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(54) Title: R-ENANTIOMER OF N-PROPARGYL-1-AMINOINDAN, SALTS, COMPOSITIONS AND USES THEREOF (57) Abstract <p>The subject invention provides R(+)-N-propargyl-1-aminoindan and pharmaceutically acceptable salts thereof, as well as pharmaceutical compositions containing same. The subject invention also provides methods of treating a subject afflicted with Parkinson's disease, a memory disorder, dementia, depression, hyperactive syndrome, an affective illness, a neurodegenerative disease, a neurotoxic injury, brain ischemia, a head trauma injury, a spinal trauma injury, schizophrenia, an attention deficit disorder, multiple sclerosis, or withdrawal symptoms, using R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt of the subject invention. The subject invention further provides a method of preventing nerve damage in a subject. Finally, the subject invention provides methods of preparing R(+)-N-propargyl-1-aminoindan, a salt thereof, and racemic N-propargyl-1-aminoindan.</p>		

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5 R-ENANTIOMER OF N-PROPARGYL-1-AMINOINDAN, SALTS, COMPOSITIONS AND USES THEREOF

This is a continuation of U.S. Application Serial No. 08/139,517, filed October 18, 1993, the contents of which
10 are hereby incorporated by reference.

Background of the Invention

I.

15 The subject invention is in the field of selective irreversible inhibitors of the enzyme monoamine oxidase (hereinafter MAO) and provides the R(+) enantiomer of N-propargyl-1-aminoindan (also referred to herein as PAI) which is a selective irreversible inhibitor of the B-form of
20 monoamine oxidase enzyme (hereinafter MAO-B). The subject invention also provides pharmaceutical compositions containing [R](+)PAI which are particularly useful for the treatment of Parkinson's disease, a memory disorder, dementia, depression, hyperactive syndrome, an affective
25 illness, a neurodegenerative disease, a neurotoxic injury, brain ischemia, a head trauma injury, a spinal trauma injury, schizophrenia, an attention deficit disorder, multiple sclerosis, and withdrawal symptoms.

30 II.

Parkinson's disease is widely considered to be the result of degradation of the pre-synaptic dopaminergic neurons in the brain, with a subsequent decrease in the amount of the neurotransmitter dopamine being released. Inadequate
35 dopamine release, therefore, leads to the onset of disturbances of voluntary muscle control, which disturbances are symptomatic of Parkinson's disease.

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Various methods of treating Parkinson's disease have been established and are currently in widespread use, including, for example, the administration of L-DOPA together with a decarboxylase inhibitor such as L-carbidopa or benserazide.

5 The decarboxylase inhibitor protects the L-DOPA molecule from peripheral decarboxylation and thus ensures L-DOPA uptake by the remaining dopaminergic neurons in the striatum of the brain. Here, the L-DOPA is converted into dopamine resulting in increased levels of dopamine in these neurons.
10 In response to physiological impulses, these neurons are therefore capable of releasing larger amounts of dopamine at levels which approximate the normal required levels. L-DOPA treatment thus alleviates the symptoms of the disease and contributes to the well-being of the patient.

15 However, L-DOPA treatment has its drawbacks, the main one being that its effectiveness is optimal only during the first few years of treatment. After this period, the clinical response diminishes and is accompanied by adverse
20 side effects which include dyskinesia, fluctuation in efficacy throughout the day ("on-off effect") and psychiatric symptoms such as confusional states, paranoia, and hallucinations. This decrease in the effect of L-DOPA treatment is attributed to a number of factors, including
25 the natural progression of the disease, alteration in dopamine receptors as a consequence of increased dopamine production or increased levels of dopamine metabolites, and pharmacokinetic problems of L-DOPA absorption (reviewed by Youdim, et al., Progress in Medicinal Chemistry, 21, 138-167
30 (1984)).

In order to overcome the drawbacks of L-DOPA treatment, various treatments have been devised in which L-DOPA is combined with MAO inhibitors with the aim of reducing the
35 metabolic breakdown of newly formed dopamine (see for

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example, Chiese, P., U.S. Patent No. 4,826,875, issued May 2, 1989).

MAO exists in two forms known as MAO-A and MAO-B which are selective for different substrates and inhibitors. For example, MAO-B more efficiently metabolizes substrates such as 2-phenylethylamine, and is selectively and irreversibly inhibited by (-)-deprenyl as described below.

It should be noted, however, that treatments combining L-DOPA with an inhibitor of both MAO-A and MAO-B are undesirable, as they lead to adverse side effects related to an increased level of catecholamines throughout the neuraxis. Furthermore, complete inhibition of MAO is also undesirable as it potentiates the action of sympathomimetic amines such as tyramine, leading to the so-called "cheese effect" (reviewed by Youdim et al., Handbook of Experimental Pharmacology, ed. by Trendelenburg and Weiner, Springer-Verlag, 90, ch. 3 (1988)). As MAO-B was shown to be the predominant form of MAO in the brain, selective inhibitors for this form are thus considered to be a possible tool for achieving a decrease in dopamine breakdown on the one hand, together with a minimization of the systemic effects of total MAO inhibition on the other.

Many inhibitors of MAO are chiral molecules. Although one enantiomer often shows some stereoselectivity in relative potency towards MAO-A and -B, a given enantiomeric configuration is not always more selective than its mirror image isomer in discriminating between MAO-A and MAO-B.

Table I lists the IC_{50} (mmol/L) of enantiomeric pairs of propargyl amines in a rat brain preparation of MAO. These results show small differences in potency in MAO-B

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inhibition between the R and S enantiomers. (B. Hazelhoff, et al., Naunyn-Schmeideberg's Arch. Pharmacol., 330, 50 (1985)). Both enantiomers are selective for MAO-B. In 1967, Magyar, et al. reported that R-(-)-deprenyl is 500 times more potent than the S-(+) enantiomer in inhibiting the oxidative deamination of tyramine by rat brain homogenate. (K. Magyar, et al., Act. Physiol. Acad. Sci., Hung., 32, 377 (1967)).

- 10 In rat liver homogenate, R-deprenyl is only 15 times as potent as the S enantiomer. In other pharmacological activity assays, such as for the inhibition of tyramine uptake, deprenyl shows different stereoselectivities. The S form is in certain cases the more potent epimer. (J. Knoll and K. Magyar, Advances in Biochemical Psychopharmacology, 5, 393 (1972)).

N-Methyl-N-propargyl-1-aminotetralin (2-MPAT) is a close structural analogue of deprenyl. The absolute stereochemistry of 2-MPAT has not been assigned. However, the (+) isomer is selective for MAO-B and the (-) isomer is selective for MAO-A. The difference in potency between the 2-MPAT enantiomers is less than 5-fold. (B. Hazelhoff, et al., id.). The enantiomers of N-propargyl-1-aminotetralin (1-PAT) are also similar in activity. The lack of data in Table I showing clear structure-activity relationships between isolated (+) or (-)-2-MPAT makes it impossible to predict the absolute stereochemistry thereof.

- 30 After extensive computer modeling, Polymeropoulos recently predicted that (R)-N-methyl-N-propargyl-1-aminoindan (R-1-MPAI) would be more potent than (S) as a MAO-B inhibitor. (E. Polymeropoulos, Inhibitors of Monoamine Oxidase B, I. Szelenyi, ed., Birkhauser Verlag, p. 110 (1993)). However, experiments described show that R-1-MPAI is a slightly more

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potent inhibitor of MAO-B than S-1-MPAI, but is an even more potent inhibitor of MAO-A. Both the selectivity between MAO-A and -B and the relative potency of the R and S epimers are low. Thus, contrary to expectations in the art, 1-MPAI
5 is useless as a pharmaceutical agent.

