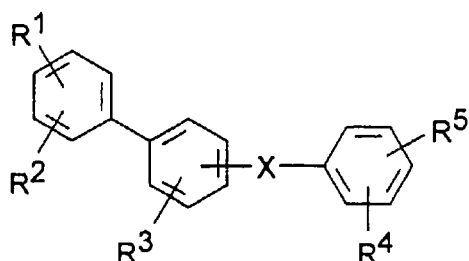


**Biphenyl derivatives as NHE-3 inhibitors**

The invention relates to compounds of the formula I

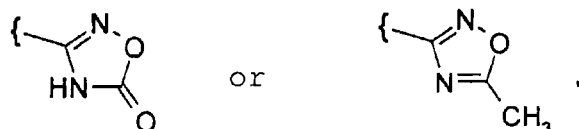


5

in which

10 R^1, R^4 in each case independently of one another are
 $-C(=NH)-NH_2$, which can also be
monosubstituted by $-COA$, $-CO-[C(R^6)_2]_n-Ar$,
 $-COOA$, $-OH$ or by a conventional amino
protective group,
 $NH-C(=NH)-NH_2$, $-CO-N=C(NH_2)_2$,

15



20

R^2, R^3, R^5 in each case independently of one another are
 H , A , OR^6 , $N(R^6)_2$, NO_2 , CN , Hal , $NHCOA$,
 $NHCOAr$, $NHSO_2A$, $NHSO_2Ar$, $COOR^6$, $CON(R^6)_2$,
 $CONHAr$, COR^6 , $COAr$, $S(O)_nA$, $S(O)_nAr$,
 $-O-[C(R^6)_2]_m-COOR^6$, $-[C(R^6)_2]_p-COOR^6$,
 $-O-[C(R^6)_2]_m-CON(R^6)_2$, $-[C(R^6)_2]_p-CON(R^6)_2$,
 $-O-[C(R^6)_2]_m-CONHAr$ or $-[C(R^6)_2]_p-CONHAr$,

25 X

is $-[C(R^6)_2]_n-$, $-CR^6=CR^6-$, $-[C(R^6)_2]_n-O-$,
 $-O-[C(R^6)_2]_n-$, $-COO-$, $-OOC-$, $-CONR^6-$ or
 $-NR^6CO-$,

R^6 is H , A or benzyl,

30



- A is alkyl having 1-20 C atoms, in which one or two CH₂ groups can be replaced by O or S atoms or by -CR⁶=CR⁶- groups and/or 1-7 H atoms can be replaced by F,
- 5 Ar is phenyl or naphthyl, which is unsubstituted or mono-, di- or trisubstituted by A, Ar', OR⁶, OAr', N(R⁶)₂, NO₂, CN, Hal, NHCOA, NHCOAr', NHSO₂A, NHSO₂Ar', COOR⁶, CON(R⁶)₂,
10 CONHAr', COR⁶, COAr', S(O)_nA or S(O)_nAr',
- Ar' is phenyl or naphthyl, which is unsubstituted or mono-, di- or trisubstituted by A, OR⁶, N(R⁶)₂, NO₂, CN, Hal, NHCOA, COOR⁶, CON(R⁶)₂,
15 COR⁶ or S(O)_nA,
- Hal is F, Cl, Br or I,
- n is 0, 1 or 2,
- 20 m is 1 or 2,
- p is 1 or 2,
- 25 and their salts and solvates as NHE-3 inhibitors.

Other inhibitors of the sodium/proton exchanger subtype 3 are described, for example, in EP 0 825 178.

30 The invention was based on the object of finding novel compounds having valuable properties, in particular those which can be used for the production of medicaments.

35 DE 19819548 describes that the compounds of the formula I and their salts have factor Xa-inhibiting properties and can therefore be employed for the control and prevention of thromboembolic disorders such

as thrombosis, myocardial infarct, arteriosclerosis, inflammation, apoplexy, angina pectoris, restenosis after angioplasty and intermittent claudication.

5 Surprisingly, it has been found that the compounds of the formula I and their salts inhibit the sodium/proton exchanger subtype 3 and have good tolerability. The compounds of the formula I can be employed as pharmaceutical active compounds in human and veterinary
10 medicine.

It is known that the Na^+/H^+ exchanger is a family having at least 6 different isoforms (NHE-1 to NHE-6), which have now all been cloned. While the subtype NHE-1 is
15 distributed ubiquitously in all tissues in the whole body, the other NHE subtypes are expressed selectively in specific organs such as in the kidney or in the lumen wall and contraluminal wall of the small intestine. This distribution reflects the specific
20 functions served by the various isoforms, namely on the one hand the regulation of the intracellular pH and of the cell volume by the subtype NHE-1 and on the other hand the Na^+ absorption and reabsorption in the intestine and kidney by the isoforms NHE-2 and NHE-3.
25 The isoform NHE-4 was mainly found in the stomach. The expression of NHE-5 is restricted to the brain and neuronal tissue. NHE-6 is the isoform which forms the sodium proton exchanger in the mitochondria.

30 The isoform NHE-3 is expressed in particular in the apical membrane of the proximal renal tubuli; an NHE-3 inhibitor therefore exerts, inter alia, a renal protective action.

35 The therapeutic use of a selective inhibitor for NHE-3 isoforms is varied. NHE-3 inhibitors inhibit or reduce tissue damage and cell necroses after pathophysiological hypoxic and ischemic events which lead to an

activation of the NHE activity, such as is the case during renal ischemia or during the removal, transport and reperfusion of a kidney in kidney transplantation.

5 The compounds of the formula I have a hypotensive action and are suitable as pharmaceutical active compounds for the treatment of hypertension. They are furthermore suitable as diuretics.

10 The compounds of the formula I have an anti-ischemic action on their own or in combination with NHE inhibitors of different subtype specificity and can be used in thromboses, atherosclerosis, vasospasms, for the protection of organs, e.g. the kidney and liver, before and during operations, as well as chronic or
15 acute kidney failure.

They can furthermore be used for the treatment of stroke, of cerebral edema, ischemia of the nervous system, various forms of shock as well as for improving the respiratory drive in, for example, the following
20 conditions: central sleep apnea, sudden infant death, post-operative hypoxia and other respiratory disorders. The respiratory activity can be further improved by combination with a carboanhydrase inhibitor.

The compounds of the formula I have an inhibitory
25 action on the proliferation of cells, for example fibroblast cell proliferation and the proliferation of smooth vascular muscle cells and can therefore be used for the treatment of diseases in which cell proliferation is a primary or secondary cause.

30 The compounds of the formula I can be used against diabetic late complications, carcinomatous disorders, fibrotic disorders, endothelial dysfunction, organ hypertrophy and hyperplasia, in particular in prostate hyperplasia or prostate hypertrophy.

35 They are further suitable as diagnostics for the determination and differentiation of certain forms of hypertension, atherosclerosis, diabetes and proliferative disorders.

Since the compounds of the formula I also advantageously influence the level of the serum lipoproteins, they can be employed on their own or in combination with other medicaments for the treatment of
5 an increased blood fat level.

The invention relates to the use of compounds of the formula I as claimed in claim 1 and their physiologically acceptable salts and/or solvates for the
10 production of a medicament for the treatment of thromboses, ischemic conditions of the heart, of the peripheral and central nervous system and of stroke, ischemic conditions of peripheral organs and limbs and for the treatment of states of shock.

15 The invention further relates to the use of compounds of the formula I as claimed in claim 1 and their physiologically acceptable salts and/or solvates for the production of a medicament for use in surgical
20 operations and organ transplantation and for the preservation and storage of transplants for surgical measures.

The invention also relates to the use of compounds of
25 the formula I as claimed in claim 1 and their physiologically acceptable salts and/or solvates for the production of a medicament for the treatment of diseases in which cell proliferation is a primary or secondary cause, for the treatment or prophylaxis of
30 disorders of the lipid metabolism or disturbed respiratory drive.

The invention further relates to the use of compounds of the formula I as claimed in claim 1 and their
35 physiologically acceptable salts and/or solvates for the production of a medicament for the treatment of ischemic kidney, ischemic intestinal disorders or for the prophylaxis of acute or chronic kidney disorders.

Methods for the identification of substances which inhibit the sodium/proton exchanger subtype 3 are described, for example, in US 5,871,919.

5 For all radicals in the compounds of the formula I which occur a number of times, such as, for example, R^6 , it is a condition that their meanings are independent of one another.

10 Hydrates and solvates are understood as meaning, for example, the hemi-, mono- or dihydrates, solvates are understood as meaning, for example, alcohol addition compounds such as, for example, with methanol or ethanol.

15

In the above formulae, A is alkyl, is linear or branched, and has 1 to 20, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 C atoms. A is preferably methyl, furthermore ethyl, propyl, isopropyl, butyl, isobutyl, 20 sec-butyl or tert-butyl, furthermore also pentyl, 1-, 2- or 3-methylbutyl, 1,1-, 1,2- or 2,2-dimethylpropyl, 1-ethylpropyl, hexyl, 1-, 2-, 3- or 4-methylpentyl, 1,1-, 1,2-, 1,3-, 2,2-, 2,3- or 3,3-dimethylbutyl, 1- or 2-ethylbutyl, 1-ethyl-1-methylpropyl, 1-ethyl- 25 2-methylpropyl, 1,1,2- or 1,2,2-trimethylpropyl, heptyl, octyl, nonyl or decyl.

