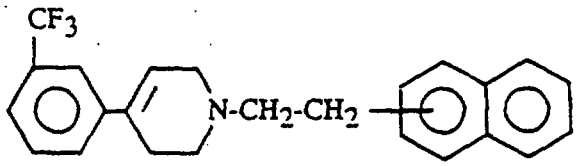
Use of a 1-(2-naphthylethyl)-4-(3-trifluoromethylphenyl)-1,2,3,6-tetrahydropyridine for the preparation of medicaments intended for the treatment of cerebral and neuronal disorders

The present invention relates to the use of a 1-(2-naphthylethyl)-4-(3-trifluoromethylphenyl)-1,2,3,6-tetrahydropyridine of the following formula:



(I)

or to the pharmaceutically acceptable acid addition salts thereof for the preparation of medicaments intended for the treatment and/or prophylaxis of cerebral and neuronal diseases.

More particularly, the present invention relates to the use of a compound of formula (I) or to the pharmaceutically acceptable addition salts thereof for the preparation of medicaments intended for the treatment of diseases associated with a neuronal degeneration.

In the above formula (I), the group 1-[4-(3-trifluoromethylphenyl)-1,2,3,6-tetrahydropyridinyl]ethyl may be bonded in the 1- or 2-position of the naphthalene. According to a preferred aspect of the present invention, this group is bonded in the 2-position of the naphthalene.

The compounds of formula (I), as free bases or addition salts, as well as their preparation, have been described in European patent application EP-A-101381. Among the pharmaceutically acceptable addition salts of the compound of formula (I), those formed with the mineral acids, such as for example hydrochloric, hydrobromic, phosphoric or sulphuric acids or with the organic acids such as acetic, formic, propionic, benzoic, maleic, succinic, tartaric, fumaric, citric, glyoxylic, aspartic, methanesulfonic, ethanesulfonic, benzenesulfonic, p-toluenesulfonic acids and the like may be cited.

In the above European patent application, an anorexigenic activity of the products is indicated.

It has now unexpectedly been found that the compounds of formula (I) do exert a neurotrophic action in the nervous system, similar to that of NGF (Nerve Growth Factor) and restore functioning of the nerve cells which are damaged or present anomalies in their physiological function. Such neurotrophic activity have been demonstrated at first by means of a neuritogenesis test in vitro.

In vitro pharmacological assessment

The neuritogenesis test in vitro has been carried out on isolated neurons which are obtained from dissections of the septal region of rat embryos by conventional procedures which afford enriched neuronal suspensions (from 95 to 98%). (S.E. Bottenstein: "Growth and differentiation of neural cells in defined media" in Cell Culture in the Neuroscience, p. 3–43, 1985, Ed. S.E. Bottenstein, G. Sato).

More particularly, the septal region of 17–day–old rat embryos was removed under a dissection microscope while keeping, throughout the removal procedure, the structures at 4°C in the following medium:

DME/F_{12} (v/v)	
glucose	5%
amphotericin B	1%
gentamicin	0.5%

The cells are dissociated by treatment with trypsin–EDTA at 37°C for 20 minutes, then centrifuged twice and washed with PBS. Dissociation is then completed with a pasteur pipette in Hanks' medium. This step is followed by three centrifugations and the obtained pellet is then taken up in a serum medium:

DME/F_{12} (v/v)	
34 mM KCl	
fetal calf serum	5%
horse serum	5%
glutamine	0.1%
amphotericin B	1%
gentamicin	0.5%

This cell suspension is placed into a culture flask and kept in the oven at 37°C under 5% CO_2 for 90 minutes.

Non–nerve cells soon stick to the plastic walls of the flask, thus affording a suspension enriched in neurons (95 to 98%). Said suspensions

were centrifuged and the pellets taken up in a serum–free culture medium (H. W. Muller and N. Seifert, J. Neurosc. Res., 8, 195–204, 1982):

DME/F_{12} (v/v)	
transferrin	1 μg/ml
triiodothyronine	3 mM
insulin	5 μg/ml
hydrocortisone	20 μM
glutamine	0.1%
amphotericin B	1%
gentamicin	0.5%

Neurons were cultured in 96 well plates (5 x 10^4 viable cells per well).