The data presented below demonstrate that high selectivity for MAO of one enantiomer versus the other cannot be predicted. The structure of the MAO active site is not well
10 enough understood to permit the prediction of the relative potency or selectivity of any given compound or pair of enantiomers thereof.

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Table IA IC₅₀ (mmol/L) Data for Rat Brain MAO Inhibition by Propargylamines

COMPOUND	REF	EPIMER	INHIBITION		RELATIVE POTENCY A/B	POTENCY +/-	
			A	B		A	B
2-MPAI	a	+	140	16	8.8		
		-	46	88	0.5	3	0.2
DEPRENYL	a	S	3600	16	120		R/S
		R	450	6	75	80	2.6
1-MPAI	b	S	70	50	1.4		
		R	3	10	0.3	23	5
1-PAT	c	S	3800	50	76		
		R	900	90	10	4	0.5

a. B. Hazelhoff, et al., Naunyn-Schmeideberg's Arch. Pharmacol., 330, 50 (1985).

b. European Patent Application 436,492 A2, published July 10, 1991.

c. Present inventors.

One selective MAO-B inhibitor, (-)-deprenyl, has been extensively studied and used as a MAO-B inhibitor to augment L-DOPA treatment. This treatment with (-)-deprenyl is generally favorable and does not cause the "cheese effect" at doses causing nearly complete inhibition of MAO-B

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(Elsworth, et al., Psychopharmacology, 57, 33 (1978)). Furthermore, the addition of (-)-deprenyl to a combination of L-DOPA and a decarboxylase inhibitor administered to Parkinson's patients leads to improvements in akinesia and overall functional capacity, as well as the elimination of "on-off" type fluctuations (reviewed by Birkmayer & Riederer in "Parkinson's Disease," Springer-Verlag, pp. 138-149 (1983)). Thus, (-)-deprenyl (a) enhances and prolongs the effect of L-DOPA, and (b) does not increase the adverse effects of L-DOPA treatment.

However, (-)-deprenyl is not without its own adverse side effects, which include activation of pre-existing gastric ulcers and occasional hypertensive episodes. Furthermore, (-)-deprenyl is an amphetamine derivative and is metabolized to amphetamine and methamphetamines, which substances may lead to undesirable side effects such as increased heart rate (Simpson, Biochemical Pharmacology, 27, 1951 (1978); Finberg, et al., in "Monoamine Oxidase Inhibitors - The State of the Art," Youdim and Paykel, eds., Wiley, pp. 31-43 (1981)).

Other compounds have been described that are selective irreversible inhibitors of MAO-B but which are free of the undesirable effects associated with (-)-deprenyl. One such compound, namely N-propargyl-1-aminoindan·HCl (racemic PAI·HCl), was described in GB 1,003,686 and GB 1,037,014 and U.S. Patent No. 3,513,244, issued May 19, 1970. Racemic PAI·HCl is a potent, selective, irreversible inhibitor of MAO-B, is not metabolized to amphetamines, and does not give rise to unwanted sympathomimetic effects.

In comparative animal tests, racemic PAI was shown to have considerable advantages over (-)-deprenyl. For example,

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racemic PAI produces no significant tachycardia, does not increase blood pressure (effects produced by doses of 5mg/kg of (-)-deprenyl), and does not lead to contraction of nictitating membrane or to an increase in heart rate at
5 doses of up to 5mg/kg (effects caused by (-)-deprenyl at doses over 0.5 mg/kg). Furthermore, racemic PAI·HCl does not potentiate the cardiovascular effects of tyramine (Finberg, et al., in "Enzymes and Neurotransmitters in Mental Disease," pp. 205-219 (1980), Usdin, et al., Eds.,
10 Wiley, New York; Finberg, et al. (1981), in "Monoamine Oxidase Inhibitors - The State of the Art," *ibid.*; Finberg and Youdim, British Journal Pharmacol., 85, 451 (1985)).

One underlying object of this invention was to separate the
15 racemic PAI compounds and to obtain an enantiomer with MAO-B inhibition activity which would be free of any undesirable side effects associated with the other enantiomer.

Since deprenyl has a similar structure to PAI and it is
20 known that the (-)-enantiomer of deprenyl, i.e. (-)-deprenyl, is considerably more pharmaceutically active than the (+)-enantiomer, the (-) enantiomer of PAI would be expected to be the more active MAO-B inhibitor.

25 However, contrary to such expectations, upon resolution of the enantiomers, it was found that the (+)-PAI enantiomer is in fact the active MAO-B inhibitor while the (-)-enantiomer shows extremely low MAO-B inhibitory activity. Furthermore, the (+)-PAI enantiomer also has a degree of selectivity for
30 MAO-B inhibition surprisingly higher than that of the corresponding racemic form, and should thus have fewer undesirable side effects in the treatment of the indicated diseases than would the racemic mixture. These findings are based on both in vitro and in vivo experiments as discussed
35 in greater detail infra.

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It was subsequently shown that (+)-PAI has the R absolute configuration. This finding was also surprising based on the expected structural similarity of (+)-PAI analogy with deprenyl and the amphetamines.

5 The high degree of stereoselectivity of pharmacological activity between [R](+)PAI and the S(-) enantiomer as discussed hereinbelow is also remarkable. The compound [R](+)PAI is nearly four orders of magnitude more active
10 than the S(-) enantiomer in MAO-B inhibition. This ratio is significantly higher than that observed between the two deprenyl enantiomers (Knoll and Magyar, Adv. Biochem. Psychopharmacol., 5, 393 (1972); Magyar, et al., Acta Physiol. Acad. Sci. Hung., 32, 377 (1967)). Furthermore, in
15 some physiological tests, (+)-deprenyl was reported to have activity equal to or even higher than that of the (-) enantiomer (Tekes, et al., Pol. J. Pharmacol. Pharm., 40, 653 (1988)).

20 MPAI is a more potent inhibitor of MAO activity, but with lower selectivity for MAO-B over A (Tipton, et al., Biochem. Pharmacol., 31, 1250 (1982)). As only a small degree of difference in the relative activities of the two resolved enantiomers was surprisingly observed with MPAI, the
25 remarkable behavior of [R](+)PAI is further emphasized (See Table 1B).

The subject invention also provides methods of using the pharmaceutically active PAI-enantiomer alone (without L-
30 DOPA) for treatment of Parkinson's disease, a memory disorder, dementia, depression, hyperactive syndrome, an affective illness, a neurodegenerative disease, a neurotoxic injury, brain ischemia, a head trauma injury, a spinal trauma injury, schizophrenia, an attention deficit disorder,
35 multiple sclerosis, or withdrawal symptoms (see review by

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Youdim, et al., in Handbook of Experimental Pharmacology, Trendelenberg and Wiener, eds., 90/I, ch. 3 (1988)).