A is furthermore, for example, trifluoromethyl, pentafluoroethyl, allyl or crotyl.

30 COR^6 is acyl and is preferably formyl, acetyl, propionyl, furthermore also butyryl, pentanoyl or hexanoyl.

$COOR^6$ is preferably methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl or butoxycarbonyl.

35

Hal is preferably F, Cl or Br, but also I.

R^2 , R^3 and R^5 are, in each case independently of one

another, preferably H, fluorine, chlorine, bromine, iodine, hydroxyl, methoxy, ethoxy, propoxy, nitro, amino, methylamino, dimethylamino, ethylamino, diethylamino, acetamido, sulfonamido, methylsulfonamido, phenylsulfonamido, methylthio, ethylthio, methylsulfanyl, ethylsulfanyl, methylsulfonyl, ethylsulfonyl, phenylsulfanyl, phenylsulfonyl, cyano, carboxyl, methoxycarbonyl, ethoxycarbonyl, carboxymethoxy, methoxycarbonylmethoxy, carboxymethyl, methoxycarbonylmethyl, aminocarbonylmethoxy, aminocarbonylmethyl, N-phenylaminocarbonylmethoxy, N-phenylaminocarbonylmethyl, furthermore also acyl or benzoyl.

In particular, R^2 , R^5 are H.

R^3 is, in particular, for example, H, COOA or $-OCH_2COOR^6$, where R^6 is H or alkyl having 1-4 C atoms.

R^6 is H, A or benzyl, but in particular H or alkyl having 1-4 C atoms.

X is preferably, for example, $-CH_2-$, $-CH=CH-$, $-CH_2O-$, $-O-CH_2-$, $-COO-$, $-OOC-$, $-CONH-$ or $-NHCO-$; $-CH_2O-$, $-O-CH_2-$ or $-CH_2-CH_2-$ is very particularly preferred.

Ar is preferably unsubstituted phenyl or naphthyl, furthermore preferably, for example, phenyl or naphthyl, which is mono-, di- or trisubstituted by A, fluorine, chlorine, bromine, iodine, hydroxyl, methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, benzyloxy, phenethyloxy, methylthio, ethylthio, methylsulfanyl, ethylsulfanyl, methylsulfonyl, ethylsulfonyl, phenylsulfanyl, phenylsulfonyl, nitro, amino, methylamino, ethylamino, dimethylamino, diethylamino, formamido, acetamido, propionylamino, butyrylamino, methylsulfonamido, ethylsulfonamido, propylsulfonamido, butylsulfonamido, phenylsulfonamido, (4-methylphenyl)-sulfonamido, carboxymethoxy, carboxyethoxy, methoxycarbonylmethoxy, methoxycarbonylethoxy, hydroxymethoxy, hydroxyethoxy, methoxyethoxy, carboxyl, methoxycarbonyl, ethoxycarbonyl, cyano, phenylaminocarbonyl,

acyl or benzoyl, furthermore also biphenyl.

Ar is therefore preferably, for example, o-, m- or p-tolyl, o-, m- or p-ethylphenyl, o-, m- or p-propylphenyl, o-, m- or p-isopropylphenyl, o-, m- or p-tert-butylphenyl, o-, m- or p-hydroxyphenyl, o-, m- or p-nitrophenyl, o-, m- or p-aminophenyl, o-, m- or p-(N-methylamino)phenyl, o-, m- or p-acetamidophenyl, o-, m- or p-methoxyphenyl, o-, m- or p-ethoxyphenyl, o-, m- or p-carboxyphenyl, o-, m- or p-methoxycarbonylphenyl, o-, m- or p-(N,N-dimethylamino)phenyl, o-, m- or p-(N-ethylamino)phenyl, o-, m- or p-(N,N-diethylamino)phenyl, o-, m- or p-acetylphenyl, o-, m- or p-formylphenyl, o-, m- or p-fluorophenyl, o-, m- or p-bromophenyl, o-, m- or p-chlorophenyl, o-, m- or p-methylsulfonylphenyl, o-, m- or p-(phenylsulfonamido)phenyl, o-, m- or p-(methylsulfonamido)phenyl, o-, m- or p-methylthiophenyl, furthermore preferably 2,3-, 2,4-, 2,5-, 2,6-, 3,4- or 3,5-difluorophenyl, 2,3-, 2,4-, 2,5-, 2,6-, 3,4- or 3,5-dichlorophenyl, 2,3-, 2,4-, 2,5-, 2,6-, 3,4- or 3,5-dibromophenyl, 2,4- or 2,5-dinitrophenyl, 2,5- or 3,4-dimethoxyphenyl, 3-nitro-4-chlorophenyl, 3-amino-4-chloro-, 2-amino-3-chloro-, 2-amino-4-chloro-, 2-amino-5-chloro- or 2-amino-6-chlorophenyl, 2-nitro-4-N,N-dimethylamino- or 3-nitro-4-N,N-dimethylaminophenyl, 2,3-diaminophenyl, 2,3,4-, 2,3,5-, 2,3,6-, 2,4,6- or 3,4,5-trichlorophenyl, 2,4,6-trimethoxyphenyl, 2-hydroxy-3,5-dichlorophenyl, p-iodophenyl, 3,6-dichloro-4-aminophenyl, 4-fluoro-3-chlorophenyl, 2-fluoro-4-bromophenyl, 2,5-difluoro-4-bromophenyl, 3-bromo-6-methoxyphenyl, 3-chloro-6-methoxyphenyl, 3-chloro-4-acetamidophenyl, 3-fluoro-4-methoxyphenyl, 3-amino-6-methylphenyl, 3-chloro-4-acetamidophenyl or 2,5-dimethyl-4-chlorophenyl.

Ar' is in particular, for example, phenyl or naphthyl, furthermore preferably, for example, o-, m- or p-tolyl,

o-, m- or p-ethylphenyl, o-, m- or p-propylphenyl, o-,
m- or p-isopropylphenyl, o-, m- or p-tert-butylphenyl,
o-, m- or p-hydroxyphenyl, o-, m- or p-nitrophenyl, o-,
m- or p-aminophenyl, o-, m- or p-(N-methylamino)phenyl,
5 o-, m- or p-acetamidophenyl, o-, m- or p-methoxyphenyl,
o-, m- or p-ethoxyphenyl, o-, m- or p-carboxyphenyl,
o-, m- or p-methoxycarbonylphenyl, o-, m- or p-(N,N-di-
methylamino)phenyl, o-, m- or p-(N-ethylamino)phenyl,
o-, m- or p-(N,N-diethylamino)phenyl, o-, m- or
10 p-acetylphenyl, o-, m- or p-formylphenyl, o-, m- or
p-fluorophenyl, o-, m- or p-bromophenyl, o-, m- or
p-chlorophenyl or o-, m- or p-methylsulfonylphenyl.

Accordingly, the invention relates in particular to the
15 use of those compounds of the formula I in which at
least one of the radicals mentioned has one of the
preferred meanings indicated above. Some preferred
groups of compounds can be expressed by the following
subformulae Ia to Ii, which correspond to the formula I
20 and in which the radicals not designated in greater
detail have the meaning indicated in the formula I, but
in which

in Ia R^1, R^4 in each case independently of one
25 another are $-C(=NH)-NH_2$, which can also
be monosubstituted by OH or
 $-CO-N=C(NH_2)_2$;

in Ib R^2, R^5 are H;

in Ic R^1, R^4 in each case independently of one
30 another are $-C(=NH)-NH_2$, which can also
be monosubstituted by OH or
 $-CO-N=C(NH_2)_2$,

R^2, R^5 are H and

R^3 is H or $COOR^6$;

35 in Id R^1, R^4 in each case independently of one
another are $-C(=NH)-NH_2$, which can also
be monosubstituted by OH or
 $-CO-N=C(NH_2)_2$,

- 5 R^2, R^5 are H and
 R^3 is H, COOR^6 or $-\text{O}-(\text{CH}_2)\text{COOR}^6$;
 in Ie X is $-\text{CH}_2-\text{O}-$ or $-\text{O}-\text{CH}_2-$;
 in If R^1, R^4 in each case independently of one
 another are $-\text{C}(=\text{NH})-\text{NH}_2$, which can also
 be monosubstituted by OH or
 $-\text{CO}-\text{N}=\text{C}(\text{NH}_2)_2$,

 10 R^2, R^5 are H,
 R^3 is H or COOR^6 and
 in Ig X is $-\text{CH}_2-\text{O}-$ or $-\text{O}-\text{CH}_2-$;
 R^1, R^4 in each case independently of one
 another are $-\text{C}(=\text{NH})-\text{NH}_2$, which can also
 be monosubstituted by OH or
 $-\text{CO}-\text{N}=\text{C}(\text{NH}_2)_2$,

 15 R^2, R^5 are H,
 R^3 is H, COOR^6 or $-\text{O}-(\text{CH}_2)\text{COOR}^6$, and
 X is $-\text{CH}_2-\text{O}-$, $-\text{O}-\text{CH}_2-$ or $-\text{CH}_2-\text{CH}_2-$;
 in Ih R^1, R^4 in each case independently of one
 another are $-\text{C}(=\text{NH})-\text{NH}_2$, which can also
 20 be monosubstituted by OH or
 $-\text{CO}-\text{N}=\text{C}(\text{NH}_2)_2$,

