, therapy of malignant diseases, preclinical

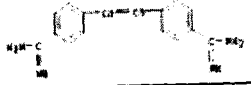
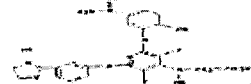
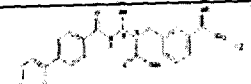
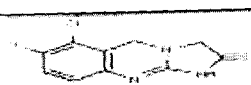
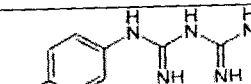
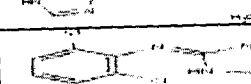
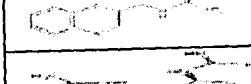
	CT50728 (Portolla Pharmaceuticals)		glycoprotein IIb/IIIa antagonist as coagulation inhibitor
	CVS-3983		matriptase inhibitor, malignant diseases, preclinical
	DX-9065a	155204-81-2	FXa inhibitor, anticoagulant, Phase III
	Lamifiban (Roche)	103577-45-3	glycoprotein IIb/IIIa antagonist as coagulation inhibitor
	LB-30870 (LG LifeSciences Ltd)		thrombin inhibitor, anticoagulant, Phase I
	LY-178550 (Lilly)		thrombin inhibitor, anticoagulant, preclinical
	PHA-927F and analogues		factor Va inhibitor, anticoagulant
	RO-44-3888 (Roche)		glycoprotein IIb/IIIa antagonist as coagulation inhibitor
	sepimostat, FUT-187 (Torii)	103926-64-3	C3/C5 convertase inhibitor, complement activation, antiphlogistic, Phase II
	stilbamidine (analogue diminazene, berenil)	140-59-0	trypanosomiasis
	viramidine (Ribapharm)	119567-79-2	virustatic agent (hepatitis C), Phase III
	WX-FX4 (Wilex)		FXa inhibitor, anticoagulant, preclinical
	YM-60828 (Yamanouchi Pharmaceutical Co. Ltd.)		FXa inhibitor, anticoagulant, preclinical
	ZK-707191 (Berlex Biosciences)		FXa inhibitor, clinical testing

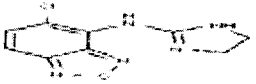
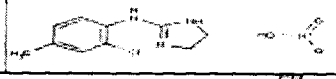
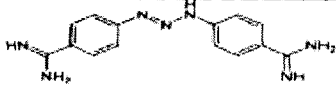
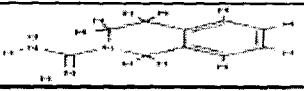
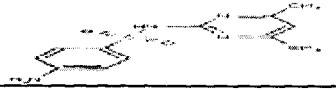
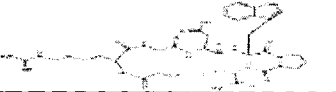
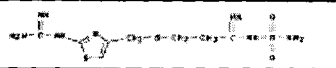
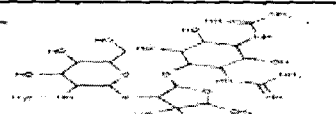
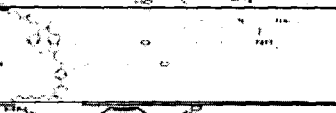
	NAPAP, SR25477	86845-59-2	analgesic
	BIIL 315 (Boehringer Ingelheim)	204974-94-7	inflammation inhibitor
	BIIL 284/260 (Boehringer Ingelheim)	204974-93-6	inflammation inhibitor
	tanogitran	637328-69-9	thrombin inhibitor
	moxilubant	146978-48-5	antiasthmatic agent, antiinfective agent
	stilbamidine	122-06-5	antiprotozoal agent, antiinfective agent, fungistatic agent
	panamidine	104-32-5	antiprotozoal agent, antiinfective agent
	fradafiban	148396-36-5	thrombin inhibitor
	orbofiban	163250-90-6	glycoprotein IIb/IIIa antagonist as coagulation inhibitor
	roxifiban	170902-47-3	glycoprotein IIb/IIIa antagonist as coagulation inhibitor
	lamifiban (Roche)	144412-49-7	thrombin inhibitor
	furamidine	73819-26-8	antiprotozoal agent, antiinfective agent
	PD0313052	861244-44-2	thrombin inhibitor
	PHA 927 F	648943-12-8	specific TF/VIIa inhibitor