Each well was treated with poly–L–Lysine (10 μg/ml) in order to form a matrix which is necessary to neuronal adhesion, survival and differentiation. Aliquots (130 μl) of serum–free medium containing either suitably selected doses of the test compounds of formula (I) or the corresponding concentrations of the solvent of the product (dimethylsulfoxide) were distribued in the wells. After seeding the neurons, the plates were maintained in the oven (37°C – 5% CO_2) for 18 hours. After fixation in a glutaraldehyde/paraformaldehyde mixture, the cells were counted as follows:

- for a given microscopic field in each seeded well, the total number of observed cells is counted as well as the number of cells having at least one neurite (neurite=outgrowth) ≥ than twice the cell diameter,
- five fields for each well are counted and for each dose of test compound two wells are seeded, thus obtaining ten data for each dose,
- the results are expressed as percentage of cells with neurites relative to total counted cells. Each batch is compared to its control by means of a non–parametric Kruskall–Wallis analysis.

The results obtained with 1–[2–(2–naphthyl)ethyl]–4–(3–trifluoromethylphenyl)–1,2,3,6–tetrahydropyridine hydrochloride (Compound A) are as follows:

TABLE I

	percentage of cells with neurites
Compound A: 2.4 nM	44.8 ± 3.45
Control (DMSO 10^{-6})	30.2 ± 1.68
Compound A: 24 nM	39.2 ± 2.39
Control (DMSO 10^{-5})	29.4 ± 1.64
Compound A: 240 nM	44.1 ± 2.02
Control (DMSO 10^{-4})	34.3 ± 1.82

NGF in the same test, gave the following results:

	percentage of cells with neurites
NGF: 1.6 nM	51.7 ± 1.61
Control	40.6 ± 2.08

The mechanism through which Compound A elicits said neurotrophic activity has not been cleared up.

Anyway it may be excluded that a serotoninergic effect is involved because Compound A has no affinity for the different serotonine receptors others than the receptor 5-HT_{1A} (i.e. the receptors 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2, and 5-HT_3) and moreover, compounds known as 5-HT_{1A} agonists or partial agonists, including buspirone, ipsapirone, and 8-hydroxy-2-(di-n-propylamino)tetralin (8OH-DPAT) showed to be completely inactive in the above model.

Compound A also is very active (at concentrations ranging from 250 nM to 2.5 μM) in a protection test of the neuronal survival in which the neurons are placed in a very poor medium free from growth factors.

In vivo pharmacological assessment

To confirm the significance of the above in vitro very positive results, a new experimental model has been set up which allows the in vivo assessment of the neurotrophic/neuroprotective activity of the compounds of formula (I) in neuronal degeneration processes.

An experimental model for this type of assessment has recently been proposed by Y. Nakagawa et al (Brain research, 1987, 408, 57–64).

In the study conducted by these authors, the resemblance between the neurochemical and behavioural modifications caused by injection of a neurotoxicant, in particular colchicine, in the hippocampus and those observed in patients affected by Alzheimer's disease has been revealed. On the basis of the results published by Y. Nakagawa and bearing in mind the bibliographical evidences of the role played by the hippocampus in mnemonic phenomena, it has been attempted to set up an experimental model of improved feasibility and even closer to the physiopathology found in patients with Alzheimer's disease.

The model which is herein provided complies with the following requirements: good feasibility and irreversible lesions highly specific for the septohippocampal cholinergic system.

In particular, lesions of the septal neurons have been caused by local injection of vincristine as a tubuline polymerisation inhibitor.

With respect to other similar products (colchicine and vinblastine) and different injection sites (intraventricular and intrahippocampal) which have been tested, optimum results, both in terms of specific blockade of the septohippocampal cholinergic route and irreversible lesions, have been obtained.

EXPERIMENTAL PROCEDURES

Method for producing lesions

The animals (male Sprague–Dawley rats weighing about 250 g) are anesthetised with pentobarbital (10 mg/kg i.p.) and placed in a stereotaxic apparatus. The injection in the medial septum is made at the following coordinates which are calculated according to the atlas of Paxinos and Watson:

anteriority	8.9
laterality	0
ventrality	6.4

(the point 0 corresponds to lambda)

Vincristine is dissolved in artificial cerebrospinal fluid (ACSF) having the following composition:

NaCl	150 mM
$CaCl_2$	1.8 mM

$MgSO_4$	1.2 mM
K_2HPO_4	2 mM
glucose	10 mM
pH 7.4	

at a concentration of 0.6 µmole of vincristine per ml.

