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EUROPEAN PATENT APPLICATION

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Copy of the English translation of the specification and claims as originally filed.

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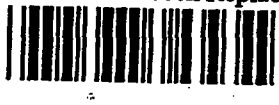

PATENT OF INVENTION

Use of 4-amino-1-(2-pyridyl)piperidines for the preparation of medicaments for the treatment and prophylaxis of conditions deriving from a dysfunction of the serotoninergic systems mediated by the 5-HT_3 receptors.

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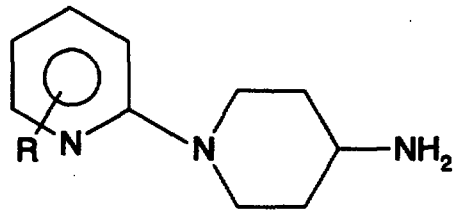
The present invention concerns a new therapeutical use of some 4–amino–1–(2–pyridyl)piperidines.

EP–B–0021973 describes the class of 4–amino–1–(2–pyridyl)piperidines of general formula (I)



(I)

in which R may represent hydrogen, a halogen atom, a methyl, trifluoromethyl, (C_1–C_3)alkoxy, trifluoromethoxy, 2,2,2–trifluoroethoxy, (C_1–C_3)alkythio, trifluoromethylthio, or phenoxy or phenylthio group optionally substituted with a halogen atom, a trifluoromethyl, (C_1–C_3)alkyl, (C_1–C_3)alkoxy, (C_1–C_3)alkylthio, or cyano group,

and the pharmaceutically acceptable salts thereof, as well as a process for their preparation.

The compounds of formula (I) are described therein as anorectic agents, essentially devoid of important side effects.

Among the compounds of formula (I), the 4–amino–1–(6–chloro–2–pyridyl)piperidine hydrochloride, described in Example 1 of EP–B–0021973 and indicated as CM 57227 was subjected to the tests necessary for entering clinical trials, and tested in humans, showed to be well tolerated up to a dose of 10 mg.

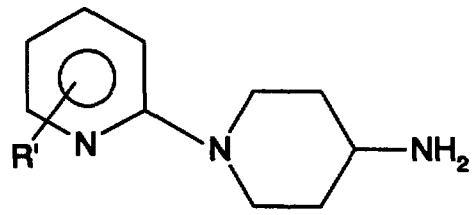
It has now been found that the compounds of formula (I) where R represents hydrogen, a halogen atom, a methyl, methylthio, trifluoromethylthio, trifluoromethyl, (C_1–C_3)alkoxy, trifluoromethoxy, 2,2,2–trifluoroethoxy or phenoxy group optionally substituted with a halogen atom, a trifluoromethyl, (C_1–C_3)alkyl, (C_1–C_3)alkoxy, (C_1–C_3)alkylthio, or cyano group, as well as their pharmaceutically acceptable salts, described in EP–B–0021973 as inhibiting the serotonin uptake, have a selective agonist activity with respect to the 5–HT_3 receptors at the peripheral and central level.

More particularly, it has been found that the compounds of formula (I) where R is as defined above and their pharmaceutically

acceptable salts have an affinity for the 5-HT_3 sites which is markedly higher than that of serotonin and 2-methylserotonin.

It has also been found by means of ex-vivo binding tests, carried out on representative compounds of said class, that the affinity for the central 5-HT_3 sites is markedly higher than that for the serotonin uptake sites.

A first object of the present invention is therefore the use of at least one compound of formula (I')



(I')

where R' represents hydrogen, a halogen atom, a methyl, trifluoromethyl, (C_1-C_3)alkoxy, trifluoromethoxy, 2,2,2-trifluoroethoxy, methylthio, trifluoromethylthio, or phenoxy group optionally substituted with a halogen atom, a (C_1-C_3)alkyl, (C_1-C_3)alkoxy, (C_1-C_3)alkylthio, trifluoromethyl or cyano group, or a pharmaceutically acceptable salt thereof, for the preparation of medicaments for the treatment and the prophylaxis of all conditions deriving from a dysfunction of the serotoninergic systems and in which a serotoninergic action selectively mediated by the 5-HT_3 receptors is considered to be beneficial.