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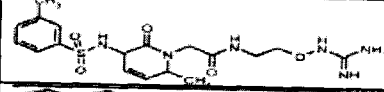
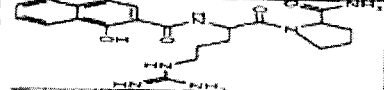
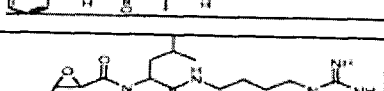
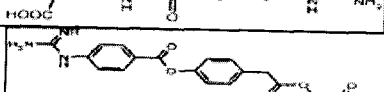
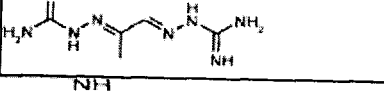
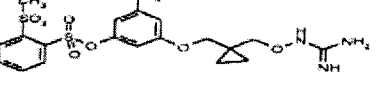
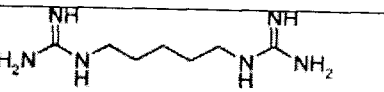
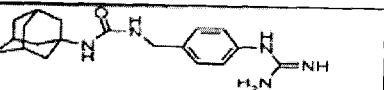
	PHA 798	508173-28-2	specific TF/VIIa inhibitor
	stilbamidine	122-06-5	antiprotozoal agent, antiinfective agent
	fidexaban (ZK807834)	183305-24-0	anticoagulant
	otamixaban	193153-04-7	factor Xa inhibitor
	thrombostop	117091-16-4	thrombin inhibitor
	amiloride hydrochloride	2016-88-8	diuretic
	anagrelide hydrochloride	58579-51-4	reduces thrombocytes
	proguanil	537-21-3	prophylactic anti-malaria agent (against plasmodium)
	cimetidine	51481-61-9	H2 receptor antagonist (against heartburn)
	clonidine hydrochloride	4205-91-8	direct α_2 adrenergic agonist treatment of hypertension * treatment of drug withdrawal symptoms (alcohol, opioids etc) *No monotherapy for alcohol
	guanoxan	19694-60-1	blood pressure reducing agent
	peramivir	229614-55-5	neuramidase inhibitor (influenza), approved for emergencies with H1N1 infections, intravenous
	romifidine	65896-14-2	α_2 adrenergic receptor antagonist, veterinary medicine: sedative, anesthetic, analgesic for large animals like horses
	tirapazamine	27314-97-2	experimental anti-tumor agent, releases small amounts of toxic radicals, chemical lead for other cancer drugs

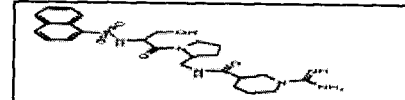
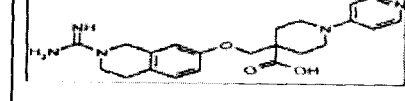
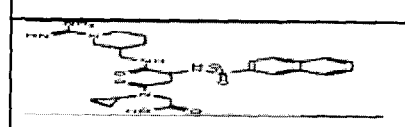
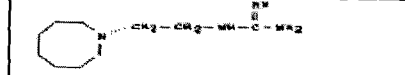
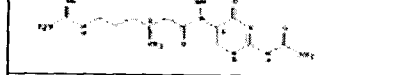
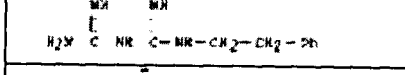
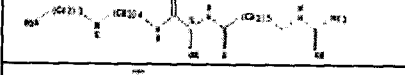
	tizanidine	51322-75-9	α 2 adrenergic receptor antagonist, muscle relaxant against spasms, cramps etc.
	tolondine nitrate	57524-15-9	
	metformin	657-24-9	diabetes mellitus type 2
	diminazene	536-71-0	antiprotozoal agent, antiinfective agent
	debrisoquine	1131-64-2	antihypertension agent
	sulfamethazine	57-68-1	additive in animal feed
	eptifibatide	188627-80-7 148031-34-9	anticoagulant
	famotidine	76824-35-6	Na ⁺ H ⁺ transport inhibitor
	zanamivir	139110-80-8	neuramidase inhibitor (influenza)
	Bayer pharmaceutical	25836-74-2	
	streptomycin A	57-92-1	antibiotic
	nafamostat	81525-10-2	
	nafamostat, FUT-175	81525-10-2	C3/C5 convertase inhibitor, complement, activation, antiphlogistic, Phase II
	inogatran	155415-08-0	