1 µl of this solution (0.6 nmole of vincristine) is locally injected in the medial septum over 1 minute.

Method for assessing the lesions

- **Morphological assessment (histoenzymologic observation of AchE)**

The animals are perfused with a fixative mixture (glutaraldehyde/paraformaldehyde) via the aorta, with a perfusion rate of 25 ml/min for 5 minutes.

The brain is removed, fixation being continued for 1 hour, then washed and cryoprotected with 20% sucrose in phosphate buffer. The brain is then cut on a cryostat and the sections (30 µm thick) are removed at the level of the septum and the hippocampus and mounted on slides which are incubated for about 15 hours in the following mixture:

200 ml of a stock solution:

925 ml	distilled water
781 mg	CuSO4
750 mg	glycine
2.89 g	sodium acetate

to which are added just before use:

230 mg	acetylthiocholine iodide
10 mg	ethopropazine.

The reaction is then developed by passing in 2% ammonium sulfide and amplified by 0.25 % $AgNO_3$.

The acetylcholinesterase sites (generally associated with cholinergic synapses) are developed in brown precipitate form.

- **Biochemical assessment (determination of the ChAT activity)**

ChAT activity is determined according to the method described by Fonnum (J. Neurochem., 24, 1975, 407–409). The tissues are homogenised in a potter (at 4°C). Each sample is brought to a concentration of 1 mg of protein per ml. 10 µl of homogenate are incubated for 7 minutes 30 seconds at 37°C, in the presence of choline

(1.5 mM), acetylCoA (70 μM), ^{14}C–acetylCoA (30 μM) and eserine (0.15 mM).

The reaction is stopped by means of an ice–bath and adding phosphate buffer (5 ml).

After addition of tetraphenylboron/acetone (2 ml) and of a scintillating agent (5 ml), ^{14}C–acetylcholine is counted in a scintillation counter. Each sample is tested three times. Each batch is compared with the corresponding control batch by the Student test.

- **Behavioural assessment**

This study deals with groups of animals reserved for this purpose, the rats having been put in an inversed cycle. The rats used in these tests are Winstar rats lesioned as described above.

- **Social memory**

(A. Perio et al., Psychopharmacology, 1989, 97, 262–268). In this test a juvenile rat is placed in the cage of an adult rat and the duration of the social relationship is measured in seconds (T1). The animals are then separated for 15 minutes and then the adult rat is again exposed to the same juvenile rat and the time of contact (T2) is also measured. In the case of normal animals, the recognition of the first meeting gives a ratio of T2/T1<1. When there is a memory impairment the T2/T1 ratio is $\geq$1.

- **T–maze test**

This test has been carried out according to the method described by P. Soubrié et al., in J. Pharmacol. (Paris), 1977, 8, 3, 393–403 for the Y–maze test.

- **Holeboard test**

(S.E. File et al., Pharmacol. Biochem. Behav., 1985, 22, 941–944).

Assessment of the lesions

Intraseptal vincristine administration affords a rapid and significant drop (from 60 to 70 % within one week after the injection) of the cholinergic markers of the hippocampus (choline acetyltransferase (ChAT) and acetylcholine esterase (AChE), as well as a degeneration of the medial septum neurons which reaches its maximum two weeks after the injection, and which is associated with a reduction of the cholinergic markers. Said degeneration seems to be irreversible as it is still present three months after vincristine injection and involves functional disorders.

Among those functional alterations which result from vincristine administration, the major finding is a consistent and irreversible deficiency of the mnemonic functions (social memory test). Parallel assessments have revealed i.a. a reduction of the explorative capabilities ("T-maze" and "holeboard")

TREATMENTS

In this model, the effects of administering Compound A to the lesioned animals have been assessed by comparison with those obtained by administering NGF.

Compound A is administered orally, 2 to 3 hours after vincristine injection, as a 1% carboxymethylcellulose suspension, at a rate of 10 ml of suspension per kg. The control batch is treated with the vehicle. The treatment is chronic, the product is administered once a day for 11 days. Compound A is administered at three different doses: 2.5 mg/kg, 5 mg/kg, and 10 mg/kg to groups of 8 animals each, and the animals are sacrificed 24 hours after the end of the treatment.