In the present specification, the term "halogen" identifies one of the four common halogens, fluorine, chlorine and bromine being particularly preferred.

The terms "(C_1-C_3)alkyl", "(C_1-C_3)alkoxy", and "(C_1-C_3) alkylthio" designate groups containing the saturated aliphatic hydrocarbon residue containing 1, 2, or 3 carbon atoms, namely methyl, ethyl, propyl and isopropyl.

The pharmaceutically acceptable salts of the compounds of formula (I') comprise the non-toxic salts derived from mineral or organic acids which salify one or both basic functions present in the molecules of the compounds of formula (I') above, such as hydrochlorides,

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hydrobromides, sulfates, phosphates, succinates, tartrates, fumarates, maleates, pamoates, napsylates, mesylates, tosylates, etc.

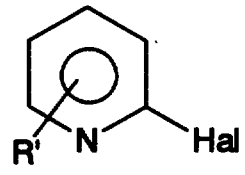
A preferred class of compounds of formula (I') comprises the compounds (I') where R' is at position 6– of the 2–pyridyl radical.

An even more advantageous class comprises the compounds of formula (I') where R' is hydrogen, a halogen atom, a methyl or (C_1–C_3)alkoxy group at position 6–.

Another preferred class of the compounds of formula (I') comprises those compounds of formula (I') where R' represents a halogen atom at position 3–.

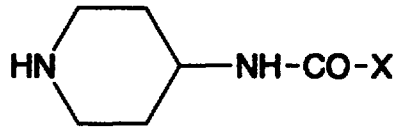
4–Amino–1–(6–chloro–2–pyridyl)piperidine and the pharmaceutically acceptable salts thereof, particularly the hydrochloride, 4–amino–1–(6–bromo–2–pyridyl)piperidine and the pharmaceutically acceptable salts thereof, particularly the hydrochloride, 4–amino–1–(6–methyl–2–pyridyl)–piperidine and the pharmaceutically acceptable salts thereof, particularly the dihydrochloride, and 4–amino–1–(6–methoxy–2–pyridyl)piperidine and the pharmaceutically acceptable salts thereof, particularly its hydrochloride, are particularly preferred compounds.

The compounds of formula (I') are easily prepared by the general process described in EP–B–0021973, by reacting the corresponding 2–halopyridine of formula (II)



(II)

in which R' is as defined above and Hal represents a halogen atom, with a blocked 4–aminopiperidine of formula (III)



(III)

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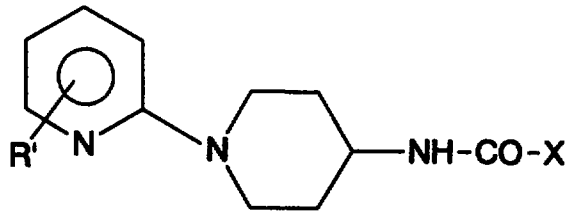
in which X represents a hydrogen atom or a (C_1–C_4)alkyl group, and hydrolysing the thus obtained N–acylated 4–amino–1–(2–pyridyl)piperidine of formula (IV)



(IV)

under basic or acid conditions.

A more detailed description of the general process for the preparation of compounds (I'), as well as some specific examples, are contained in the above cited European Patent EP–B–0021973.

The affinity of the compounds of formula (I') for 5–HT_3 receptors has been evaluated at first by means of an in vitro binding test using 5–HT_3 binding sites present in the rat cerebral cortical tissue (cfr. G.J. Kilpatrick, B.J. Jones and M.B. Tyers. Identification and distribution of 5–HT3 receptors in rat brain using radioligand binding. Nature, 1987, 330, 746–8) and [^3H] BRL 43694 (granisetron), a potent and specific 5–HT_3 receptor antagonist as the labelled ligand. The potency of the compounds of formula (I') in displacing [^3H] BRL 43694 was compared to that of serotonin and to that of other 5–HT_3 agonists (2–methylserotonin and m–chlorophenyl–biguanide), as well as to that of the antagonist ICS 205930.