The subject invention further provides a method of using the
5 pharmaceutically active PAI-enantiomer alone for pre-treatment of Parkinson's disease. The subject invention also provides pharmaceutical compositions comprising [R](+)PAI and synergistic agents such as levodopa. The use of such agents has been studied with respect to (-)-deprenyl
10 which was shown to be effective when administered alone to early Parkinson's patients, and may also have a synergistic effect in these patients when administered together with α -tocopherol, a vitamin E derivative (The Parkinson's Study Group, New England J. Med., 321(20), 1364-1371 (1989)).

15 In addition to its usefulness in treating Parkinson's disease, (-)-deprenyl has also been shown to be useful in the treatment of patients with dementia of the Alzheimer type (DAT) (Tariot, et al., Psychopharmacology, 91, 489-495
20 (1987)), and in the treatment of depression (Mendelewicz and Youdim, Brit. J. Psychiat. 142, 508-511 (1983)). The [R](+)PAI compound of this invention, and particularly the mesylate salt thereof, has been shown to restore memory. [R](+)PAI thus has potential for the treatment of memory
25 disorders, and dementia, especially of the Alzheimer's type, in children.

Finally, the subject invention provides highly stable salts of [R](+)PAI with superior pharmaceutical properties. The
30 mesylate salt is especially stable, shows unexpectedly greater selectivity, and shows significantly fewer side effects than do the corresponding racemic salts.

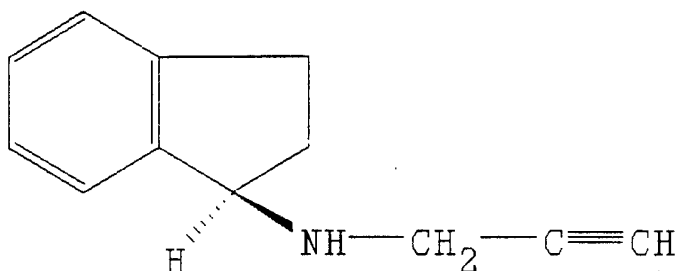
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Summary of the Invention

The subject invention provides R(+)-N-propargyl-1-aminoindan having the structure:

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The subject invention further provides a pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan.

The subject invention further provides a pharmaceutical composition which comprises a therapeutically effective amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

The subject invention further provides a method of treating a subject afflicted with Parkinson's disease which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat Parkinson's disease in the subject.

The subject invention further provides a method of treating a subject afflicted with a memory disorder which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the memory disorder in the subject.

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The subject invention further provides a method of treating a subject afflicted with dementia which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat dementia in the subject. In one embodiment, the dementia is of the Alzheimer type (DAT).

10 The subject invention further provides a method of treating a subject afflicted with depression which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat depression in the subject.

15 The subject invention further provides a method of treating a subject afflicted with hyperactive syndrome which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat hyperactive syndrome in the subject.

20 The subject invention further provides a method of treating a subject afflicted with an affective illness which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the affective illness in the subject.

25 The subject invention further provides a method of treating a subject afflicted with a neurodegenerative disease which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the neurodegenerative disease in the subject.

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The subject invention further provides a method of treating a subject afflicted with a neurotoxic injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof
5 of the subject invention effective to treat the neurotoxic injury in the subject.

The subject invention further provides a method of treating a subject afflicted with brain ischemia which comprises
10 administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat brain ischemia in the subject.

15 The subject invention further provides a method of treating a subject afflicted with a head trauma injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the
20 head trauma injury in the subject.

The subject invention further provides a method of treating a subject afflicted with a spinal trauma injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable
25 salt thereof of the subject invention effective to treat the spinal trauma injury in the subject.

The subject invention further provides a method of treating
30 a subject afflicted with schizophrenia which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat schizophrenia in the subject.

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The subject invention further provides a method of treating a subject afflicted with an attention deficit disorder which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the attention deficit disorder in the subject.

The subject invention further provides a method of treating a subject afflicted with multiple sclerosis which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat multiple sclerosis in the subject.

The subject invention further provides a method of preventing nerve damage in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to prevent nerve damage in the subject.

The subject invention further provides a method of treating a subject suffering from symptoms of withdrawal from an addictive substance which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the symptoms of withdrawal in the subject.

The subject invention further provides a method for preparing R(+)-N-propargyl-1-aminoindan which comprises contacting, in the presence of an organic or inorganic base, R(-)-aminoindan with either propargyl bromide or propargyl chloride or propargyl benzenesulfonate so as to form R(+)-N-propargyl-1-aminoindan, and isolating the R(+)-N-propargyl-

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1-aminoindan formed thereby.

The subject invention further provides a method for preparing racemic N-propargyl-1-aminoindan which comprises
5 contacting, in the presence of an organic or inorganic base, racemic 1-aminoindan with propargyl bromide or propargyl chloride so as to form racemic N-propargyl-1-aminoindan, and isolating the racemic N-propargyl-1-aminoindan formed thereby.

10

Finally, the subject invention provides a method of preparing an R(+)-N-propargyl-1-aminoindan salt which comprises contacting racemic N-propargyl-1-aminoindan with an optically active acid so as to form two diastereomeric N-
15 propargyl-1-aminoindan salts, and isolating R(+)-N-propargyl-1-aminoindan salt from the diastereomeric N-propargyl-1-aminoindan salts so formed.

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Brief Description of the Figures

Figure 1 is a graphic representation of the results according to Example 22 showing in vitro MAO-A inhibitory activity.

Figure 2 is a graphic representation of the results according to Example 22 showing in vitro MAO-B inhibitory activity.

Figure 3A is a graphic representation of the results according to Example 22 showing MAO activity in human cortical tissue. Substrate is ^{14}C -labelled phenylethylamine (PEA).

Figure 3B is a graphic representation of the results according to Example 22 showing MAO activity in human cortical tissue. Substrate is ^{14}C -labelled 5-hydroxytryptamine (5-HT).

Figure 4 is a graphic representation of the results according to Example 23 showing acute inhibition (i.p.) of MAO-A in brain.

Figure 5 is a graphic representation of the results according to Example 23 showing acute inhibition (i.p.) of MAO-B in brain.

Figure 6 is a graphic representation of the results according to Example 23 showing acute inhibition (i.p.) of MAO-A in liver.

Figure 7 is a graphic representation of the results according to Example 23 showing acute inhibition (i.p.) of MAO-B in liver.

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Figure 8 is a graphic representation of the results according to Example 23 showing acute inhibition (per os) of MAO-A in brain.

5 Figure 9 is a graphic representation of the results according to Example 23 showing acute inhibition (per os) of MAO-B in brain.

Figure 10 is a graphic representation of the results
10 according to Example 23 showing acute inhibition (per os) of MAO-A in liver.

Figure 11 is a graphic representation of the results according to Example 23 showing acute inhibition (per os) of
15 MAO-B in liver.

Figure 12 is a graphic representation of the results according to Example 24 showing chronic inhibition of MAO-A in brain.
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Figure 13 is a graphic representation of the results according to Example 24 showing chronic inhibition of MAO-B in brain.

25 Figure 14 is a graphic representation of the results according to Example 24 showing chronic inhibition of MAO-A in liver.

Figure 15 is a graphic representation of the results
30 according to Example 24 showing chronic inhibition of MAO-B in liver.

Figure 16 is a graphic representation of the results according to Example 25 showing MAO-B activity in rat brain
35 as a function of time following i.p. administration of [R] (+) PAI.