On the contrary NGF is administered by intraventricular perfusion, dissolved in ACSF containing 0.01 % rat albumin and gentamicin (1 ml/15 ml) according to the method described by W. Fisher et al. in Nature, 1987, 329 (6134), 65-68. The NGF concentration in the solution is calculated in view of the diffusion flow rate (0.44 $\pm$ 0.02 μl/h) so as to provide the animals (7 rats) with an overall amount of 0.105 μg, 1.05 μg, or 10.5 μg of NGF over two weeks of perfusion. In the control batch, NGF is replaced by a protein of similar molecular weight (about 130,000) which has no neurotrophic activity: cytochrome C. Two weeks after the lesion and the beginning of the treatment, the animals are sacrificed. One group of sacrificed animals is perfused for histoenzymologic assessment of AChE, the other animals are employed for the assay of ChAT in both hippocampus and septum.

RESULTS

Morphological assessment

In the lesioned, untreated, animals almost no brown precipitate is seen while the hippocampal buddings observed in the animals treated with 5 mg/kg of Compound A give an image of the structure which is quite similar to that seen in normal animals. These results are analogous to those obtained in the NGF-treated animals.

Biochemical assessment

The lesion causes a significant drop in the ChAT activity in the hippocampus whose increase depends on the dose of the administered Compound A, up to a complete recovery at the dose of 10 mg/kg. Analogous results are obtained in the group of NGF–perfused animals. The results are summarized in the following table

TABLE II

	ChAT activity (pmol/mg/mn)
non–lesioned normal animals	277 ± 13
lesioned controls	115 ±18
NGF 0.105 μg/rat/2 weeks	168 ± 27
NGF 1.05 μg/rat/2 weeks	336 ± 28
NGF 10.05 μg/rat/2 weeks	293 ± 10
non–lesioned normal animals	242 ± 19
lesioned controls	97 ± 18
Compound A 2.5 mg/kg/day	164 ± 42
Compound A 5 mg/kg/day	204 ± 24
Compound A 10 mg/kg/day	242 ± 27

In the septum, the lesion causes a drop in the ChAT activity whose recovery depends on the dose of the administered Compound A. In the NGF–perfused animals the results are not significant probably because in addition to the septal necrosis produced by the vincristine injection, an additional necrosis associated with the implantation of a cannula in a ventricle near the septum develops.

Behavioural assessment

- **Social memory**

The vincristine–lesioned animals show alterations in social memory which seem to be irreversible (said alterations are still present 50 days after the vincristine injection). Compound A has been tested at the dose of 10 mg/kg per os in comparison with NGF at the dose of 10.5 μg and the test has been performed on day 7 from the lesion. In both cases a

very important protecting effect has been observed with a T2/T1 ratio comprised between 0.6 and 0.7.

- **T–maze**

  The results obtained in the lesioned, untreated, animals are significant of an alteration in the exploratory capacities which are however restored to the normal level by treatment with Compound A at the dose of 10 mg/kg. This test has been carried out blind, 7 days after the end of the treatment.

- **Holeboard**

  In the control batch, most of the animals (6 rats) completely lost their exploratory capacity, while only two rats showed an exploratory hyperactivity. Treatment with Compound A at the dose of 10 mg/kg leads to a normalisation of the explorative behaviour compared with the average results obtained with normal animals. Said test too has been carried out blind, 11 days after the end of the treatment.

In the light of the results obtained in this model, the use of the compounds of formula (I), as well as of their pharmaceutically acceptable addition salts in the treatment and/or prophylaxis of diseases involving neuronal degeneration can be envisaged. More particularly the compounds of formula (I) as well as their pharmaceutically acceptable addition salts can be employed mainly in the following indications: memory disorders, vascular dementia, post–encephalitic disorders, post–apoplectic disorders, post–traumatic syndromes caused by injury to the head, disorders associated with cerebral anoxia, Alzheimer's disease, senile dementia, sub–cortical dementia, such as Huntington's chorea and Parkinson's disease, AIDS dementia, neuropathies caused by morbidity or lesions of the sympathetic or sensory nerves and cerebral diseases such as cerebral oedema, and spinocerebellar degenerations.

