

HYPERFORIN DERIVATIVES AND THEIR USE IN ALZHEIMER'S DISEASE

Field of invention

The present invention relates to hyperforin derivatives, their use in the pharmaceutical and/or nutritional field, in particular for the prevention and treatment of Alzheimer's disease, and pharmaceutical formulations containing said
5 derivatives.

Technical background

The flowering tops of *Hypericum perforatum* (St John's wort) contain a large number of structurally different substances which act directly or indirectly on the central nervous system. Among said substances, hyperforin, a phloroglucinol
10 derivative, is one of the main constituents, together with adhyperforin, of the lipophilic fraction obtained from the flowering tops of the plant (Erdelmeier C.A.J., Pharmacopsychiatry 31, 2, 1998).

Hyperforin has formed the subject of numerous studies, which have demonstrated a potent antidepressant activity (Laakman G. *et al.*,
15 Pharmacopsychiatry 31, 54, 1998; Butterweck V. *et al.*, Life Science 73, 627, 2003).

Moreover, salts with inorganic or ammonium cations of hyperforin and adhyperforin have been described as having an important action for the prophylaxis and treatment of Alzheimer's disease (WO99/41220).

20 It is also known from the literature that hyperforin is highly unstable under the usual extraction and storage conditions, and derivatives have been devised to improve its stability (WO99/41220, WO99/64388).

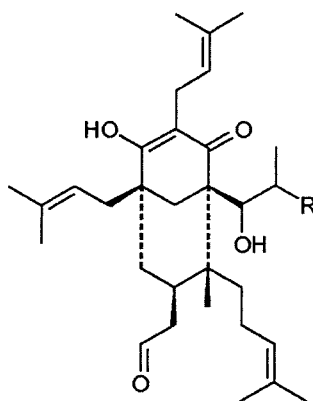
In particular, more stable hyperforin and adhyperforin derivatives have been developed by total reduction of the double bonds of the isoprene chains and
25 reduction to hydroxyl groups of the keto groups in the 1 and 10 positions (Bystrov

N.S. *et al.*, Bioorg. Khim. 4, 791, 1978). These derivatives have proved not only more stable, but also much more effective as antidepressants, anxiolytics and anti-neurodegenerative drugs (WO03/091194).

It has now surprisingly been found that the hyperforin and adhyperforin derivatives obtainable by reduction to hydroxy groups of the ketones in the 1 and 10 position described in WO03/091194 can in turn, by hydroxylation followed by deisopropylation, give rise to novel products which cross the blood-brain barrier more efficiently and inhibit neuropathological damage induced by A β fibrils in different experimental models.

Description of the invention

The present invention relates to the following hyperforin and adhyperforin derivatives of formula a) and b) respectively:



a: R = CH₃
b: R = CH₂CH₃

Compound a) is deisopropyl-dehydro tetrahydro hyperforin.

Compound b) is deisopropyl-dehydro tetrahydro adhyperforin.

The two compounds can be used in the medical and/or nutritional field, in particular for the treatment of Alzheimer's disease. The products according to the invention have proved able to penetrate the blood-brain barrier, and are particularly effective in inhibiting neuropathological damage, such as that which develops in Alzheimer's disease.

The subject of the present invention is therefore the use of said compounds in the prevention and treatment of Alzheimer's disease.

A further subject of the present invention is pharmaceutical formulations containing the compounds of formulas a) and b). Said formulations can, for example, take the form of soft gelatin capsules, hard gelatin capsules, tablets, suppositories and controlled-release formulations, prepared by known methods such as those reported in Remington's Pharmaceutical Sciences Handbook, 17th ed., Mack Pub., NY, USA.

The preferred pharmaceutical formulations are soft or hard gelatin capsules, tablets and transdermal patches.

In the latter case, controlled-release compounds can be administered by applying the patch in the area proximal to the arterial branches of the cerebral carotids.

The dose of the compounds in the formulations can range between 10 and 100 mg/dose/day.

Example 1

Determination of the plasma and brain levels of hyperforin and the derivatives according to the invention in mice treated subacutely by intraperitoneal administration.

In mice treated subacutely (twice a day for 4 days) by intraperitoneal administration at the dose of 20 mg/kg of the products according to the invention, their brain and plasma levels were determined by a combined HPLC/MS/MS analysis technique developed on the basis of a method already described by Keller J.H. *et al.* (Anal. Chem. 75, 6084, 2003).

The results set out in Table 1 below demonstrate the presence of the products according to the invention in the brain. The levels observed exceed those measured after administration of an equal dose of hyperforin, this finding being in line with those reported in the literature (Keller J.H. *et al.*, Anal. Chem. 75, 6084,

2003; Rozio M. *et al.*, J. Chromatogr. B. 816, 21, 2005).