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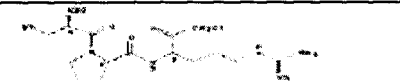
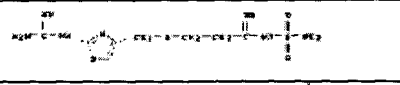
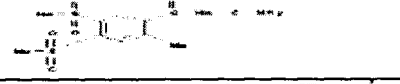
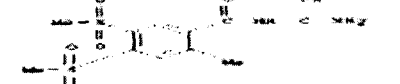
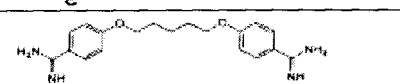
	guanethidine (Thilodigon)	645-43-2	hypertension agent, glaucoma
	3DP-10017	226566-43-4	dual FXa and thrombin inhibitor, clinical development
	APC-366	178925-65-0	tryptase inhibitor, allergies, asthma, Phase II
	CVS-1123		thrombin inhibitor, clinical testing
	diphenyl phosphonate derivatives	5945-33-5	urokinase inhibitor, malignant diseases, preclinical
	E-64	66701-25-5	Cathepsin L inhibitor, malignant diseases, preclinical
	FOY-305	59721-28-7	broad spectrum serin protease inhibitors (pancreatitis, anticoagulation), clin. development
	MBGB		lymphomas, leukemia, clinic
	MIBG	103346-16-3	neuroblastoma, clinic
	RWJ-422521		dual FXa and thrombin inhibitor, clinical development
	Synthalin	301-15-5	antidiabetic agent
	WX-293		urokinase inhibitor, malignant diseases, preclinical
	WX-340		amyotrophic lateral sclerosis, malignomas, Phase I

	BMS-189090		thrombin inhibitor, clinical testing
	JTV-803 (Japan Tobacco)		FXa inhibitor, anticoagulant, Phase II
	napsagatran	159668-20-9	thrombin inhibitor, anticoagulant, clinical development
	Abapressin, azocine, Dopom	55-65-2	hypertension agent
	TAN 1057A	128126-44-3	antibiotic
	Hydikal, MK 870	2609-46-3	cardiac rhythm disorder
	Phenformin, Retardo	114-86-3	diabetes mellitus II
	netropsin, Sinanomycin	1438-30-8	antibiotic
	BIIB, sabiporide	261505-80-0	Na+ T+ transport inhibitor
	deoxyspergualin	89149-10-0	immunosuppressant
	BMS 262084	253174-92-4	tryptase inhibitor
	Apophage, Siamformet	1115-70-4	diabetes mellitus II

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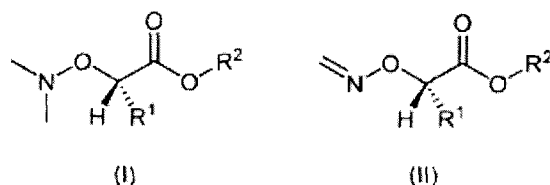
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	Pebac, L-prolinamide	71142-71-7	thrombin inhibitor
	MERGETPA	77102-28-4	thrombin inhibitor
	BCX 1812, peramivir	330600-85-6	neuramidase inhibitor (influenza)
	apogastine, famogast	76824-35-6	proton pump inhibitor
		65113-67-9	
	pentamidine	100-33-4	antiprotozoal agent, antiinfective agent

CLAIMS

1. A prodrug, comprising a partial structure having the general formula (I) or (II)

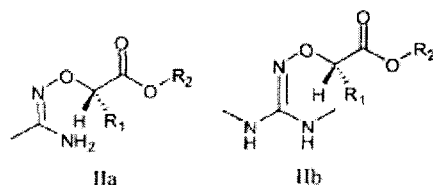


where R^1 and R^2 are hydrogen, alky radicals or aryl radicals.

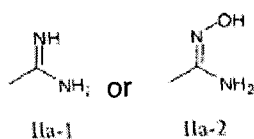
2. The prodrug according to claim 1, characterized in that the partial structure which the prodrug comprises is part of a hydroxylamine, an *N*-oxide, a nitron, a diazeniumdiolate (NONOat) or a similar N-O-containing nitric oxide donor, a hydroxamic acid, a hydroxyurea, an oxime, an amidoxime (*N*-hydroxyamidine), an *N*-hydroxyamidinohydrazone or an *N*-hydroxyguanidine.
3. A prodrug according to any one of the preceding claims, characterized in that the prodrug is metabolized into a pharmaceutical, which is a pharmaceutical for treating diseases associated with nitric oxide deficiency.
4. A prodrug according to any one of the preceding claims, characterized in that the prodrug or the corresponding pharmaceutical is selected from the group consisting of protease inhibitors, DNA- and RNA-intercalating compounds, inhibitors of viral enzymes, and *N*-methyl-D-aspartate receptor antagonists.
5. A prodrug according to any one of claims 1 to 4, characterized in that the partial