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Figure 17 is a graphic representation of the results according to Example 32 showing restoration of normokinesia in mice that had received haloperidol 6 mg/kg s.c. Mice received each of the test drugs i.p. at the indicated dose. 2 hours later they received haloperidol. Kinetic scores were taken 3 hours after haloperidol. These scores consisted of the ability to move horizontally along a rod, the ability to descend a vertical rod, and the shortening of catalepsia. In the absence of haloperidol, the maximum score is 12, with haloperidol alone, 6.8 ± 0.03 . Statistical significance was calculated by the Student's "t" test: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ with respect to haloperidol alone. The scores of [R](+)PAI are significantly different from those of racemic-PAI at 5 mg/kg ($p \leq 0.05$), at 10 mg/kg ($p \leq 0.01$), and at 15 mg/kg ($p \leq 0.05$), ($n=5.6$). The dosage shown is for the free base of PAI (and not the mesylate salt).

Figure 18 is a graphic representation of the results according to Example 32 showing restoration of motor activity in rats treated with α -methyl-p-tyrosine at 100 mg/kg i.p. Rats received the test drug i.p. at the indicated doses. After two hours they received α -Mpt and were immediately placed in activity cages. Total motor activity was recorded for the duration of 10 hours. Control rats, treated with saline, only scored $15,862 \pm 1424$. With α -Mpt alone, they scored $8,108 \pm 810$. Statistical significance by the Student's "t" test: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ with respect to α -Mpt alone. The scores of [R](+)PAI are significantly different from racemic-PAI at 2 mg/kg ($p \leq 0.01$), ($n=6$). Dosage shown is for the free base of PAI and not the mesylate salt.

Figure 19 is a graph showing the NADH response to 2 minutes of anoxia measured 30 minutes after injury and at half-hour intervals thereafter.

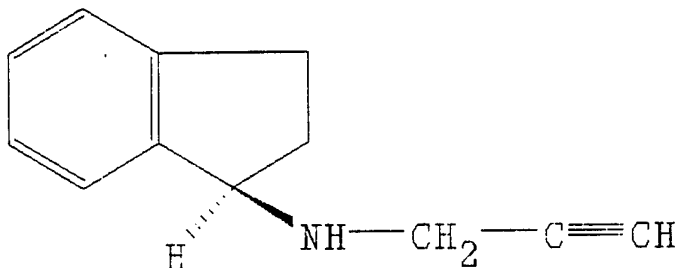
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Detailed Description of the Invention

The subject invention provides R(+)-N-propargyl-1-aminoindan having the structure:

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As demonstrated in the Experimental Examples hereinbelow,
15 [R] (+)PAI is nearly 7,000 times more active as an inhibitor
of MAO-B than is [S] (-)PAI. In view of known MAO-B
inhibitors in the art which possess low selectivity between
MAO-A and MAO-B, and which do not show predictable trends in
potency as a function of R or S configuration, the
20 selectivity and potency of [R] (+)PAI are unexpected.

[R] (+)PAI may be obtained by optical resolution of racemic
mixtures of R- and S-enantiomers of PAI. Such a resolution
can be accomplished by any conventional resolution method
25 well known to a person skilled in the art, such as those
described in J. Jacques, A. Collet and S. Wilen,
"Enantiomers, Racemates and Resolutions," Wiley, New York
(1981). For example, the resolution may be carried out by
preparative chromatography on a chiral column. Another
30 example of a suitable resolution method is the formation of
diastereomeric salts with a chiral acid such as tartaric,
malic, mandelic acid or N-acetyl derivatives of amino acids,
such as N-acetyl leucine, followed by recrystallisation to
isolate the diastereomeric salt of the desired R enantiomer.

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The racemic mixture of R and S enantiomers of PAI may be prepared, for example, as described in GB 1,003,676 and GB 1,037,014. The racemic mixture of PAI can also be prepared by reacting 1-chloroindan with propargylamine.

- 5 Alternatively, this racemate may be prepared by reacting propargylamine with 1-indanone to form the corresponding imine, followed by reduction of the carbon-nitrogen double bond of the imine with a suitable agent, such as sodium borohydride.

10

- In accordance with this invention, the R enantiomer of PAI can also be prepared directly from the optically active R-enantiomer of 1-aminoindan by reaction with propargyl bromide or propargyl chloride or propargyl benzenesulfonate
15 in the presence of an organic or inorganic base, and optionally in the presence of a suitable solvent.

- Suitable organic or inorganic bases for use in the above reaction include, by way of example, triethylamine,
20 pyridine, alkali metal carbonates, and bicarbonates. If the reaction is conducted in the presence of a solvent, the solvent may be chosen from, e.g., toluene, methylene chloride, and acetonitrile. One method of preparing [R] (+) PAI is to react R-1-aminoindan with propargyl chloride
25 using potassium bicarbonate as a base and acetonitrile as solvent.

- The above-described reaction of 1-aminoindan generally results in a mixture of unreacted primary amine, the desired
30 secondary amine and the tertiary amine N,N-bispropargylamino product. The desired secondary amine, i.e., N-propargyl-1-aminoindan, can be separated from this mixture by a conventional separation method including, by way of example, chromatography, distillation and selective extraction.

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The R-1-aminoindan starting material can be prepared by methods known in the art which include, by way of example, the method of Lawson and Rao, Biochemistry, 19, 2133 (1980), methods in references cited therein, and the method of
5 European Patent No. 235,590.

R-1-aminoindan can also be prepared by resolution of a racemic mixture of the R and S enantiomers, which involves, for example, the formation of diastereomeric salts with
10 chiral acids, or any other known method such as those reported in J. Jacques, et al., ibid. Alternatively, R-1-aminoindan may be prepared by reacting 1-indanone with an optically active amine, followed by reduction of the carbon nitrogen double bond of the resulting imine by hydrogenation
15 over a suitable catalyst, such as palladium on carbon, platinum oxide or Raney nickel. Suitable optically active amines include, for example, one of the antipodes of phenethylamine or an ester of an amino acid, such as valine or phenylalanine. The benzylic N-C bond may be cleaved
20 subsequently by hydrogenation under non-vigorous conditions.

An additional method for preparing R-1-aminoindan is the hydrogenation of indan-1-one oxime ethers as described above, wherein the alkyl portion of the ether contains an
25 optically pure chiral center. Alternatively, a non-chiral derivative of indan-1-one containing a carbon-nitrogen double bond, such as an imine or oxime, can be reduced with a chiral reducing agent, e.g., a complex of lithium aluminum-hydride and ephedrine.

30

The subject invention further provides a pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan.

In the practice of this invention, pharmaceutically
35 acceptable salts include, but are not limited to, the

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mesylate, maleate, fumarate, tartrate, hydrochloride, hydrobromide, esylate, p-toluenesulfonate, benzoate, acetate, phosphate and sulfate salts.

- 5 In one embodiment, the salt is selected from the group consisting of the mesylate salt of R(+)-N-propargyl-1-aminoindan, the esylate salt of R(+)-N-propargyl-1-aminoindan, and the sulfate salt of R(+)-N-propargyl-1-aminoindan.

10

As demonstrated in the Experimental Examples hereinbelow, the mesylate salt is highly stable to thermal degradation, and shows unexpectedly superior selectivity for MAO-B over the racemic salt.

15

- For the preparation of pharmaceutically acceptable acid addition salts of the compound of [R](+)PAI, the free base can be reacted with the desired acids in the presence of a suitable solvent by conventional methods. Similarly, an acid addition salt may be converted to the free base form in a known manner.