 R^2, R^5 are H,
 R^3 is H, COOR^6 , $-\text{O}-\text{CH}_2-\text{COOR}^6$, $\text{CH}_2-\text{COOR}^6$,
 $-\text{O}-\text{CH}_2-\text{CON}(\text{R}^6)_2$, $\text{CH}_2-\text{CON}(\text{R}^6)_2$,
 25 $-\text{O}-\text{CH}_2-\text{CONHAr}$ or $\text{CH}_2-\text{CONHAr}$,
 X is $-\text{CH}_2-\text{O}-$, $-\text{O}-\text{CH}_2-$ or $-\text{CH}_2-\text{CH}_2-$,
 R^6 is H or A,
 A is alkyl having 1-4 C atoms;
 in Ii R^1, R^4 in each case independently of one
 30 another are $-\text{C}(=\text{NH})-\text{NH}_2$, which can also
 be monosubstituted by OH or
 $-\text{CO}-\text{N}=\text{C}(\text{NH}_2)_2$,

 R^2, R^5 are H,
 R^3 is H, COOR^6 , $-\text{O}-\text{CH}_2-\text{COOR}^6$, $\text{CH}_2-\text{COOR}^6$,
 35 $-\text{O}-\text{CH}_2-\text{CON}(\text{R}^6)_2$ or $\text{CH}_2-\text{CON}(\text{R}^6)_2$,
 X is $-\text{CH}_2-\text{O}-$, $-\text{O}-\text{CH}_2-$ or $-\text{CH}_2-\text{CH}_2-$,
 R^6 is H or A,
 A is alkyl having 1-4 C atoms.

The compounds of the formula I and also the starting substances for their preparation are otherwise prepared by methods known per se, such as are described in the literature (e.g. in the standard works such as
5 Houben-Weyl, Methoden der organischen Chemie [Methods of Organic Chemistry], Georg-Thieme-Verlag, Stuttgart), namely under reaction conditions which are known and suitable for the reactions mentioned. Use can also be made in this case of variants which are known per se,
10 but not mentioned here in greater detail.

If desired, the starting substances can also be formed in situ such that they are not isolated from the reaction mixture, but are immediately reacted further
15 to give the compounds of the formula I.

Compounds of the formula I can preferably be obtained by liberating compounds of the formula I from one of their functional derivatives by treating them with a
20 solvolyzing or hydrogenolyzing agent.

Preferred starting substances for the solvolysis or hydrogenolysis are those which otherwise correspond to the formula I, but instead of one or more free amino
25 and/or hydroxyl groups contain corresponding protected amino and/or hydroxyl groups, preferably those which, instead of an H atom which is bonded to an N atom, carry an amino protective group, in particular those which, instead of an HN group, carry an R'-N- group, in
30 which R' is an amino protective group, and/or those which, instead of the H atom of a hydroxyl group, carry a hydroxyl protective group, e.g. those which correspond to the formula I, but instead of a group -COOH carry a group -COOR'' in which R'' is a hydroxyl
35 protective group.

Preferred starting substances are also the oxadiazole derivatives which can be converted into the corresponding amidino compounds.

The introduction of the oxadiazole group is carried out, for example, by reaction of the cyano compounds with hydroxylamine and reaction with phosgene, dialkyl carbonate, chloroformic acid ester, N,N'-carbonyl-
5 diimidazole or acetic anhydride.

It is also possible for a number of - identical or different - protected amino and/or hydroxyl groups to be present in the molecule of the starting substance.
10 If the protective groups present are different from one another, in many cases they can be removed selectively.

The expression "amino protective group" is generally known and relates to groups which are suitable for
15 protecting (or blocking) an amino group from chemical reactions, but which are easily removable after the desired chemical reaction has been carried out at other positions in the molecule. Typical groups of this type are in particular unsubstituted or substituted acyl,
20 aryl, aralkoxymethyl or aralkyl groups. Since the amino protective groups are removed after the desired reaction (or reaction sequence), their size and nature are otherwise not critical; however, those having 1-20, in particular 1-8, C atoms are preferred. The
25 expression "acyl group" is to be interpreted in the widest sense in connection with the present process. It includes acyl groups derived from aliphatic, araliphatic, aromatic or heterocyclic carboxylic acids or sulfonic acids, and, in particular, alkoxycarbonyl,
30 aryloxy-carbonyl and especially aralkoxycarbonyl groups. Examples of acyl groups of this type are alkanoyl such as acetyl, propionyl, butyryl; aralkanoyl such as phenylacetyl; aroyl such as benzoyl or toluyl; aryloxy-alkanoyl such as POA; alkoxycarbonyl such as methoxy-
35 carbonyl, ethoxycarbonyl, 2,2,2-trichloroethoxy-carbonyl, BOC (tert-butyloxycarbonyl), 2-iodoethoxy-carbonyl; aralkyloxycarbonyl such as CBZ ("carbo-benzoyl"), 4-methoxybenzyloxycarbonyl, FMOC; aryl-

sulfonyl such as Mtr. Preferred amino protective groups are BOC and Mtr, furthermore CBZ, Fmoc, benzyl and acetyl.

5 The expression "hydroxyl protective group" is likewise generally known and relates to groups which are suitable for protecting a hydroxyl group from chemical reactions, but which are easily removable after the
10 desired chemical reaction has been carried out at other positions in the molecule. Typical groups of this type are the abovementioned unsubstituted or substituted aryl, aralkyl or acyl groups, furthermore also alkyl groups. The nature and size of the hydroxyl protective groups are not critical, as they are removed again
15 after the desired chemical reaction or reaction sequence; groups having 1-20, in particular 1-10, C atoms are preferred. Examples of hydroxyl protective groups are, inter alia, benzyl, p-nitrobenzoyl, p-toluenesulfonyl, tert-butyl and acetyl, benzyl and
20 tert-butyl being particularly preferred.

The liberation of the compounds of the formula I from their functional derivatives is carried out - depending on the protective group used - for example using strong
25 acids, expediently using TFA or perchloric acid, but also using other strong inorganic acids such as hydrochloric acid or sulfuric acid, strong organic carboxylic acids such as trichloroacetic acid or sulfonic acids such as benzene- or p-toluenesulfonic
30 acid. The presence of an additional inert solvent is possible, but not always necessary. Suitable inert solvents are preferably organic solvents, for example carboxylic acids such as acetic acid, ethers such as tetrahydrofuran or dioxane, amides such as DMF,
35 halogenated hydrocarbons such as dichloromethane, furthermore also alcohols such as methanol, ethanol or isopropanol, and also water. In addition, mixtures of the abovementioned solvents are possible. TFA is

preferably used in an excess without addition of a further solvent, perchloric acid in the form of a mixture of acetic acid and 70% perchloric acid in the ratio 9:1. The reaction temperatures for the cleavage
5 are expediently between approximately 0 and approximately 50°; the reaction is preferably carried out between 15 and 30° (room temperature).

The groups BOC, OBut and Mtr can preferably be removed,
10 for example, using TFA in dichloromethane or using approximately 3 to 5 N HCl in dioxane at 15-30°; the Fmoc group using an approximately 5 to 50% solution of dimethylamine, diethylamine or piperidine in DMF at 15-30°.

15 Hydrogenolytically removable protective groups (e.g. CBZ, benzyl or the liberation of the amidino group from its oxadiazole derivative) can be removed, for example, by treating with hydrogen in the presence of a catalyst
20 (e.g. of a noble metal catalyst such as palladium, expediently on a support such as carbon or such as moist Raney nickel with addition of, for example, acetic acid). Suitable solvents here are those indicated above, in particular, for example, alcohols
25 such as methanol or ethanol or amides such as DMF. As a rule, the hydrogenolysis is carried out at temperatures between approximately 0 and 100° and pressures between approximately 1 and 200 bar, preferably at 20-30° and 1-10 bar. Hydrogenolysis of the CBZ group is readily
30 carried out, for example, on 5 to 10% Pd/C in methanol or on Pd/C in methanol/DMF at 20-30° using ammonium formate (instead of hydrogen).

Compounds of the formula I in which R¹ and R⁴ are
35 -C(=NH)-NH₂ can preferably be obtained from the corresponding cyano compound.

A cyano group is converted into an amidino group by reaction with, for example, hydroxylamine and

subsequent reduction of the N-hydroxyamidine with hydrogen in the presence of a catalyst such as, for example, Pd/C or Raney nickel.

For the preparation of an amidine of the formula I
5 ($R^1 = -C(=NH)-NH_2$), it is also possible to add ammonia to a nitrile of the formula I ($R^1 = CN$). The addition is preferably carried out in a number of stages by, in a manner known per se, a) converting the nitrile using H_2S into a thioamide which is converted using an
10 alkylating agent, e.g. CH_3I , into the corresponding S-alkyl imidothioester, which for its part reacts with NH_3 to give the amidine, b) converting the nitrile using an alcohol, e.g. ethanol in the presence of HCl , into the corresponding imidoester and treating this
15 with ammonia, or c) reacting the nitrile with lithium bis(trimethylsilyl)amide and then hydrolyzing the product.

Preparation of the cyano compounds is carried out by
20 methods known per se.

Compounds of the formula I in which R^1 and R^4 are $-CON(=NH)-NH_2$ can preferably be obtained from the corresponding alkoxycarbonyl compounds by reacting with
25 guanidine.

It is furthermore possible to convert a compound of the formula I into another compound of the formula I by converting one or more radicals R^1 , R^2 , R^3 , R^4 and/or R^5
30 into one or more radicals R^1 , R^2 , R^3 , R^4 and/or R^5 , e.g. by acylating an amino group or reducing nitro groups to amino groups (for example by hydrogenation on Raney nickel or Pd/carbon in an inert solvent such as methanol or ethanol).