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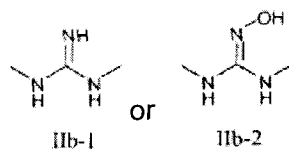
structure has the general formula IIa or IIb



6. The prodrug according to claim 5, characterized in that the prodrug is a prodrug of a pharmaceutical, wherein the partial structure of the general formula IIa, after metabolization, comprises a structure having the formula

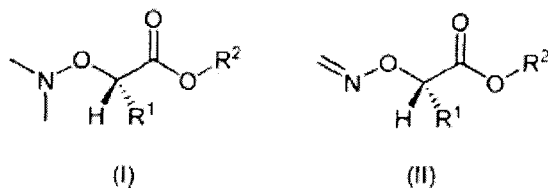


and the partial structure of the general formula IIb, after metabolization, comprises a structure having the formula



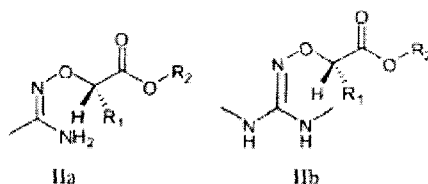
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7. Use of a partial structure forming the general formula (I) or (II)



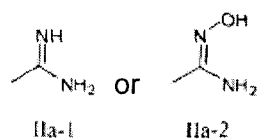
as part of the overall structure of a prodrug which is prodrug of a pharmaceutical, where R^1 and R^2 are hydrogen, alkyl radicals or aryl radicals.

8. Use according to claim 7, wherein the partial structure has the general formula (II), which is part of a higher-level partial structure IIa or IIb



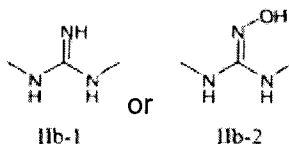
in the place of an amidine or guanidine group of a pharmaceutical to improve solubility, oral bioavailability, blood-brain barrier crossing, the flavor and/or the physical-chemical stability.

9. Use according to claim 7 or 8, wherein the prodrug is a prodrug of a pharmaceutical which has the same structure as the prodrug, except that instead of the higher-level partial structure IIa it comprises one of the partial structures IIa-1 or IIa-2



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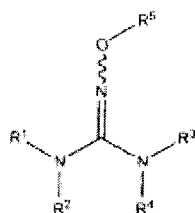
or instead of the higher-level partial structure IIb it comprises one of the partial structures IIb-1 or IIb-2



10. Use according to claim 8 or 9 for activating the pharmaceutical by means of peptidylglycine α -amidating monooxygenase (PAM).
11. Use according to any one of claims 7 to 10, characterized in that the partial structure is part of a hydroxylamine, an *N*-oxide, a nitron, a diazeniumdiolate (NONOat) or a similar N-O-containing nitric oxide donor, a hydroxamic acid, a hydroxyurea, an oxime, an amidoxime (*N*-hydroxyamidine), an *N*-hydroxyamidinohydrazone or an *N*-hydroxyguanidine.
12. A method for introducing a pharmaceutical comprising a free amidine or guanidine function into the PAM activation path, comprising the production of a prodrug of the pharmaceutical according to any one of claims 1 to 6.
13. A method for treating a patient, comprising the administration of a prodrug according to any one of claims 1 to 6 to the patient.

14. Use of a prodrug according to any one of the claims 1 to 6 for producing a pharmaceutical.
15. Use according to any one of claims 7 to 11 and claim 13, or the method according to claim 14, wherein the use or the method is a use or a method for treating diseases associated with nitric oxide deficiency.
16. Use according to any one of claims 7 to 11 and 15, characterized in that the pharmaceutical or the prodrug is selected from the group consisting of protease inhibitors, DNA- and RNA-intercalating compounds, inhibitors of viral enzymes, and N-methyl-D-aspartate receptor antagonists.
17. Use according to any one of claims 7 to 11 and 15 to 17, wherein the use is a use for the prophylaxis and/or treatment of visceral and/or cutaneous leishmaniasis, trypanosomiasis, phase 2 of trypanosomiasis or pneumonia caused by *Pneumocystis carinii*, for inhibiting the growth of malignant tumors, for inhibiting blood coagulation, for lowering blood pressure, for neuroprotection, or for combating viral infections, including influenza and HIV infections.
18. A method for producing an *N*-alkoxy guanidine of the formula