20

- A preferred mode of preparing the mesylate salt of [R](+)PAI comprises (a) adding an aqueous solution of 15% sodium hydroxide to a solution of propargyl benzenesulfonate (or tosylate or mesylate) in toluene; (b) stirring for 5 hours; (c) adding additional toluene and water; (d) separating and washing the organic phase with 10% sodium hydroxide, and then diluting with water; (e) adjusting the pH of the mixture to 3.2 by adding 10% aqueous sulfuric acid; (f) separating the aqueous phase and adjusting the pH to 7.3 with 10% sodium hydroxide; (g) extracting three times with toluene while maintaining constant pH; (h) concentrating combined organic layers in vacuo to give a yellow oil; (i) dissolving the oil and L-tartaric acid in isopropanol; (j)

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heating to reflux for 1 hour; (k) cooling to room temperature and collecting the precipitate by filtration; (l) recrystallizing the crude di-propargylaminoindan tartrate from methanol/isopropanol (1:1) to give di(R(+)-N-propargyl-1-aminoindan) tartrate; (m) dissolving the tartrate salt and methanesulfonic acid in isopropanol, and heating to reflux for 30 minutes; and (n) cooling to room temperature, and collecting the precipitated R(+)-N-propargyl-1-aminoindan.

10

The subject invention further provides a pharmaceutical composition which comprises a therapeutically effective amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. The "therapeutically effective amount" of the R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof may be determined according to methods well known to those skilled in the art.

15

Possible salts useful for such compositions include hydrochloride, phosphate, maleate, fumarate, tartrate, mesylate, esylate, and sulfate salts.

20

These compositions may be prepared as medicaments to be administered orally, parenterally, rectally, or transdermally.

25

In one embodiment, the pharmaceutically acceptable carrier is a solid and the pharmaceutical composition is a tablet. The therapeutically effective amount may be an amount from about 0.1 mg to about 100 mg. The therapeutically effective amount may also be an amount from about 1 mg to about 10 mg.

30

Suitable forms for oral administration include tablets, compressed or coated pills, dragees, sachets, hard or soft

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gelatin capsules, sublingual tablets, syrups and suspensions.

In an alternative embodiment, the pharmaceutically acceptable carrier is a liquid and the pharmaceutical composition is an injectable solution. The therapeutically effective amount may be an amount from about 0.1 mg/ml to about 100 mg/ml. The therapeutically effective amount may also be an amount from about 1 mg/ml to about 10 mg/ml. In one embodiment, the dose administered is an amount between 0.1ml and 1.0ml.

In a further alternative embodiment, the carrier is a gel and the pharmaceutical composition is a suppository.

15

For parenteral administration the invention provides ampoules or vials that include an aqueous or non-aqueous solution or emulsion. For rectal administration there are provided suppositories with hydrophilic or hydrophobic vehicles. For topical application as ointments and transdermal delivery there are provided suitable delivery systems as known in the art.

In the preferred embodiment, the pharmaceutically acceptable salt is a mesylate salt.

These compositions may be used alone to treat the above-listed disorders, or alternatively, as in the case of Parkinson's disease, for example, they may be used as an adjunct to the conventional L-DOPA treatments.

The preferred dosages of the active ingredient, i.e., [R](+)PAI, in the above compositions are within the following ranges. For oral or suppository formulations, 0.1-100 mg per dosage unit may be taken daily, and

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preferably 1-10 mg per dosage unit is taken daily. For injectable formulations, 0.1-100 mg/ml per dosage unit may be taken daily, and preferably 1-10 mg/ml per dosage unit is taken daily.

5

In one embodiment, the pharmaceutical composition further comprises a therapeutically effective amount of Levodopa. In another embodiment, the pharmaceutical composition still further comprises an effective amount of a decarboxylase inhibitor.

10

The amount of decarboxylase inhibitor administered in combination with [R](+)PAI or a pharmaceutically acceptable salt thereof is an amount effective to ensure L-DOPA uptake in the subject.

15

The decarboxylase inhibitor may be L-Carbidopa. In one embodiment, the therapeutically effective amount of R(+)-N-propargyl-1-aminoindan is about 0.1 mg to about 100 mg, the therapeutically effective amount of Levodopa is about 50 mg to about 250 mg, and the effective amount of L-Carbidopa is about 10 mg to about 25 mg.

20

The decarboxylase inhibitor may also be benserazide. In one embodiment, the therapeutically effective amount of R(+)-N-propargyl-1-aminoindan is about 0.1 mg to about 100 mg, the therapeutically effective amount of Levodopa is about 50 mg to about 200 mg, and the effective amount of benserazide is about 12.5 mg to about 50 mg.

25

30

The subject invention further provides a method of treating a subject afflicted with Parkinson's disease which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat Parkinson's

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disease in the subject.

Methods of treatment of Parkinson's disease which combine the use of [R](+)PAI with other drugs, such as dopamine
5 agonists, bromocryptine, pergolide, lisuride, as well as catecholamine oxidase methyl transferase inhibitors are within the scope of the subject invention.

In the preferred embodiment, the pharmaceutically acceptable
10 salt is a mesylate salt.

The administering may comprise orally administering, rectally administering, transdermally administering, or parenterally administering.

15 In one embodiment, the method of the subject invention further comprises administering to the subject a therapeutically effective amount of Levodopa. In another embodiment, the method of the subject invention still
20 further comprises administering to the subject an effective amount of a decarboxylase inhibitor.

The decarboxylase inhibitor may be L-Carbidopa. Alternatively, the decarboxylase inhibitor may be
25 benserazide.

The subject invention further provides a method of treating a subject afflicted with a memory disorder which comprises administering to the subject an amount of R(+)-N-propargyl-
30 1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the memory disorder in the subject.

The subject invention further provides a method of treating
35 a subject afflicted with dementia which comprises

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administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat dementia in the subject. In one embodiment, the dementia is of the
5 Alzheimer type (DAT).

The subject invention further provides a method of treating a subject afflicted with depression which comprises administering to the subject an amount of R(+)-N-propargyl-
10 1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat depression in the subject.

The subject invention further provides a method of treating
15 a subject afflicted with hyperactive syndrome which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat hyperactive syndrome in the subject.

20 The administering may comprise orally administering, rectally administering, or parenterally administering.

The subject invention further provides a method of treating
25 a subject afflicted with an affective illness which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the affective illness in the subject.

30 The subject invention further provides a method of treating a subject afflicted with a neurodegenerative disease which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable
35 salt thereof of the subject invention effective to treat the

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neurodegenerative disease in the subject.

The subject invention further provides a method of treating a subject afflicted with a neurotoxic injury which comprises
5 administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the neurotoxic injury in the subject.

10 The subject invention further provides a method of treating a subject afflicted with brain ischemia which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof
15 of the subject invention effective to treat brain ischemia in the subject.

The subject invention further provides a method of treating a subject afflicted with a head trauma injury which comprises administering to the subject an amount of R(+)-N-
20 propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the head trauma injury in the subject.

The subject invention further provides a method of treating
25 a subject afflicted with a spinal trauma injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the spinal trauma injury in the subject.

30 The subject invention further provides a method of treating a subject afflicted with schizophrenia which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof
35 of the subject invention effective to treat schizophrenia in

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the subject.

The subject invention further provides a method of treating a subject afflicted with an attention deficit disorder which
5 comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the attention deficit disorder in the subject.