35

Esters can be hydrolysed, for example, with acetic acid or with $NaOH$ or KOH in water, water/THF or water/dioxane at temperatures between 0 and 100° .

It is further possible to acylate free amino groups in a customary manner using an acid chloride or anhydride or to alkylate them using an unsubstituted or substituted alkyl halide, expediently in an inert
5 solvent such as dichloromethane or THF and/or in the presence of a base such as triethylamine or pyridine at temperatures between -60 and $+30^{\circ}$.

As a rule, the reaction is carried out in an inert
10 solvent, in the presence of an acid-binding agent, preferably of an alkali metal or alkaline earth metal hydroxide, carbonate or bicarbonate or of another salt of a weak acid of the alkali or alkaline earth metals, preferably of potassium, sodium, calcium or cesium. The
15 addition of an organic base such as triethylamine, dimethylaniline, pyridine or quinoline or an excess of the amine component of the formula II or of the alkylating derivative of the formula III can also be favorable. Depending on the conditions used, the
20 reaction time is between a few minutes and 14 days, the reaction temperature between approximately 0° and 150° , normally between 20° and 130° .

Suitable inert solvents are, for example, hydrocarbons
25 such as hexane, petroleum ether, benzene, toluene or xylene; chlorinated hydrocarbons such as trichloroethylene, 1,2-dichloroethane, carbon tetrachloride, chloroform or dichloromethane; alcohols such as methanol, ethanol, isopropanol, n-propanol, n-butanol
30 or tert-butanol; ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran (THF) or dioxane; glycol ethers such as ethylene glycol monomethyl or monoethyl ether (methyl glycol or ethyl glycol), ethylene glycol dimethyl ether (diglyme); ketones such as acetone or
35 butanone; amides such as acetamide, dimethylacetamide, N-methylpyrrolidone (NMP) or dimethylformamide (DMF); nitriles such as acetonitrile; sulfoxides such as dimethyl sulfoxide (DMSO); carbon disulfide; carboxylic

acids such as formic acid or acetic acid; nitro compounds such as nitromethane or nitrobenzene; esters such as ethyl acetate or mixtures of the solvents mentioned.

5

A base of the formula I can be converted with an acid into the associated acid addition salt, for example by reaction of equivalent amounts of the base and of the acid in an inert solvent such as ethanol and subsequent
10 evaporation. Suitable acids for this reaction are in particular those which yield physiologically acceptable salts. Thus inorganic acids can be used, e.g. sulfuric acid, nitric acid, halohydric acids such as hydrochloric acid or hydrobromic acid, phosphoric acids such
15 as orthophosphoric acid, sulfamic acid, furthermore organic acids, in particular aliphatic, alicyclic, araliphatic, aromatic or heterocyclic mono- or polybasic carboxylic, sulfonic or sulfuric acids, e.g. formic acid, acetic acid, propionic acid, pivalic acid,
20 diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, lactic acid, tartaric acid, malic acid, citric acid, gluconic acid, ascorbic acid, nicotinic acid, isonicotinic acid, methane- or ethanesulfonic acid, ethanedisulfonic acid,
25 2-hydroxyethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, naphthalenemono- and -disulfonic acids and laurylsulfuric acid. Salts with physiologically unacceptable acids, e.g. picrates, can be used for the isolation and/or purification of the
30 compounds of the formula I.

On the other hand, compounds of the formula I can be converted into the corresponding metal salts, in particular alkali metal salts or alkaline earth metal
35 salts, or into the corresponding ammonium salts using bases (e.g. sodium or potassium hydroxide or carbonate).

Physiologically acceptable organic bases, such as, for

example, ethanolamine, can also be used.

The invention furthermore relates to the use of the compounds of the formula I as NHE-3 inhibitors and/or
5 their physiologically acceptable salts for the production of pharmaceutical preparations, in particular by a non-chemical route. In this context, they can be brought into a suitable dose form together with at least one solid, liquid and/or semi-liquid
10 vehicle or excipient and if appropriate in combination with one or more further active compounds.

The invention furthermore relates to pharmaceutical preparations comprising at least one NHE-3 inhibitor of
15 the formula I and/or one of its physiologically acceptable salts and solvates.

These preparations can be used as medicaments in human or veterinary medicine. Possible vehicles are organic
20 or inorganic substances which are suitable for enteral (e.g. oral) or parenteral administration or topical application and do not react with the novel compounds, for example water, vegetable oils, benzyl alcohols, alkylene glycols, polyethylene glycols, glycerol
25 triacetate, gelatin, carbohydrates such as lactose or starch, magnesium stearate, talc and petroleum jelly. In particular, tablets, pills, coated tablets, capsules, powders, granules, syrups, juices or drops are used for oral administration, suppositories are
30 used for rectal administration, solutions, preferably oily or aqueous solutions, furthermore suspensions, emulsions or implants, are used for parenteral administration and ointments, creams or powders are used for topical application, or transdermally in
35 patches.

The novel compounds can also be lyophilized and the lyophilizates obtained can be used, for example, for the production of injection preparations. The

preparations indicated can be sterilized and/or can contain excipients such as lubricants, preservatives, stabilizers and/or wetting agents, emulsifiers, salts for influencing the osmotic pressure, buffer
5 substances, colorants, flavorings and/or [lacuna] more further active compounds, e.g. one or more vitamins. Pharmaceutical preparations which are suitable for administration in the form of aerosols or sprays are, for example, solutions, suspensions or emulsions of the
10 active compound of the formula I in a pharmaceutically acceptable solvent.

The compounds of the formula I and their physiologically acceptable salts and solvates can be used for
15 the treatment and/or prophylaxis of the diseases or disease conditions indicated above.

In this context, the substances according to the invention are as a rule preferably administered in
20 doses between approximately 0.1 and 100 mg, in particular between 1 and 10 mg, per dose unit. The daily dose is preferably between approximately 0.001 and 10 mg/kg of body weight. The specific dose for each patient, however, depends on all sorts of factors, for
25 example on the efficacy of the specific compound employed, on the age, body weight, general state of health, sex, on the diet, on the time and route of administration, on the excretion rate, pharmaceutical combination and severity of the particular disorder to
30 which the therapy applies. Oral administration is preferred.

Above and below, all temperatures are indicated in °C. In the following examples, "customary working up"
35 means: if appropriate, water is added, the mixture is adjusted, if necessary, depending on the constitution of the final product, to a pH of between 2 and 10 and extracted with ethyl acetate or dichloromethane, the

organic phase is separated off, dried over sodium sulfate and evaporated, and the residue is purified by chromatography on silica gel and/or by crystallization.

Mass spectrometry (MS): EI (electron impact ionization) M^+
FAB (fast atom bombardment) $(M+H)^+$

Example 1

A solution of 2.06 g of 3-bromobenzonitrile and 1.50 g
of 3-tolylboronic acid in 50 ml of dimethoxyethane is
treated with 247 mg of palladium(II) acetate, 335 mg of
tri-o-tolylphosphine, 20 ml of water and 954 mg of
anhydrous sodium carbonate and heated at 100°C for
18 hours with stirring. The mixture is worked up in the
customary manner, the residue is chromatographed on a
silica gel column using petroleum ether/ethyl acetate
9:1 and 3'-methylbiphenyl-3-carbonitrile is obtained as
a colorless solid ("A"), EI 193.

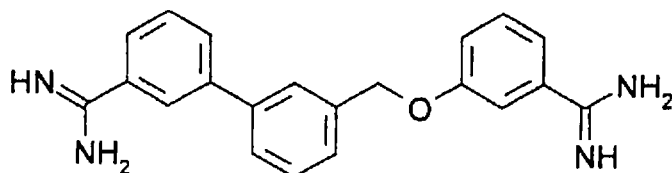
A solution of 1.17 g of "A" in 10 ml of carbon tetra-
chloride is treated with 1.09 g of N-bromosuccinimide
(NBS) and 60 mg of azobisisobutyronitrile and heated at
70°C for 18 hours. It is worked up in the customary
manner, the residue is chromatographed on a silica gel
column using petroleum ether/ethyl acetate 9:1 and
3'-bromomethylbiphenyl-3-carbonitrile is obtained as a
colorless solid ("B").

A solution of 500 mg of "B" and 238 mg of 3-hydroxy-
benzonitrile in 10 ml of acetonitrile is treated with
652 mg of cesium carbonate and stirred at room
temperature for 40 hours. After customary working up,
the residue is chromatographed on a reversed-phase
column using acetonitrile/water 65:35.
3'-(3-Cyanophenoxymethyl)biphenyl-3-carbonitrile ("C")
is obtained as a colorless solid, FAB 311.

A solution of 90 mg of "C" and 125 mg of hydroxyl-
ammonium chloride in 10 ml of ethanol is treated with
1.2 g of polymer-bound dimethylaminopyridine (DMAP) and
stirred at room temperature for 18 hours. The polymer

is filtered off, the solvent is removed and N-hydroxy-3'-[3-(N-hydroxycarbamimidoyl)phenoxyethyl]biphenyl-3-carboxamide ("D") is obtained as a colorless solid, FAB 377.