For the preparation of cerebral membranes, male rats weighing about 200 g and sacrificed by decapitation were employed. The membrane preparation and the binding test were carried out according to the method described by Nelson and Thomas (D.R. Nelson and D.R. Thomas. [^3H]BRL 43694 (Granisetron), a specific ligand for 5–HT_3 binding sites in rat brain cortical membranes. Biochem. Pharmacol., 1989, 38, 1963–65). In summary, the cortical tissue from 4 animals was homogenized in 20 volumes of HEPES and kept at 4°C until used.

The thus prepared membranes contained on the average 7.42 (± 0.11) mg of protein/ml, calculated according to the Lowry method (O.H. Lowry, N.J. Rosenbrough, A.L. Fan and R.J. Randall. Protein measurement

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with the Folin phenol reagent. J. Biol. Chem., 1951, 193, 265–75) using bovine serum albumin for the determination of the standard curve.

For the displacing experiments, 500 µl of membrane suspension was incubated in the presence of 0.5 nM [^{3}H]BRL 43694 (specific activity 61 Ci/mmol) and of different concentrations of unlabelled compounds for 30 minutes at 25°C, in a total volume of 1 ml. The incubation was terminated by filtering rapidly through Whatman GF/B filters which were then washed twice with 50 mM HEPES, pH 7.5, (10ml) at 4°C. Aspecific binding was determined in samples containing an excess of ICS 205930 (1 µM).

Preliminary saturation and kinetic studies were carried out to set up the test. For the saturation study, 9 triplicate concentrations of [^{3}H]BRL 43694 (0.05–2nM) were used, determining, for each radioligand concentration, the aspecific binding (in the presence of 1 µM ICS 205930).

The kinetics for the association–dissociation of [^{3}H]BRL 43694 to the 5–HT_3 sites was followed for 45 minutes, in the absence and in the presence of 1µM ICS 205930, respectively.

The results were calculated by means of non–linear fitting methods "Accufit saturation" for the saturation studies (H.A. Feldman. Mathematical theory of complex ligand–binding systems at equilibrium: some methods of parameter fitting. Analyt. Biochem., 1972, 48, 317–38) and "Accufit competition" for the displacing studies, (H.A. Feldman, D. Rodbard, and D. Levine. Mathematical theory of cross reactive radioimmunoassay and ligand–binding systems at equilibria. Analyt. Biochem., 1972, 45, 530–56).

The Scatchard analysis carried out on [^{3}H]BRL 43694 revealed a saturable binding site of high affinity (Kd = 0.46 ± 0.01 nM; B_{max} = 7.54 ± 0.07 mol/mg of proteins or 1.12 fmol/mg of wet tissue). The Kd obtained from the association and dissociation curves and equal to 0.5 is in agreement with that obtained by the Scatchard analysis.

The 0.5 nM concentration of [^{3}H]BRL 43694 was therefore employed in the competition studies in order to obtain the affinities of the different compounds (Ki).

In this test, the compounds of formula (I') showed to be much more potent (generally 10 to 20 times more potent) than serotonin and 2–methylserotonin in displacing [^{3}H]BRL 43694.

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By way of example, the compound of formula (I') where R' is a chloro atom at position 6- (CM 57227) showed a K_i of 193 nM, whereas serotonin and 2-methylserotonin have K_i of 1195 and 1115 nM respectively.

This affinity for the 5-HT_3 receptors is perfectly selective. In particular, the ability of the same compound (CM 57227) to displace in vitro a series of labelled ligands from different binding sites has been evaluated and the obtained results show that this product has no affinity (K_i > 10,000 nM) for the 5-HT_{1A} and 5-HT_{1B} receptors, for the serotonin uptake site ([^{3}H]paroxetine displacement), for adrenergic sites α_1, α_1, β_1 and for dopaminergic sites D_1 and D_2.

It was then confirmed that the compounds of formula (I') where R' is as defined above do penetrate the CNS, passing through the blood brain barrier.

To this purpose, ex vivo tests were carried out in mice evaluating the inhibition of [^{3}H]BRL 43694 specific binding to cortical cerebral membranes, 30 minutes after the intraperitoneal (i.p.) administration of increasing doses of representative compounds of formula (I').