Table 1

Plasma and brain levels of hyperforin and the derivatives according to the invention in mice treated subacutely (twice a day for 4 days) by intraperitoneal administration with 20 mg/kg

Product	Time (h)	Plasma (ng/ml)	Brain (ng/g)	Brain/Plasma %
Hyperforin	0	624.5±81.8	15.2±16.1	1.89±0.91
	1	1547.2±226.6	21.1±7.4	1.24±0.75
	2	1421.3±235.4	23.2±7.8	1.32±0.82
	4	1275.4±212.8	32.3±8.1	1.91±0.91
	6	1192.6±196.5	35.6±8.8	2.12±1.12
Deisopropyl-dehydro tetrahydro hyperforin (a)	0	1339.5±283.8	62.1±34.6	3.89±1.33
	1	4270.0±1237.8	78.5±16.2	2.76±1.62
	2	2947.8±961.6	63.1±9.8	2.93±0.95
	4	2870.0±426.2	256.6±112.4	12.24±7.64
	6	2235.0±264.8	367.3±76.6	17.04±1.37
Deisopropyl-dehydro tetrahydro adhyperforin (b)	0	1262.4±272.3	59.1±28.5	3.78±1.25
	1	3971.2±984.5	61.4±19.6	1.51±0.96
	2	2756.4±622.3	60.2±21.4	2.05±1.71
	4	2564.1±531.4	195.6±60.6	6.51±3.25
	6	2120.4±410.6	251.8±71.2	10.56±6.25

Each value is the mean ± S.E. of 4 animals. Time 0 corresponds to the 14th hour after the previous treatment

Example 2

Effect of hyperforin and the derivatives according to the invention on neuropathological damage induced by A β *in vivo*

The products according to the invention have proved to be particularly effective in inhibiting neuropathological damage induced by A β fibrils *in vivo*. To evaluate the potential neuroprotective effect of the products according to the invention, male rats were treated stereotactically in the dorsal hippocampus with

80 µg of Aβ fibrils in the presence or absence of the products. The injection of Aβ fibrils produces proliferation and an increase in astrocyte density, an increase in the soma and GFAP staining in the astrocytes present around the injection site.

The results set out in Table 2 below demonstrate that co-administration of the products according to the invention with Aβ fibrils significantly reduces astrocyte proliferation (compared with the group treated with Aβ fibrils alone), reduces GFAP staining and completely abolishes enlargement of the astrocytic perikaryon.

Table 2

The effect of hyperforin and the derivatives according to the invention on neuropathological damage induced by Ap *in vivo*

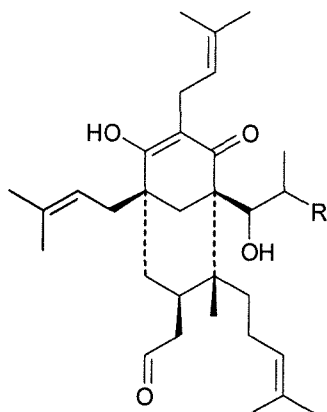
Product	Density of reactive astrocytes (GFAP ⁺ cell./4x10 ³ µm ²)	GFP intensity of the astrocyte soma (arbitrary unit)	Measurement of astrocyte soma (arbitrary unit)	Number of neurones in the dentate gyrus (cell./4x10 ³ mm ²)
Control	11±4	134±11	101±18	119±19
Aβ (fibrils only)	39±7	259±9	310±16	58±7
Hyperforin (+ fibrils)	37±6	248±7	306±14	62±8
Deisopropyl-dehydro tetrahydro hyperforin (+ fibrils)	22±3*	150±19*	105±8*	161±14**
Deisopropyl-dehydro tetrahydro adhyperforin (+ fibrils)	24±4	165±17*	117±9*	121±11*

The β -amyloid fibrils were injected stereotactically into the hippocampus, either alone or in combination with hyperforin and the derivatives according to the invention.

* $p < 0.05$; ** $p < 0.001$ [vs $A\beta$ (fibrils only)].

CLAIMS

1. A compound having the following formula a) or b):



a: R = CH₃

b: R = CH₂CH₃

5

2. The compound according to claim 1 for use as a medicament.
3. The compound according to claim 2 for use in the prevention and/or treatment of Alzheimer's disease.
- 10 4. A pharmaceutical composition containing the compound of formula a) or b) as claimed in claim 1 and a pharmaceutically acceptable excipient and/or carrier.
5. Use of the compound according to claim 1 in the manufacture of a medicament
- 15 for the prevention and/or treatment of Alzheimer's disease.