- 5 A solution of 76 mg of "D" in 10 ml of methanol is treated with 100 mg of water-moist Raney nickel and 30 mg of acetic acid and hydrogenated at room temperature and normal pressure for 18 hours. The catalyst is filtered off, the solvent is removed and
10 3'-(3-carbamimidoylphenoxyethyl)biphenyl-3-carboxamide, acetate, is obtained, EI 327 ($M^+ - NH_3$), 310 ($M^+ - 2 NH_3$)



15

The following compounds are obtained analogously

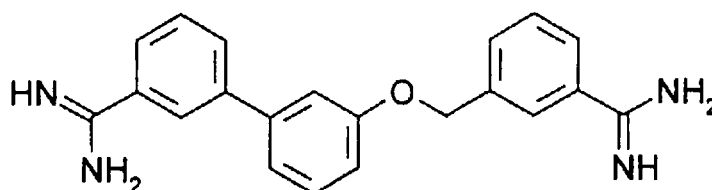
- 3'-(3-carbamimidoylphenoxyethyl)biphenyl-4-carboxamide, diacetate, FAB 345;
3'-(4-carbamimidoylphenoxyethyl)biphenyl-4-carboxamide, diacetate, FAB 345;
20 3'-(4-carbamimidoylphenoxyethyl)biphenyl-3-carboxamide, diacetate, FAB 345;
4'-(4-carbamimidoylphenoxyethyl)biphenyl-4-carboxamide,
25 4'-(4-carbamimidoylphenoxyethyl)biphenyl-3-carboxamide,
4'-(3-carbamimidoylphenoxyethyl)biphenyl-3-carboxamide and
4'-(3-carbamimidoylphenoxyethyl)biphenyl-4-carboxamide.
30

Example 2

Analogously to Example 1, the compound 3'-methoxybiphenyl-3-carbonitrile is obtained by reaction of
35

3-bromobenzonitrile with 3-methoxyphenylboronic acid.
By subsequent ether cleavage using aluminum triiodide
in acetonitrile and reaction with 3-bromomethylbenzo-
nitrile, 3'-(3-cyanobenzoyloxy)biphenyl-3-carbonitrile
5 is obtained.

By reaction with hydroxylamine and reduction with
hydrogen under Ra Ni catalysis, 3'-(3-carbamimidoyl-
benzoyloxy)biphenyl-3-carboxamide is obtained



The following are obtained analogously

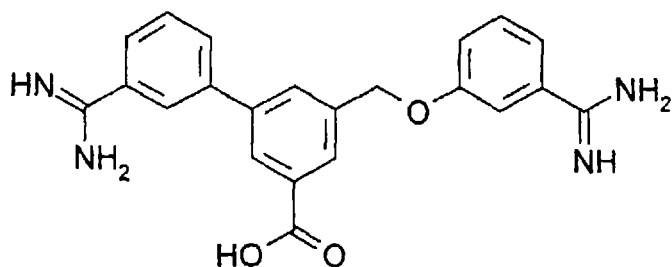
4'-(4-carbamimidoylbenzoyloxy)biphenyl-4-carboxamide,
15 diacetate, FAB 345;
4'-(3-carbamimidoylbenzoyloxy)biphenyl-4-carboxamide,
diacetate, FAB 345.

Example 3

Analogously to Example 1, the compound methyl 3'-cyano-
5-methylbiphenyl-3-carboxylate is obtained by reaction
of 3-cyanophenylboronic acid with methyl 3-bromo-
5-methylbenzoate. By bromination with NBS and reaction
25 with 3-hydroxybenzonitrile, methyl 3'-cyano-5-(3-cyano-
phenoxyethyl)biphenyl-3-carboxylate is obtained.

Reaction with hydroxylamine and reduction with H₂/Ra Ni
affords the compound methyl 3'-carbamimidoyl-
5-(3-carbamimidoylphenoxyethyl)biphenyl-3-carboxylate.

30 By hydrolysis of the ester using aqueous NaOH,
3'-carbamimidoyl-5-(3-carbamimidoylphenoxyethyl)bi-
phenyl-3-carboxylic acid is obtained therefrom.



By chromatography on a reversed-phase column using an acetonitrile/water/TFA mixture, 3'-carbamimidoyl-5-(3-carbamimidoylphenoxy)methylbiphenyl-3-carboxylic acid, bistrifluoroacetate, is obtained.

The following compounds are obtained analogously

10 methyl 4'-carbamimidoyl-4-(4-carbamimidoylphenoxy-methyl)biphenyl-3-carboxylate, FAB 403;

methyl 4'-carbamimidoyl-4-(3-carbamimidoylphenoxy-methyl)biphenyl-2-carboxylate, FAB 403;

15

methyl 3'-carbamimidoyl-4-(4-carbamimidoylphenoxy-methyl)biphenyl-2-carboxylate, FAB 403;

20 methyl 3'-carbamimidoyl-4-(3-carbamimidoylphenoxy-methyl)biphenyl-2-carboxylate, FAB 403;

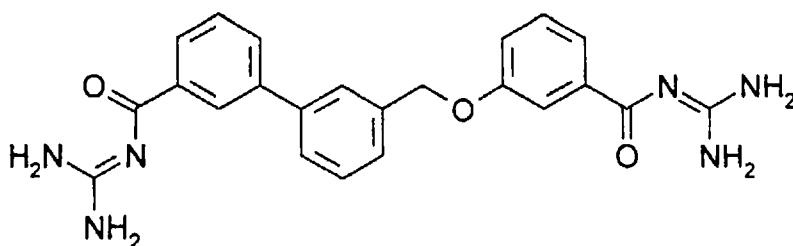
methyl 4'-carbamimidoyl-5-(3-carbamimidoylphenoxy-methyl)biphenyl-4-carboxylate, FAB 403;

25 methyl 3'-carbamimidoyl-5-(3-carbamimidoylphenoxy-methyl)biphenyl-4-carboxylate, FAB 403.

Example 4

30 Analogously to Example 1, methyl 3'-methylbiphenyl-3-carboxylate is obtained by reaction of methyl 3-bromobenzoate with 3-tolylboronic acid. By bromination with NBS and reaction with methyl

3-hydroxybenzoate, methyl 3'-(3-methoxycarbonylphenoxy-methyl)biphenyl-3-carboxylate is obtained therefrom. By reaction with guanidine hydrochloride in methanolic sodium methoxide solution, N-[3'-(3-guanidinocarbonyl-
5 phenoxy-methyl)biphenyl-3-carbonyl]guanidine is obtained therefrom



10 The compound N-[4'-(4-guanidinocarbonylphenoxy-methyl)-biphenyl-4-carbonyl]guanidine is obtained analogously.

Example 5

15 A solution of 7.0 g of 3-bromo-5-methylphenol and 5.97 g of methyl bromoacetate and also 13 g of cesium carbonate in 100 ml of acetonitrile is stirred overnight at room temperature. After customary working up, 9.70 g of methyl (3-bromo-5-methylphenoxy)acetate
20 ("AB") are obtained.

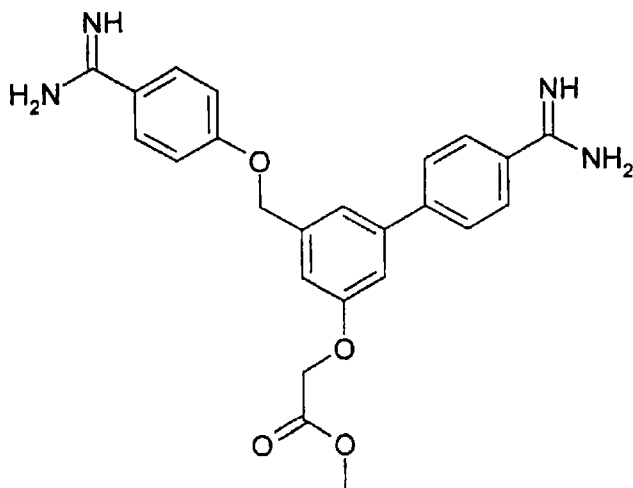
A suspension of 2.0 g of "AB", 100 mg of tetrakis-(triphenylphosphine)palladium and 0.85 g of sodium carbonate in 50 ml of toluene is heated to boiling. A solution of 2.94 g of 3-cyanophenylboronic acid in
25 30 ml of methanol is then added dropwise and the mixture is heated under reflux for 14 hours. It is worked up in the customary manner and 2.17 g of methyl (3'-cyano-5-methylbiphenyl-3-yloxy)acetate ("AC") are obtained.

30 A solution of 1.2 g of "AC" and 0.765 g of NBS in 50 ml of carbon tetrachloride is irradiated at room temperature using UV light. After customary working up, 1.54 g of methyl (3'-cyano-5-bromomethylbiphenyl-

3-yloxy)acetate ("AD") are obtained.

A solution of 185 mg of "AD", 63.1 mg of 4-hydroxybenzonitrile and 172.7 mg of cesium carbonate in 10 ml of acetonitrile is stirred at room temperature for 4 days. After customary working up, methyl [3'-cyano-5-(4-cyanophenoxymethyl)biphenyl-3-yloxy]acetate ("AE") is obtained.

A solution of 60 mg of "AE", 69.5 mg of hydroxylammonium chloride and 101 mg of triethylamine in 10 ml of ethanol is heated under reflux for 14 hours. After removal of the solvent, the residue is taken up in water. The solid is separated off and 70 mg of methyl [3'-N-hydroxyamidino-5-(4-N-hydroxyamidinophenoxy-methyl)biphenyl-3-yloxy]acetate ("AF") are obtained. By reduction using H₂/Raney nickel, methyl [3'-amidino-5-(4-amidinophenoxymethyl)biphenyl-3-yloxy]acetate, FAB 433, is obtained.