In these tests, carried out by following the methodology described by Wood and Piper (J. Psychopharmacol., 1990, 4(4), 290), ID_{50} values (the doses which inhibit by 50% the [^{3}H]BRL 43694 binding) obtained at different dilutions were calculated and the ID_{50} value at zero dilution was then extrapolated.

The compounds of formula (I') subjected to this test showed an ID_{50} (mg/kg i.p.) comparable to that of the compound GR-38032 F, a known selective 5-HT_3 antagonist, employed as reference product.

In particular, CM 57227 showed an ID_{50} i.p., extrapolated to zero dilution, of 1 mg/kg and, by repeating the test but administering the compound orally, and ID_{50} p.o., extrapolated to zero dilution, of the same magnitude order.

The ID_{50} of CM 57227 for [^{3}H]BRL 43694 is markedly lower than the ID_{50} of [^{3}H]BRL paroxetine, paroxetine being a marker of the serotonin uptake sites, evaluated under the same experimental conditions.

CM 57227 was also studied in the "turning" test carried out as described by P. Worms et al. in Life Sciences, 1986, 39, 2199-2208.

Like 2-methylserotonin, the 4-amino-1-(6-chloro-2-pyridyl)-piperidine hydrochloride, injected into the mice striatum, induces a turning

behaviour which is inhibited by 5-HT_3 antagonists. Intraperitoneally injected, this compound blocks the antagonism exerted by ondansetron (1mg/kg i.p.) to the turning behaviour induced by intrastriatal injection of 2-methylserotonin with an ED_{50} of 0.25 mg/kg, dose which is entirely in agreement with the above ex vivo data.

The selective agonist activity of the 5-HT_3 receptors of the compounds of formula (I') was confirmed in vivo in the Bezold-Jarish test. In this test, intravenous administration of the compounds of formula (I') induces in the anaesthetised rat, a short-lived reduction of heart rate (Bezold-Jarish effect). The intensity of this effect varies depending on the dose and is comparable to that obtained by administering serotonin or 2-methylserotonin.

This effect is inhibited by selective 5-HT_3 receptor antagonists (e.g. ICS 205930 and zacopride), while it is not inhibited by serotonin D receptor antagonists (e.g. methysergide).

More particularly, the Bezold-Jarish effect induced by the compounds of formula (I') was evaluated using Sprague-Dawley rats weighing from 200 to 300 g, anaesthetised with an intraperitoneal dose of 1.25 g/kg of urethane. The blood pressure was recorded from the carotid artery and heart rate was evaluated, as pulse frequency, by means of a cardiotachometer. A catheter was inserted into the jugular vein for the administration of the substances.

Different doses of the compounds to be tested were administered intravenously in a volume of 0.5 ml/kg and the ID_{50}, i.e. the dose which inhibits by 50% the heart rate in the treated animals, was calculated.

In this test the compounds of formula (I') where R' is as defined above showed an ID_{50} lower or comparable to that of serotonin and to that of 2-methylserotonin.

By way of example, the ID_{50} i.v. of CM 57227 is 8.29 µg/kg while the ID_{50} of serotonin and 2-methylserotonin is 13.61 and 5.00 µg/kg respectively.

The compounds of formula (I') where R' is as defined above also showed an intestinal prokinetic activity related to their 5-HT_3 agonist action.

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Said activity was demonstrated by means of a test in the course of which evaluation of faecal excretion in rats was studied.

According to this test, male rats weighing from 220 to 250 g were fasted for 3–4 hours in individual cages while water was provided without restriction. The compounds of formula (I') to be studied were administered subcutaneously. In the treatment schedule 8 animals in each group were used.

After the treatment, the rectal ampoule was manually emptied from any residual faeces and the animals were then placed again into their respective cages.

90 minutes after the treatment the faecal pellets were collected, counted and weighed so as to determine the wet weight. They were then put in an oven and dried for 10 hours at 40°C for the determination of dry weight.