The compounds of formula (I) or their pharmaceutically acceptable addition salts may advantageously be administered orally, parenterally, sublingually or transdermally. The amount of active principle to be administered in the treatment of cerebral and neuronal disorders according to the method of the present invention will vary, as usually, depending on the nature and severity of the disease to be treated as well as on the weight of the patients. Nevertheless, preferred unit dosages will contain from 2 to 300 mg, preferably from 5 to 150 mg, comprised by way of

example between 5 and 50 mg, e.g. 5, 10, 20, 30, 40, and 50 mg, of product. Said unit dosages are generally administered one or more times a day, e.g. 2, 3, 4, or 5 times a day, preferably from 1 to 3 times a day, the overall daily dosage in humans being from 2 to 900 mg a day, typically from 3 to 500 mg, more advantageously from 10 to 300 mg a day.

For a therapeutic and/or prophylactic use according to the present invention, the compounds of formula (I) and their pharmaceutically acceptable addition salts are preferably formulated in pharmaceutical compositions. The pharmaceutical compositions of the invention contain an amount of at least one product selected from the compounds of formula (I) and their pharmaceutically acceptable addition salts which is effective for the treatment or prophylaxis of cerebral and neuronal disorders, in admixture with an inert pharmaceutical vehicle.

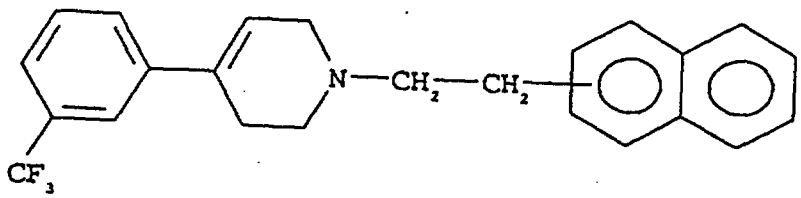
As for the oral or sublingual administration, in particular tablets, optionally sugar-coated, granule capsules, optionally with a delayed release, drops or liposomes may be used. As for the intravenous, subcutaneous, or intramuscular administration, sterile or sterilisable solutions are employed, while conventional patches for transdermal administration can be used.

The pharmaceutical compositions according to the present invention can be prepared by conventional techniques such as those described in EP-101381 or in Remington's Pharmaceutical Sciences, 18th Ed, Mack Publishing Company. The active principle may be incorporated therein to excipients usually employed in said pharmaceutical compositions, such as talc, arabic gum, lactose, starch, magnesium stearate, aqueous or non aqueous vehicles, animal or vegetable fats, paraffin derivatives, glycols, wetting, dispersing or emulsifying agents, preservatives, etc.

The pharmaceutical compositions according to the invention may advantageously contain a compound of formula (I) or a pharmaceutically acceptable addition salt thereof in association with one or more other medicaments, known and commonly employed for the same therapeutic indications.

CLAIMS

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



(I)

or of a pharmaceutically acceptable acid addition salt thereof for the preparation of medicaments intended for the treatment and/or the prophylaxis of cerebral and neuronal diseases.

2. Use according to claim 1 for the preparation of medicaments intended for the treatment and/or the prophylaxis of diseases which involve neuronal degeneration.

3. Use according to claim 2 for the preparation of medicaments intended for the treatment and/or the prophylaxis of memory disorders, vascular dementia, post-encephalitic disorders, post-apoplectic disorders, post-traumatic syndromes caused by injury to the head, disorders associated with cerebral anoxia, Alzheimer's disease, senile dementia, sub-cortical dementia, such as Huntington's chorea and Parkinson's disease, AIDS dementia, neuropathies caused by morbidity or lesions of the sympathetic or sensory nerves, cerebral oedema or spinocerebellar degenerations.

4. Use according to claim 3 for the preparation of medicaments intended for the treatment of vascular dementia, senile dementia or Alzheimer's disease.

5. Use according to any one of claims 1 to 4, in which the compound of formula (I) is chosen from 1-[2-(2-naphthyl)ethyl]-4-(3-trifluoromethylphenyl)-1,2,3,6-tetrahydropyridine and the pharmaceutically acceptable acid addition salts thereof.

6. Use according to any one of claims 1 to 5, where the medicament is a pharmaceutical composition in unit dosage form.

7. Use according to claim 6, in which the unit dosage form comprises from 2 to 300 mg of active principle.

* C L M S *

8. Use according to claim 7, in which the unit dosage form comprises from 5 to 150 mg of active principle.

9. Use according to claim 8, in which the unit dosage form comprises from 5 to 50 mg of active principle.

* C L M S *