The following compounds are obtained analogously

methyl [4'-amidino-5-(4-amidinophenoxymethyl)biphenyl-3-yloxy]acetate, FAB 433

methyl [3'-amidino-5-(3-amidinophenoxymethyl)biphenyl-3-yloxy]acetate, FAB 433

methyl [4'-amidino-5-(3-amidinophenoxy)methyl]biphenyl-3-yloxy]acetate, FAB 433.

5 If methyl bromoacetate is replaced in the first stage by tert-butyl bromoacetate, the tert-butyl esters obtained in the last stage can be cleaved using trifluoroacetic acid and the corresponding carboxylic acids

10 [3'-amidino-5-(4-amidinophenoxy)methyl]biphenyl-3-yloxy]acetic acid, bistrifluoroacetate, FAB 419;

[4'-amidino-5-(4-amidinophenoxy)methyl]biphenyl-3-yloxy]acetic acid;
15

[3'-amidino-5-(3-amidinophenoxy)methyl]biphenyl-3-yloxy]acetic acid;

[4'-amidino-5-(3-amidinophenoxy)methyl]biphenyl-3-yloxy]acetic acid
20

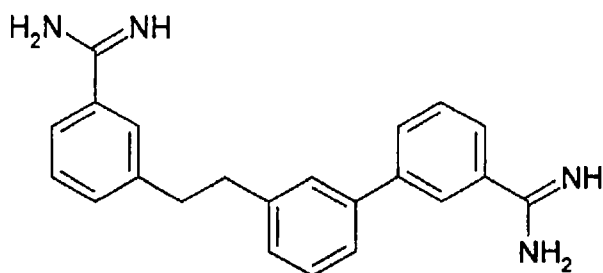
are obtained.

Example 6

25 A solution of 5.0 g of 3'-bromomethylbiphenyl-3-carbonitrile and 5 ml of triethyl phosphite are combined and slowly heated to 150°. The mixture is stirred at 150° for 6 h and 6.05 g of diethyl (3'-cyanobiphenyl-3-ylmethyl)phosphonate ("BA") are
30 obtained after customary working up.

150 mg of sodium hydride are added with ice-cooling and under nitrogen to a solution of 1.0 g of "BA" and 3-cyanobenzaldehyde in 20 ml of ethylene glycol dimethyl ether. The mixture is subsequently stirred for
35 4 hours, worked up in the customary manner and 0.93 g of 3'-[2-(3-cyanophenyl)vinyl]biphenyl-3-carbonitrile ("BB") is obtained.

After hydrogenation of 360 mg of "BB" using Pd-C 5% in methanol, 360 mg of 3'-[2-(3-cyanophenyl)ethyl]-biphenyl-3-carbonitrile ("BC") are obtained. After reaction with hydroxylammonium chloride and hydrogenation using Raney nickel, the compound 3'-[2-(3-amidinophenyl)ethyl]biphenyl-3-carboxamide, FAB 343, is obtained analogously to Example 1



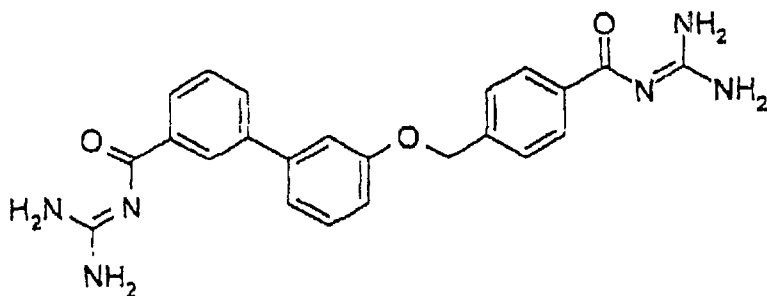
10

The compound 3'-[2-(4-amidinophenyl)ethyl]biphenyl-3-carboxamide, FAB 343, is obtained analogously.

Example 7

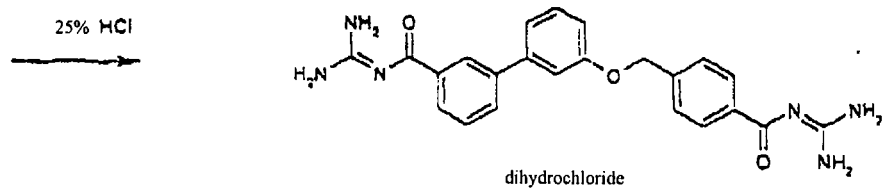
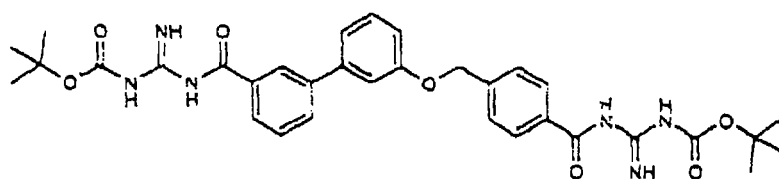
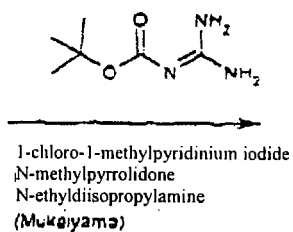
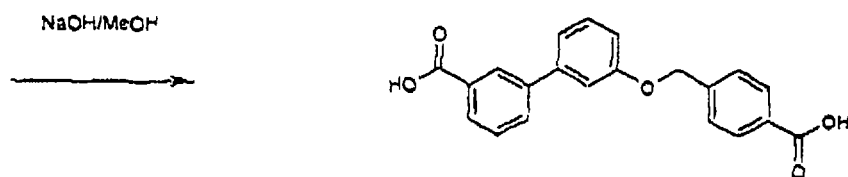
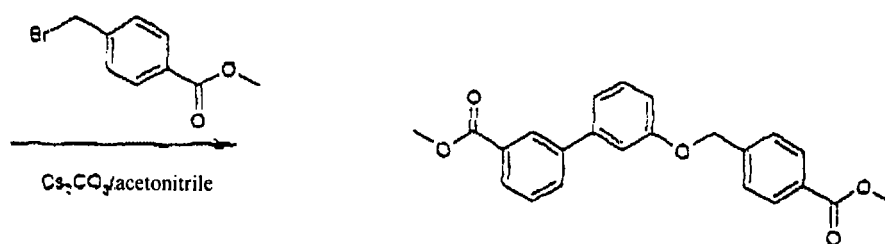
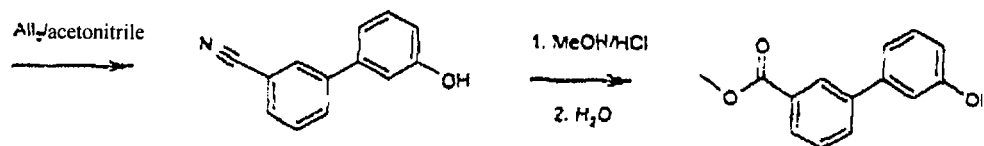
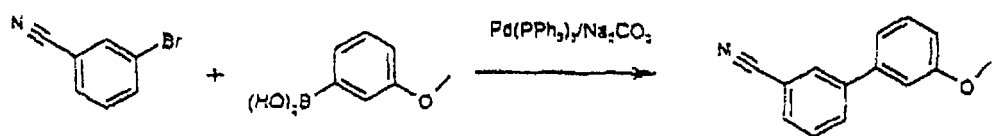
15

The compound N-(3'-(4-guanidinocarbonylbenzyloxy)-biphenyl-3-carbonyl]guanidine, dihydrochloride, FAB 431



20

is obtained for example according to the following reaction scheme:



The following compounds are obtained analogously

N-[3'-(3-guanidinocarbonylbenzyloxy)biphenyl-
3-carbonyl]guanidine, dihydrochloride, FAB 431;

5

N-[3'-(4-guanidinocarbonylbenzyloxy)biphenyl-
4-carbonyl]guanidine, dihydrochloride, FAB 431;

10 N-[3'-(3-guanidinocarbonylbenzyloxy)biphenyl-
4-carbonyl]guanidine, dihydrochloride, FAB 431.

Pharmacological tests

15 The methodology which was used for the characterization
of the compounds of the formula I as NHE-3 inhibitors
is presented below.

20 The compounds of the formula I were characterized with
respect to their selectivity to the isoforms NHE-1 to
NHE-3. The three isoforms were stably expressed in
mouse fibroblast cell lines. The inhibitory action of
the compounds was assessed by determination of the
EIPA-sensitive $^{22}\text{Na}^+$ absorption into the cells after
intracellular acidosis.

25 For the characterization of the Na^+/H^+ exchange
inhibitors with respect to their isoform selectivity,
we investigated the compounds for their inhibition of
the NHE isoforms NHE-1, -2 and -3, which were stably
expressed in a mouse fibroblast cell line (see
30 procedure section) by determining the EIPA-sensitive
 $^{22}\text{Na}^+$ absorption into the cells after intracellular
acidosis.

Material and methods

35

LAP1 cell lines which express the different NHE
isoforms

The LAP1 cell lines which express the isoforms NHE-1, -2 and -3 (a mouse fibroblast cell line), were obtained from Prof. J. Pouyssegur (Nice, France). The transfections were carried out by the procedure of Franchi et al. (1986). The cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 10% inactivated foetal calf serum (FCS). For the selection of the NHE-expressing cells, the so-called "acid destruction procedure" of Sardet et al. (1989) was used. The cells were first removed to an NH_4Cl -containing bicarbonate- and sodium-free buffer by washing with a bicarbonate-, NH_4Cl - and sodium-free buffer and incubation was carried out with a bicarbonate-free NaCl -containing buffer. Only those cells which functionally express NHE were able to survive in the intracellular acidification to which they were exposed.