The parameter used to evaluate the activity was the dry weight (in grams) of the faeces cumulatively collected during the 90 minutes following the subcutaneous administration of the product.

Almost no faecal excretion was observed during the same period in the control animals which received the vehicle only.

In the animals treated with high doses, a maximum excretion varying from 12 to 16 faecal pellets with a dry weight of from about 1.4 to about 1.8g, could be attained.

With total excretions exceeding the above values, diarrhoea is clearly observed and a correct quantification is impossible.

In this test the compounds of formula (I') showed a fairly good faecal excretion stimulating activity at very low doses.

By way of example, in this test the 4–amino–1–(6–chloro–2–pyridyl)piperidine hydrochloride (the compound of formula (I') where R' = 6–Cl), at a dose of 2.5 mg/kg s.c. induced the excretion of about 1.3 g of faeces.

Said activity is related to the 5–HT_3 agonist activity as it is antagonised by selective 5–HT_3 antagonists like zacopride or ICS 205930.

On the basis of the found properties, the compounds of formula (I') are indicated for the treatment of those pathologies related to disorders of the CNS and in particular of the serotoninergic systems, which are selectively mediated by the 5–HT_3 receptors.

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The compounds of formula (I') where R' may represent hydrogen, a halogen atom, a methyl, trifluoromethyl, (C_1–C_3)alkoxy, trifluoromethoxy, 2,2,2–trifluoroethoxy, methylthio, trifluoromethylthio or phenoxy group optionally substituted with a halogen atom, a (C_1–C_3)alkyl, (C_1–C_3)alkoxy, (C_1–C_3)alkylthio, trifluoromethyl or cyano group, as well as their pharmaceutically acceptable salts, are therefore very interesting potential psychotropic agents, which may act through the 5–HT_3 receptors.

More particularly the above compounds, owing to their original mechanism of action, are mainly useful in the treatment of dysthymic disorders and may also be useful in cases of anxiety or psychotic disorders. They may be useful also for the treatment of intestinal motoricity troubles and in particular for the treatment of constipation.

In general, they may be employed in the treatment of all pathological disorders in which a serotonin–like action selectively mediated by the 5–HT_3 receptors may be beneficial.

The invention provides therefore for the use of compounds (I') where R' is as defined above for the preparation of medicaments suitable for the treatment and the prophylaxis of the above indicated pathologies.

Furthermore, the compounds of formula (I') are poorly toxic, their toxicity being well compatible with a therapeutical utilisation.

For the use in the above pathologies, the compounds of formula (I') as well as their pharmaceutically acceptable salts, may conveniently be administered orally, parenterally, sublingually, rectally or transdermally, suitably formulated in pharmaceutical compositions.

The amount of active principle to be daily administered, will depend, on the particular therapeutical indication, on the severity of the disorders to be treated, as well as on the weight of the patient and the administration route.

In general, however, the daily overall dosage in humans varies between 0.05 and 100 mg, e.g. between 0.1 and 50 mg, and more conveniently between 0.5 and 20 mg.

The pharmaceutical compositions prepared by using the compounds of formula (I') and suitable for fighting against the above indicated pathologies, comprise at least one product selected from the compounds of formula (I') and their pharmaceutically acceptable addition salts in combination with a pharmaceutically inert vehicle.

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They may be prepared according to conventional methods well-known in the galenic field.

The active principle may be incorporated into the excipients conventionally employed in these pharmaceutical compositions, such as talc, gum arabic, lactose, starch, magnesium stearate, aqueous or non-aqueous vehicles, animal or vegetable fats, paraffin derivatives, glycols, different wetting, dispersing, emulsifying or preservative agents, etc.

For oral administration, which is, anyway, the preferred route of administration, suitable pharmaceutical dosage forms comprise tablets, delayed release tablets, sugar coated tablets, gelatin capsules, suspensions, solutions or also liposomes.

As for the intravenous, subcutaneous or intramuscular administration, sterile or sterilisable solutions may be employed, whilst conventional suppositories, or gelatin capsules or microoenemas can be used for rectal administration.

For transdermal administration, conventional patches prepared according to techniques well known to the person skilled in the art can be employed.