10 The subject invention further provides a method of treating a subject afflicted with multiple sclerosis which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof
15 of the subject invention effective to treat multiple sclerosis in the subject.

The subject invention further provides a method of preventing nerve damage in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-
20 1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to prevent nerve damage in the subject.

In one embodiment, the nerve damage is structural nerve
25 damage. In another embodiment, the structural nerve damage is optic nerve damage.

The subject invention further provides a method of treating a subject suffering from symptoms of withdrawal from an
30 addictive substance which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the symptoms of withdrawal in the subject.

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As used herein, the term "symptoms of withdrawal" refers to physical and/or psychological symptoms, including drug craving, depression, irritability, anergia, amotivation, appetite change, nausea, shaking and sleep irregularity.

5

As used herein, the term "addictive substance" includes, by way of example, (a) addictive opiates such as opium, heroin and morphine, (b) psychostimulants such as cocaine, amphetamines and methamphetamines, (c) alcohol, (d) 10 nicotine, (e) barbiturates and (f) narcotics such as fentanyl, codeine, diphenoxylate and thebaine.

In one embodiment, the addictive substance is cocaine. In another embodiment, the addictive substance is alcohol.

15

The subject invention further provides a method for preparing R(+)-N-propargyl-1-aminoindan which comprises contacting, in the presence of an organic or inorganic base, R(-)-aminoindan with either propargyl bromide or propargyl 20 chloride or propargyl benzenesulfonate so as to form R(+)-N-propargyl-1-aminoindan, and isolating the R(+)-N-propargyl-1-aminoindan formed thereby.

The subject invention further provides a method for 25 preparing racemic N-propargyl-1-aminoindan which comprises contacting, in the presence of an organic or inorganic base, racemic 1-aminoindan with propargyl bromide or propargyl chloride so as to form racemic N-propargyl-1-aminoindan, and isolating the racemic N-propargyl-1-aminoindan formed 30 thereby.

Finally, the subject invention provides a method of preparing an R(+)-N-propargyl-1-aminoindan salt which comprises contacting racemic N-propargyl-1-aminoindan with 35 an optically active acid so as to form two diastereomeric N-

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propargyl-1-aminoindan salts, and isolating R(+)-N-propargyl-1-aminoindan salt from the diastereomeric N-propargyl-1-aminoindan salts so formed.

- 5 In one embodiment, the isolating comprises isolating by fractional crystallization.

The following Experimental Details are set forth to aid in an understanding of the invention, and are not intended, and
10 should not be construed, to limit in any way the invention set forth in the claims which follow thereafter.

Experimental Details

EXAMPLE 1

5 Racemic N-propargyl-1-aminoindan hydrochloride

10.0g of racemic 1-aminoindan and 10.4g of potassium carbonate were added to 75 ml of acetonitrile. The resulting suspension was heated to 60°C and 4.5g of
10 propargyl chloride was added dropwise.

The mixture was stirred at 60°C for 16 hours, whereafter most of the volatiles were removed by distillation in vacuo. The residue was partitioned between 10% aqueous sodium
15 hydroxide and methylene chloride.

The organic phase was dried and the solvent removed by distillation. The residue was flash chromatographed on silica gel, eluting with 40% ethyl acetate/60% hexane. The
20 fractions containing the title compound as a free base were combined and the eluant replaced by ether. The ethereal solution was treated with gaseous HCl; the precipitate formed was isolated by suction filtration and recrystallized from isopropanol to yield 7.3g of the title compound, m.p.
25 182-4°C.

Chromatographic and spectroscopic data were in accordance with U.S. Patent No. 3,513,244, issued May 19, 1970, and an authentic sample, and were as follows: NMR δ (CDCl₃): 2.45
30 (2H, m), 2.60 (1H, t), 2.90 (1H, m), 3.45 (1H, m), 3.70 (2H, d), 4.95 (1H, t), 7.5 (4H, m) ppm.

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EXAMPLE 2**S-(-)-N-Propargyl-1-aminoindan hydrochloride**

The title compound in free base form was isolated by
5 resolving the racemic mixture of the free base of Example 1
on a Chiracel OJ (cellulose tris [p-methylbenzoate])
preparative HPLC column eluting with 10% isopropanol/90%
hexane and collecting the first eluted major peak. The
10 resulting oil was converted to the title compound
(hydrochloride) by treatment of a 10% diethyl ether solution
of the oil with gaseous HCl, and the resulting precipitate
was collected by suction filtration. $[\alpha]_D -29.2^\circ$ (1%,
ethanol), m.p. 182-184°C. Other chromatographic and
spectroscopic properties were identical with the
15 hydrochloride salt of Example 1.

EXAMPLE 3**R-(+)-N-Propargyl-1-aminoindan hydrochloride**

20 The title compound was prepared as in Example 2 above,
except that the second eluted peak from the preparative HPLC
was collected: $[\alpha]_D +29.1^\circ$ (0.8%, ethanol), m.p. 179-181°C.
Other chromatographic and spectroscopic properties were
identical with the hydrochloride salt of Example 1.

25

EXAMPLE 4**R-(+)-N-Propargyl-1-aminoindan hydrochloride**

12.4g of R-(-)-1-Aminoindan and 12.9g of potassium carbonate
30 were added to 95 ml of acetonitrile. The resulting
suspension was heated to 60°C and 5.6g of propargyl chloride
was added dropwise. The mixture was stirred at 60°C for 16
hours, whereafter most of the volatiles were removed by
distillation in vacuo. the residue was partitioned between

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10% aqueous sodium hydroxide and methylene chloride.

The organic phase was dried and the solvent removed in vacuo. The residue was flash chromatographed on silica gel eluting with 40% ethyl acetate/60% hexane. Fractions containing the free base of the title compound were combined and the solvent replaced by ether. The ethereal solution was treated with gaseous HCl and the resulting precipitate was isolated by suction filtration and recrystallized from isopropanol to yield 6.8g of the title compound, m.p. 183-185°C, $[\alpha]_D +30.90$ (2% ethanol). Spectral properties were identical to those reported for the compound of Example 1.

EXAMPLE 5

15 S-(-)-N-Propargyl-1-aminoindan hydrochloride

The title compound was prepared by the method of Example 4, except that S-(+)-1-aminoindan was used as starting material. The product exhibited $[\alpha]_D -30.3$ (2% ethanol), m.p. 183-5°C. Spectral properties were identical to those reported for the compound of Example 1.

EXAMPLE 6A

Di(R-(+)-N-propargyl-1-aminoindan) L-tartrate

25

To a solution of tartaric acid (4.4g) in 48 ml of boiling methanol was added a solution of R-(+)-N-propargyl-1-aminoindan free base (5.0g) in methanol (48 ml). The solution was heated to reflux and 284 ml of t-butylmethyl ether was added over 20 minutes. The mixture was heated for an additional 30 minutes, cooled, and the resulting precipitate was isolated by suction filtration to yield 6.7g of the title compound: m.p. 175-177°C; $[\alpha]_D (1.5, H_2O) = +34.3$; Anal. calcd. for $C_{28}H_{32}O_6N_2$; C, 68.26, H, 6.56, N, 5.69.

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Found: C, 68.76; H, 6.57; N, 5.61.