Characterization of NHE inhibitors with respect to their isoform selectivity

Using the abovementioned mouse fibroblast cell lines which express the isoforms NHE-1, NHE-2 and NHE-3, compounds were tested for selectivity to the isoforms according to the procedure described by Counillon et al. (1993) and Scholz et al. (1995). The cells were acidified intracellularly by the NH_4Cl prepulse procedure and then by incubation in a bicarbonate-free $^{22}\text{Na}^+$ -containing buffer. On account of the intracellular acidification, NHE was activated and sodium was absorbed into the cells. The effect of the test compound was expressed as the inhibition of the EIPA (ethylisopropylamiloride)-sensitive $^{22}\text{Na}^+$ absorption. The cells which expressed NHE-1, NHE-2 and NHE-3 were transferred to microtiter plates having 24 wells by inoculation at a density of $5-7.5 \times 10^4$ cells/well and cultured to confluence for 24 to 48 hours. The medium was aspirated and the cells were incubated at 37°C for

60 minutes in the NH_4Cl buffer (50 mM NH_4Cl , 70 mM choline chloride, 15 mM MOPS, pH 7.0). The buffer was then removed and the cells were rapidly covered twice with a layer of the choline chloride wash buffer
5 (120 mM choline chloride, 15 mM PIPES/tris, 0.1 mM ouabain, 1 mM MgCl_2 , 2 mM CaCl_2 , pH 7.4); the cells were incubated in this buffer for 6 minutes. After the incubation time was over, the incubation buffer was aspirated. For the purpose of removal of extracellular
10 radioactivity, the cells were washed rapidly four times with ice-cold phosphate-buffered saline solution (PBS). The cells were then solubilized by addition of 0.3 ml of 0.1 N NaOH per well. The cell-fragment-containing solutions were transferred to scintillation tubes. Each
15 well was additionally washed twice with 0.3 ml of 0.1 N NaOH and the wash solutions were likewise added to the corresponding scintillation tubes. The tubes containing the cell lyzate were mixed with scintillation cocktail and the radioactivity absorbed into the cells was
20 determined by determination of the β radiation.

Inhibition of the $^{22}\text{Na}^+$ absorption in rabbit erythrocytes

25 The Na^+/H^+ exchange activity was also determined by observation of the absorption of $^{22}\text{Na}^+$ ions in acidified rabbit erythrocytes. Rabbit erythrocytes have found wide application in investigations on Na^+/H^+ exchange activity (Escobales & Fugueroa, 1991; Morgan & Canessa,
30 1990). The EIPA-sensitive portion of the $^{22}\text{Na}^+$ absorption in acidified erythrocytes was regarded as Na^+/H^+ -dependent $^{22}\text{Na}^+$ absorption.

Cell preparation

35

The preparation of the red blood corpuscles and the internal acidification of the red blood corpuscles were carried out closely following the procedures of Morgan

and Canessa (1990).

The blood was obtained from rabbits (e.g. New Zealand White). It was collected in 50 ml Falcon centrifuge tubes which contained 5 ml of sodium heparin solution (250 U/ml). The blood and the heparin solution were well mixed. The red blood corpuscles were recovered by centrifugation at $2000 \times g$ at 4°C ; the plasma and buffy coat were removed. The remaining solution was filtered through $200 \mu\text{m}$ gauze. The filtrate was again suspended in the original volume with wash buffer (140 mM KCl, 0.15 mM MgCl_2 , 10 mM TRIS/MOPS, pH 7.4). The red blood corpuscles were again recovered by centrifugation ($2000 \times g$, 4°C). The washing process was repeated twice.

Intracellular acidification

For intracellular acidification, 5 ml of the deposited collected red blood corpuscles were again suspended using 45 ml of acidification buffer (170 mM KCl, 0.15 mM MgCl_2 , 0.1 mM ouabain, 10 mM glucose, 10 mM sucrose, 20 mM tris/Mes, pH 6.2). The suspension of the red blood corpuscles was incubated at 37°C for 10 minutes (with occasional mixing). In order to fix the internal pH, treatment is carried out with up to $200 \mu\text{M}$ and 1 mM DIDS or DIAMOX (acetazolamide). The mixture was additionally incubated at 37°C for 30 minutes.

The red blood corpuscles were then recovered by centrifugation (4 minutes at $2000 \times g$, 4°C); they were again suspended using ice-cold unbuffered wash solution (170 mM KCl, 40 mM sucrose, 0.15 mM MgCl_2) and washed four times therewith.

Incubation and measurement of the $^{22}\text{Na}^+$ absorption

The incubation was carried out in macrowell tube strips in a format of 8×12 . The incubation was begun by

mixing 200 μ l of incubation buffer (160 mM KCl, 22 NaCl (37 MBq/well), 10 mM NaCl, 0.15 mM MgCl_2 , 0.1 mM ouabain, 10 mM glucose, 40 mM sucrose, 10 mM tris/MOPS, pH 8.0, 0.5 mM Diamox, 1% DMSO) with 20 μ l of the
5 (prewarmed) acidified solution of the red blood corpuscles. The test substances were first dissolved using 100% DMSO and the solution was then diluted to the corresponding concentrations using incubation buffer. Incubation was carried out at 37°C for
10 5 minutes. (In preliminary experiments it was shown that under these incubation conditions the 22 Na absorption rate was linear during the 5-minute incubation period). The incubation was stopped by addition of 800 μ l of ice-cold stop solution (112 mM
15 MgCl_2 , 0.1 mM ouabain). The tubes were briefly stored on ice. The tubes were then covered with Parafilm and the red blood corpuscles were recovered by centrifugation at 2000 $\times$ g and 4°C for 7 minutes. The supernatant was aspirated with a home-made aspiration
20 apparatus, with which it was possible to aspirate 4 tubes next to one another simultaneously; spacer rings on the further ends of the tips prevented the tips dipping too deeply into the tubes and the pellet of the red blood corpuscles being aspirated. All
25 22 Na-containing supernatants and wash solutions were stored and disposed of as radioactive waste.

The red blood corpuscles were washed three times with 900 μ l of ice-cold stop solution, namely by repeating the suspension/centrifugation step described above.
30 After the last wash, the pellet of the red blood corpuscles was mixed with 200 μ l of water. The tubes were then treated with ultrasound for 2 $\times$ 30 minutes. The macrowell tube strips were then taken apart and each tube was put headfirst into an individual
35 scintillation tube; the hemolyzed solution of the red blood corpuscles emptied into the scintillation vial as a result of gentle shaking. Each vial was treated with 3 ml of the scintillation fluid Aquasafe 300 PS, and

the vials were provided with closures and well mixed. The radioactivity absorbed into the red blood corpuscles was determined in a scintillation counter by monitoring the β decomposition.

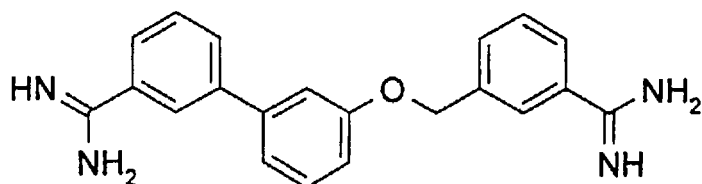
- 5 Per substance concentration, the determination was carried out in triplicate. The mean of the count determination in the presence of 10 μ M EIPA was subtracted from each value in order to include the non- Na^+/H^+ -dependent $^{22}\text{Na}^+$ absorption into the erythrocytes.
- 10 The mean of the remaining counts in the absence of a substance was used as a 100% control; the mean values in the presence of the test compounds were expressed as a percentage of this control value. The percentage absorption data were plotted semilogarithmically; IC50
- 15 values were obtained by fitting the values to a non-linear curve using the equation $f(x) = 100/(1 + (\text{IC50}/x)^{**n})$.

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Test results

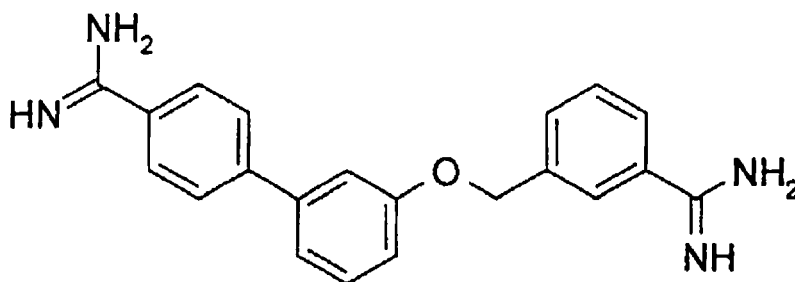
1.



Code EMD 221960

IC₅₀ (NHE-3) = 1-2 μ M

2.