Unit dosage forms for the new therapeutical use will typically comprise from 0.05 to 20 mg, preferably from 0.1 to 10 mg, including for instance from 0.5 to 5 mg (e.g. 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 mg of product). These unit dosage forms are typically administered once or more times a day, preferably from 1 to 3 times a day.

If desired, the pharmaceutical compositions of the present invention may also contain one or more other medicaments which are known and commonly employed for the same therapeutical indications.

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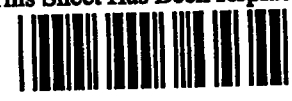

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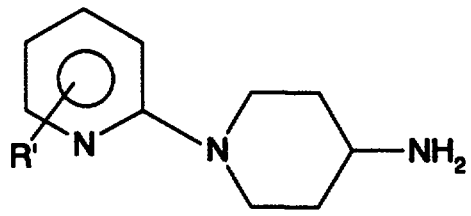
CLAIMS

1. Use of at least one compound of formula (I')



(I')

where R' represents hydrogen, a halogen atom, a methyl, trifluoromethyl, (C_1–C_3)alkoxy, trifluoromethoxy, 2,2,2–trifluoroethoxy, methylthio, trifluoromethylthio, or phenoxy group optionally substituted with a halogen atom, a (C_1–C_3)alkyl, (C_1–C_3)alkoxy, (C_1–C_3)alkylthio, trifluoromethyl or cyano group, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment and the prophylaxis of conditions deriving from a dysfunction of the serotoninergic systems in which a serotoninergic action selectively mediated by 5–HT_3 receptors is required.

2. Use according to claim 1, in which R' is at position 6– of the 2–pyridyl radical.

3. Use according to claim 1, in which R' represents a hydrogen atom, a halogen atom, a methyl or (C_1–C_3)alkoxy group at position 6–.

4. Use according to claim 3, in which the compound of formula (I') is selected from the group consisting of 4–amino–1–(6–chloro–2–pyridyl)piperidine, and 4–amino–1–(6–bromo–2–pyridyl)piperidine, 4–amino–1–(6–methyl–2–pyridyl)piperidine, 4–amino–1–(6–methoxy–2–pyridyl)piperidine and the pharmaceutically acceptable salts thereof.

5. Use according to claim 4, in which the compound of formula (I') is 4–amino–1–(6–chloro–2–pyridyl)piperidine or a pharmaceutically acceptable salt thereof.

6. Use according to claim 1, in which R' is a halogen atom at position 3– of the 2–pyridyl radical.

7. Use according to any one of claims 1 to 6, characterized in that it is a psychotropic medicament.

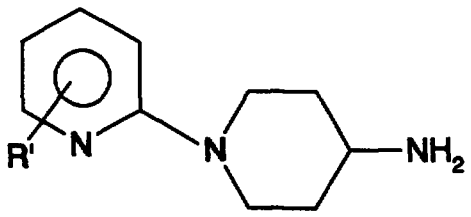
* C L M S *

8. Use according to claim 7, characterized in that the medicament is suitable for the treatment of dysthymic disorders, anxiety or psychotic disorders.

9. Use according to any one of claims 1 to 6, characterized in that the medicament is suitable for the treatment of intestinal motoricity disorders.

10. Use according to claim 9, characterized in that the medicament is suitable for the treatment of constipation.

11. Medicament with agonist action of the 5-HT_3 receptor comprising, as the active principle, at least one compound of formula (I')



(I')

where R' represents hydrogen, a halogen atom, a methyl, trifluoromethyl, (C_1-C_3)alkoxy, trifluoromethoxy, 2,2,2-trifluoroethoxy, methylthio, trifluoromethylthio, or phenoxy group optionally substituted with a halogen atom, a (C_1-C_3)alkyl, (C_1-C_3)alkoxy, (C_1-C_3)alkylthio, trifluoromethyl or cyano group, or a pharmaceutically acceptable salt thereof.

12. Medicament according to claim 11 in the form of a pharmaceutical composition containing from 0.05 to 20 mg, preferably from 0.5 to 5 mg, of active principle for each unit dosage form.

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