EXAMPLE 6B

R-(+)-N-propargyl-1-aminoindan mesylate

5

a) To a solution of propargyl benzenesulfonate (78.4g) and racemic aminoindan (63.2g) in toluene (240mL) at 20°C was added dropwise an aqueous solution of 15% sodium hydroxide (108 mL). After 5 hours of stirring, additional toluene (80
10 mL) and water (200 mL) were added with stirring. The organic phase was separated and washed with 10% aqueous sodium hydroxide and then diluted with water. The pH of the mixture was adjusted to 3.2 by the addition of 10% aqueous sulfuric acid. The aqueous phase was separated and its pH
15 was adjusted to 7.3 with 10% sodium hydroxide and extracted three times with toluene while maintaining constant pH. The combined organic layers were concentrated in vacuo to 40.7g of a yellow oil.

20 b) The above crude racemic propargylaminoindan and L-tartaric acid (10g) were dissolved in isopropanol (1 L) and heated to reflux for 1 hour. The reaction was then allowed to cool to room temperature with stirring and the precipitate collected by filtration. The crude di-
25 propargylaminoindan tartrate was recrystallized from 1 L of 1:1 methanol/isopropanol to give di(R-(+)-N-propargyl-1-aminoindan)-L-tartrate with physical and spectral properties identical to that of the compound of Example 6A.

30 c) A solution of di-(R-(+)-N-propargyl-1-aminoindan) tartrate (15g) and methanesulfonic acid (6g) in isopropanol (150 mL) was heated to reflux for 30 minutes. The reaction was allowed to cool to room temperature and the resulting precipitate isolated by suction filtration to give the title

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compound (11.1g) with m.p. 157°C and $[\alpha]_D = 22^\circ$.

EXAMPLE 7**R-(+)-N-Methyl-N-propargyl-1-aminoindan hydrochloride**

5

The free base form of R-(+)-N-propargyl-1-aminoindan from Example 4 (1.2 grams), potassium carbonate (0.97 grams) and methyl iodide (1 gram) were added to 15 ml of acetone and the resulting suspension heated to reflux under a nitrogen
10 atmosphere for 8 hours. Thereafter the volatiles were removed under reduced pressure and the residue partitioned between 10% aqueous sodium hydroxide (30 ml) and methylene chloride (30 ml). The organic phase was dried and the solvent removed in vacuo. The residue was flash
15 chromatographed on silica gel eluting with 40% ethyl acetate/60% hexane. Fractions containing the title compound as a free base were combined and the solvent replaced by diethyl ether. The ethereal solution was treated with gaseous HCl. The volatiles were removed in vacuo, and the
20 residue recrystallized from isopropanol to yield 400 mg of the title compound as a white crystalline solid, m.p. 134-136°C, $[\alpha]_D +31.40$ (ethanol). NMR δ (CDCl₃): 2.55 (2H, m); 2.7 (1H, br.s); 2.8 (3H, s); 3.0 (1H, m); 3.4 (1H, m); 3.9 (2H, br.s); 5.05 (1H, m); 7.7 (4H, m) ppm.

25

EXAMPLE 8**S-(-)-N-Methyl-N-propargyl-1-aminoindan hydrochloride**

The title compound was prepared as in Example 7 above,
30 except that S-(-)-N-propargyl-1-aminoindan (free base) from Example 5 was used as the starting material. All of the physical and spectral properties of the title compound were identical to those in Example 7 except for the $[\alpha]_D -34.9^\circ\text{C}$ (ethanol).

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EXAMPLE 9**Tablet Composition**

	N-Propargyl-1(R)-aminoindan Hydrochloride	7.81 mg*
5	Pregelatinized starch NF	47.0 mg
	Lactose NF hydrous	66.0 mg
	Microcrystalline cellulose NF	20.0 mg
	Sodium starch glycolate NF	2.99 mg
	Talc USP	1.5 mg
10	Magnesium stearate NF	0.7 mg

*Equivalent to 5.0 mg of N-propargyl aminoindan base.

EXAMPLE 10**Tablet Composition**

15	N-Propargyl-1(R)-aminoindan Hydrochloride	1.56 mg*
	Lactose hydrous	50.0 mg
	Pregelatinized starch	36.0 mg
	Microcrystalline cellulose	14.0 mg
20	Sodium starch glycolate	2.14 mg
	Talc USP	1.0 mg
	Magnesium stearate NF	0.5 mg

*Equivalent to 1.0 mg of N-propargyl aminoindan base.

EXAMPLE 11**Capsule Composition**

	N-Propargyl-1(R)-aminoindan Hydrochloride	5.0 mg
	Pregelatinized starch	10.0 mg
30	Starch	44.0 mg
	Microcrystalline cellulose	25.0 mg
	Ethylcellulose	1.0 mg
	Talc	1.5 mg

Purified water added as required for granulation.

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EXAMPLE 12Injection Composition

	N-Propargyl-1(R)-aminoindan Hydrochloride	5.0 mg
5	Dextrose anhydrous	44.0 mg
	HCl added to pH 5	
	Purified water added as required for 1 ml	

EXAMPLE 1310 Injection Composition

	N-Propargyl-1(R)-aminoindan Hydrochloride	1.0 mg
	Sodium chloride	8.9 mg
	HCl added to pH 5	
15	Purified water added as required for 1 ml	

EXAMPLE 14Injection Composition

20	N-Propargyl-1(R)-aminoindan Hydrochloride	2.0 mg
	Sodium chloride	8.9 mg
	HCl added to pH 5	
	Purified water added as required for 1 ml	

25 EXAMPLE 15Syrup Composition

	N-Propargyl-1(R)-aminoindan Hydrochloride	5.0 mg
	Sucrose	2250.0 mg
30	Saccharin sodium	5.0 mg
	Methylparaben	6.0 mg
	Propylparaben	1.0 mg
	Flavor	20.0 mg
	Glycerin USP	500 mg
35	Alcohol 95% USP	200 mg

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Purified water as required to 5.0 ml

EXAMPLE 16**Sublingual Tablets**

5	N-Propargyl-1(R)-aminoindan Hydrochloride	2.5 mg
	Microcrystalline cellulose	20.0 mg
	Lactose hydrous	5.0 mg
	Pregelatinized starch	3.0 mg
10	Povidone	0.3 mg
	Coloring agent	q.s.
	Flavor	q.s.
	Sweetener	q.s.
	Talc	0.3 mg

15

Blend the excipients and the active and granulate with an ethanol solution of Povidone. After drying and weighing, it is blended with the talc and compressed.

20 **EXAMPLE 17****PAI Sublingual Tablets**

	N-Propargyl-1(R)-aminoindan Hydrochloride	5.0 mg
	Microcrystalline cellulose	15.0 mg
25	Pregelatinized starch	12.0 mg
	Ethyl cellulose	0.3 mg
	Talc	0.3 mg

Purified water added as required for granulation.

30 **EXAMPLE 18****Tablet Composition**

	N-Propargyl-1(R)-aminoindan Hydrochloride	5.0 mg
	Levodopa	100.0 mg
35	Carbidopa	25.0 mg

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	Pregelatinized starch	24.0 mg
	Starch	40.0 mg
	Microcrystalline cellulose	49.5 mg
	Col. D & C Yellow No. 10	0.5 mg
5	Col. D & C Yellow No. 6	0.02 mg
	Alcohol USP added as required for granulation.	