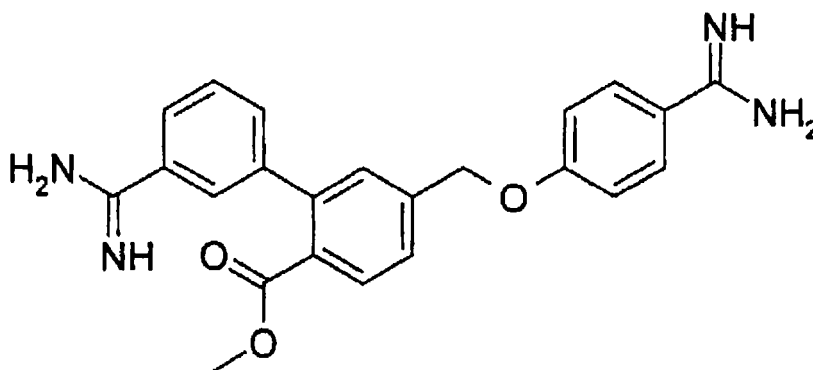
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Code EMD 221963

IC₅₀ (NHE-3) = 1 μ M

Diacetate;

3.

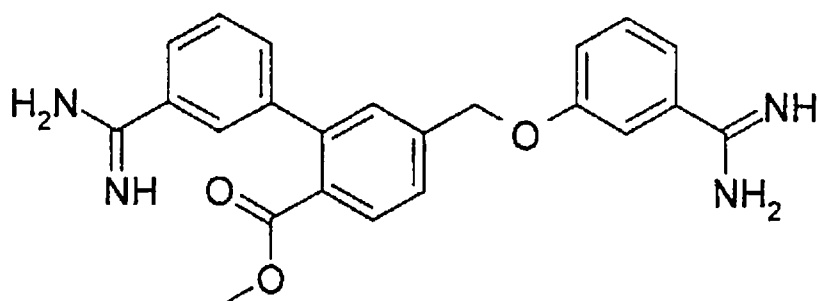


Diacetate;

10 Code EMD 246326

IC₅₀ (NHE-3) = 3 μ M

4.



Diacetate;

Code EMD 246327

IC50 (NHE-3) = 3-4 μ M.

The following examples relate to pharmaceutical preparations:

Example A: Injection vials

5

A solution of 100 g of an NHE-3 inhibitor of the formula 1 and 5 g of disodium hydrogenphosphate is adjusted to pH 6.5 using 2N hydrochloric acid in 3 l of double-distilled water, sterile filtered, dispensed
10 into injection vials, lyophilized under sterile conditions and aseptically sealed. Each injection vial contains 5 mg of active compound.

Example B: Suppositories

15

A mixture of 20 g of an NHE-3 inhibitor of the formula I is fused with 100 g of soya lecithin and 1400 g of cocoa butter, poured into molds and allowed to cool. Each suppository contains 20 mg of active
20 compound.

Example C: Solution

A solution is prepared from 1 g of an NHE-3 inhibitor
25 of the formula I, 9.38 g of $\text{NaH}_2\text{PO}_4 \cdot 2 \text{H}_2\text{O}$, 28.48 g of $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ and 0.1 g of benzalkonium chloride in 940 ml of doubled-distilled water. It is adjusted to pH 6.8, made up to 1 l and sterilized by irradiation. This solution can be used in the form of eye drops.

30

Example D: Ointment

500 mg of an NHE-3 inhibitor of the formula I are mixed with 99.5 g of petroleum jelly under aseptic
35 conditions.

Example E: Tablets

A mixture of 1 kg of an NHE-3 inhibitor of the formula I, 4 kg of lactose, 1.2 kg of potato starch, 5 0.2 kg of talc and 0.1 kg of magnesium stearate is compressed in a customary manner to give tablets such that each tablet contains 10 mg of active compound.

Example F: Coated tablets

10

Tablets are pressed analogously to Example E and then coated in a customary manner with a coating of sucrose, potato starch, talc, tragacanth and colorant.

15 **Example G: Capsules**

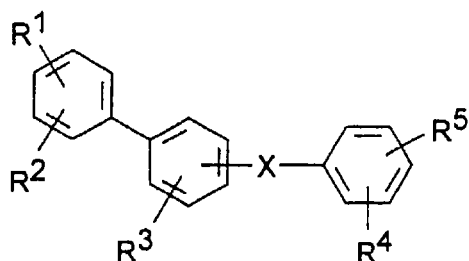
2 kg of an NHE-3 inhibitor of the formula I are filled into hard gelatin capsules in a customary manner such that each capsule contains 20 mg of the active 20 compound.

Example H: Ampoules

A solution of 1 kg of NHE-3 inhibitor of the formula I 25 in 60 l of double-distilled water is sterile filtered, dispensed into ampoules, lyophilized under sterile conditions and aseptically sealed. Each ampoule contains 10 mg of active compound.

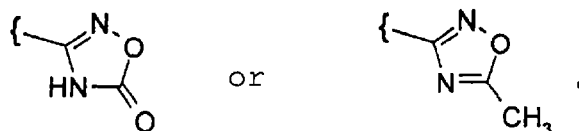
Patent Claims

1. A compound of the formula I



in which

R^1, R^4 in each case independently of one another are $-C(=NH)-NH_2$, which can also be monosubstituted by $-COA$, $-CO-[C(R^6)_2]_n-Ar$, $-COOA$, $-OH$ or by a conventional amino protective group, $NH-C(=NH)-NH_2$, $-CO-N=C(NH_2)_2$,



R^2, R^3, R^5 in each case independently of one another are H , A , OR^6 , $N(R^6)_2$, NO_2 , CN , Hal , $NHCOA$, $NHCOAr$, $NHSO_2A$, $NHSO_2Ar$, $COOR^6$, $CON(R^6)_2$, $CONHAr$, COR^6 , $COAr$, $S(O)_nA$, $S(O)_nAr$, $-O-[C(R^6)_2]_m-COOR^6$, $-[C(R^6)_2]_p-COOR^6$, $-O-[C(R^6)_2]_m-CON(R^6)_2$, $-[C(R^6)_2]_p-CON(R^6)_2$, $-O-[C(R^6)_2]_m-CONHAr$ or $-[C(R^6)_2]_p-CONHAr$,

X is $-[C(R^6)_2]_n-$, $-CR^6=CR^6-$, $-[C(R^6)_2]_n-O-$, $-O-[C(R^6)_2]_n-$, $-COO-$, $-OOC-$, $-CONR^6-$ or $-NR^6CO-$,

R^6 is H, A or benzyl,

5 A is alkyl having 1-20 C atoms, in which one or two CH_2 groups can be replaced by O or S atoms or by $-CR^6=CR^6-$ groups and/or 1-7 H atoms can be replaced by F,

10 Ar is phenyl or naphthyl, which is unsubstituted or mono-, di- or trisubstituted by A, Ar' , OR^6 , OAr' , $N(R^6)_2$, NO_2 , CN, Hal, $NHCOA$, $NHCOAr'$, $NHSO_2A$, $NHSO_2Ar'$, $COOR^6$, $CON(R^6)_2$, $CONHAr'$, COR^6 , $COAr'$, $S(O)_nA$ or $S(O)_nAr'$,

15 Ar' is phenyl or naphthyl, which is unsubstituted or mono-, di- or trisubstituted by A, OR^6 , $N(R^6)_2$, NO_2 , CN, Hal, $NHCOA$, $COOR^6$, $CON(R^6)_2$, COR^6 or $S(O)_nA$,

20 Hal is F, Cl, Br or I,

n is 0, 1 or 2,

25 m is 1 or 2,

p is 1 or 2,

and its salts and solvates as NHE-3 inhibitors.

30

2. The compound as claimed in claim 1

a) 3'-(3-carbamimidoylphenoxy)methyl)biphenyl-3-carboxamidine;

35 b) 3'-(3-carbamimidoylbenzyloxy)biphenyl-3-carboxamidine;

c) 3'-carbamimidoyl-5-(3-carbamimidoylphenoxy-methyl)biphenyl-3-carboxylic acid;

- d) N-[3'-(3-guanidinocarbonylphenoxy)methyl)-
biphenyl-3-carbonyl]guanidine;
- e) methyl [3'-amidino-5-(4-amidinophenoxy)methyl)-
biphenyl-3-yloxy]acetate;
- 5 f) [3'-amidino-5-(4-amidinophenoxy)methyl)biphenyl-
3-yloxy]acetic acid,

and its salts and solvates as NHE-3 inhibitors.

- 10 3. Use of the compound of the formula I as claimed in
claim 1 and its physiologically acceptable salts
and/or solvates for the production of a medicament
for the treatment of thromboses, ischemic
15 conditions of the heart, of the peripheral and
central nervous system and of stroke, ischemic
conditions of peripheral organs and limbs and for
the treatment of states of shock.
- 20 4. Use of the compound of the formula I as claimed in
claim 1 and its physiologically acceptable salts
and/or solvates for the production of a medicament
for use in surgical operations and organ
transplantation and for the preservation and
storage of transplants for surgical measures.
- 25 5. Use of the compound of the formula I as claimed in
claim 1 and its physiologically acceptable salts
and/or solvates for the production of a medicament
for the treatment of diseases in which cell
30 proliferation is a primary or secondary cause, for
the treatment or prophylaxis of disorders of the
lipid metabolism or disturbed respiratory drive.
- 35 6. Use of the compound of the formula I as claimed in
claim 1 and its physiologically acceptable salts
and/or solvates for the production of a medicament
for the treatment of ischemic kidney, ischemic

intestinal disorders or for the prophylaxis of acute or chronic kidney disorders.

- 5 7. A pharmaceutical preparation, characterized in that it contains at least one NHE-3 inhibitor as claimed in claim 1 and/or one of its physiologically acceptable salts and/or solvates.