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comprising the reaction of a carbodiimide of the formula $\text{R}_6\text{-N}=\text{C}=\text{N-R}_7$ with an aminoxy compound of the formula $\text{H}_2\text{N-O-R}_8$ or a salt thereof, wherein when a salt of the aminoxy compound is used, the reaction is carried out in the presence of a base, where R_1 , R_2 , R_3 and R_4 , R_6 and R_7 independently of each other are selected from the group consisting of H, optionally substituted alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aryloxycarbonyl, optionally substituted aminoacyl, optionally substituted alkoxycarbonyl, optionally substituted alkoxycarbonylalkoxy, optionally substituted heteroalkyl, optionally substituted alkylcycloalkyl, optionally substituted heteroalkyl cycloalkyl, optionally substituted aralkyl, and optionally substituted cycloalkyl,

and R_5 is selected from the group consisting of alkoxycarbonyl, (alkoxycarbonyl)alkoxy and carboxyalkoxy.

19. The method according to claim 18, wherein the reaction is carried out in the presence of a base, which is preferably selected from the group consisting of diisopropylamine and triethylamine.

Application number / numéro de demande: DE201000009.

Figures: _____

Pages: 1/2

Unscannable items
received with this application
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Documents reçu avec cette demande ne pouvant être balayés
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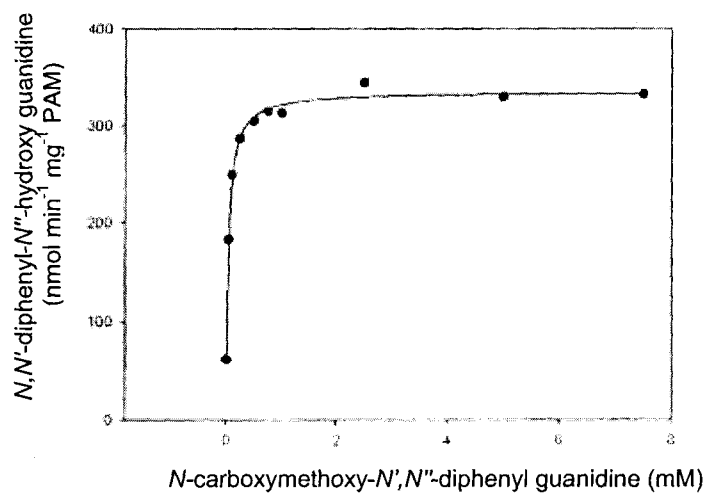
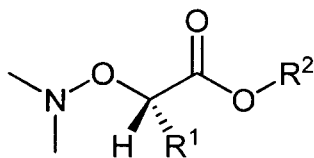
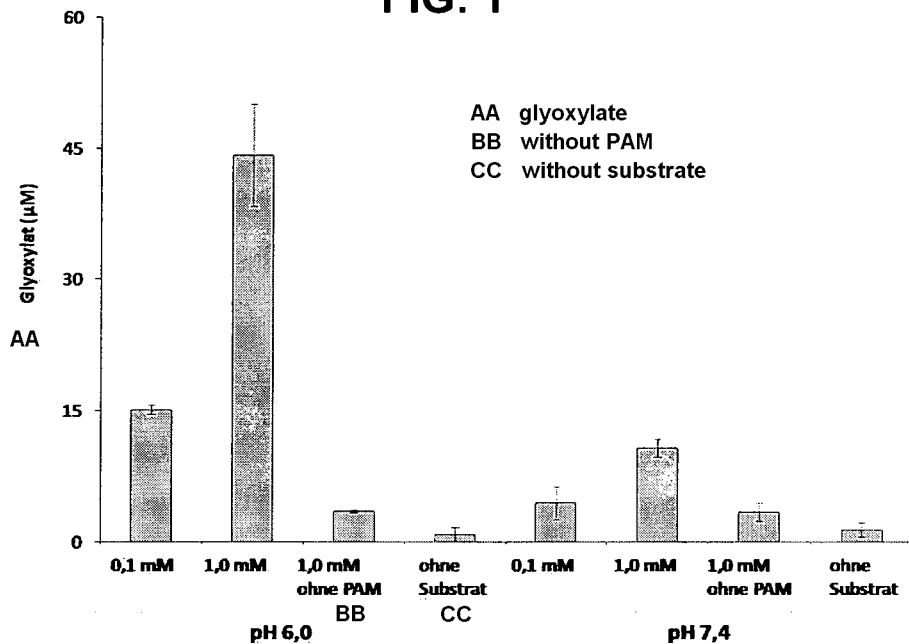
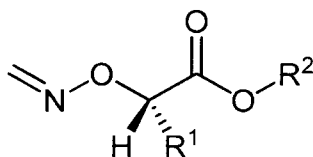


FIG. 3

FIG. 1



(I)



(II)