EXAMPLE 19**Tablet Composition**

10	N-Propargyl-1(R)-aminoindan Mesylate	7.81 mg*
	Pregelatinized starch NF	47.0 mg
	Lactose NF hydrous	66.0 mg
	Microcrystalline cellulose NF	20.0 mg
15	Sodium starch glycolate NF	2.99 mg
	Talc USP	1.5 mg
	Magnesium stearate NF	0.7 mg

*Equivalent to 5.0 mg of N-propargyl aminoindan base.

EXAMPLE 20**Tablet Composition**

	N-Propargyl-1(R)-aminoindan Mesylate	1.56 mg*
	Lactose hydrous	50.0 mg
25	Pregelatinized starch	36.0 mg
	Microcrystalline cellulose	14.0 mg
	Sodium starch glycolate	2.14 mg
	Talc USP	1.0 mg
	Magnesium stearate NF	0.5 mg

30 *Equivalent to 1.0 mg of N-propargyl aminoindan base.

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EXAMPLE 21**Capsule Composition**

	N-Propargyl-1(R)-aminoindan Mesylate	5.0 mg
5	Pregelatinized starch	10.0 mg
	Starch	44.0 mg
	Microcrystalline cellulose	25.0 mg
	Ethylcellulose	1.0 mg
	Talc	1.5 mg
10	Purified water added as required for granulation.	

The following Examples and the accompanying Tables and Figures relate to biological experiments carried out in accordance with this invention.

15

EXAMPLE 22**Inhibition of MAO activity in vitro****Experimental protocol**

- 20 The MAO enzyme source was a homogenate of rat brain in 0.3M sucrose, which was centrifuged at 600g for 15 minutes. The supernatant was diluted appropriately in 0.05M phosphate buffer, and pre-incubated with serial dilutions of compounds: [R](+)PAI, [S](-)PAI and racemic PAI for 20
- 25 minutes at 37°C. ¹⁴C-Labelled substrates (2-phenylethylamine, hereinafter PEA; 5-hydroxytryptamine, hereinafter 5-HT) were then added, and the incubation continued for a further 20 minutes (PEA), or 30-45 minutes (5-HT). Substrate concentrations used were 50uM (PEA) and
- 30 1mM (5-HT). In the case of PEA, enzyme concentration was chosen so that not more than 10% of the substrate was metabolized during the course of the reaction. The reaction was then stopped by addition of tranylcypromine (to a final concentration of 1mM), and the incubate filtered over a

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small column of Amberlite CG-50 buffered to pH 6.3. The column was washed with 1.5ml water, the eluates pooled and the radioactive content determined by liquid scintillation spectrometry. Since the amine substrates are totally retained on the column, radioactivity in the eluate indicates the production of neutral and acidic metabolites formed as a result of MAO activity. Activity of MAO in the sample was expressed as a percentage of control activity in the absence of inhibitors after subtraction of appropriate blank values. The activity determined using PEA as substrate is referred to as MAO-B, and that determined using 5-HT as MAO-A.

Results

Inhibitory activity of [R](+)PAI, [S](-)PAI and racemic-PAI were examined separately in vitro, and the results of typical experimental runs are shown in Figures 1 and 2. The entire experiment was repeated three times. Concentrations of inhibitor producing 50% inhibition of substrate metabolism (IC-50) were calculated from the inhibition curves, and are shown in Table 1B. From this data it can be seen that:

- (a) the [R](+)PAI is twice as active as the racemate for inhibition of MAO-B;
- (b) the [R](+)PAI is 29 times more active for inhibition of MAO-B than MAO-A;
- (c) the [S](-)PAI is only 1/6,800 as active as the [R](+)PAI for inhibition of MAO-B, and shows little or no selectivity between MAO-B and MAO-A.

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Table 1A

5 IC-50 (nM) VALUES FOR INHIBITION OF
MAO-A AND MAO-B BY RACEMIC-PAI AND THE
R(+) AND S(-) ENANTIOMERS THEREOF IN
RAT BRAIN HOMOGENATE IN VITRO

10	MAO-A			MAO-B		
	[S] (-) PAI	[R] (+) PAI	Rac	[S] (-) PAI	[R] (+) PAI	Rac
	26000	73	140	17000	2.5	5

15 The results of the same experiments using R(+) and S(-) MPAI
(N-methyl-N-propargyl-1-aminoindan) are reported in Table
1B. Each of the enantiomers of MPAI is less selective in
MAO-A and MAO-B inhibition than [R](+)PAI. Furthermore,
20 [R](+)MPAI is only five times as active as [S](-)MPAI in
MAO-B inhibition, in contrast to [R](+)PAI which is about
7000 times as active as [S](-)PAI in this assay.

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Table 1B

IC-50 (nM) VALUES FOR INHIBITION OF MAO-A AND MAO-B BY THE
R(+) AND S(-) ENANTIOMERS OF MPAL IN RAT BRAIN HOMOGENATE
IN VITRO

5

IC-50 (nM)

	<u>MAO-A</u>		<u>MAO-B</u>	
	S(-)MPAL	R(+)MPAL	S(-)MPAL	R(+)MPAL
10 Compound:	70	3	50	10

Some experiments were also carried out with human cerebral
cortical tissues obtained 6 hours post-mortem, and treated
15 as described above. The results of such an experiment are
shown in Figure 3, where [R](+)PAL, [S](-)PAL, and racemic
PAL are as defined herein.

EXAMPLE 23

20 Inhibition of MAO activity in vivo: acute treatment
Experimental protocol

Rats (male Sprague-Dawley-derived) weighing 250±20g were
treated with one of the enantiomers or the racemic form of
25 PAL by intraperitoneal injection (ip) or oral gavage (po)
and decapitated 1h or 2h later respectively. Groups of
three rats were used for each dose level of inhibitor, and
MAO activity determined in brain and liver using the general
technique described above. The amount of protein in each
30 incubation was determined using the Folin-Lowry method, and
enzyme activity calculated as nmol of substrate metabolized
per hour of incubation for each mg of protein. Activity of
MAO in tissues from animals treated with inhibitors was
expressed as a percentage of the enzyme activity in a group
35 of control animals administered vehicle (water for oral

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administration, 0.9% saline for ip injection) and killed as above.

Results

- 5 None of the dose levels used with the inhibitor drugs produced any obvious behavioral alteration. The results are depicted in Figures 4 to 11. Following i.p. administration, compound [R](+)PAI produced 90% inhibition of brain MAO-B activity at a dose of 0.5mg/kg. The same dose produced only
- 10 20% inhibition of MAO-A activity. By oral administration, the same dose of [R](+)PAI produced 80% inhibition of MAO-B with no detectable inhibition of MAO-A. Essentially similar results were seen for inhibition of hepatic MAO, as for brain MAO. The doses producing 50% inhibition of MAO-A and
- 15 MAO-B (IC-50) were calculated from the inhibition curves, and are shown in Table 2. These data show: (a) that MAO inhibitory activity of [R](+)PAI is maintained in vivo in the rat; (b) that selectivity for inhibition of MAO-B, as opposed to MAO-A, by [R](+)PAI is maintained in vivo; (c)
- 20 that the much greater activity of the (+)-enantiomer as opposed to the (-)-enantiomer, is maintained in vivo; (d) that the compounds are effectively absorbed after oral administration; and (e) that the compounds effectively pass the blood-brain barrier, and effectively inhibit brain MAO.
- 25 The fact that [R](+)PAI was about twice as active as the racemic compound for inhibition of MAO-B is a reflection of the extremely low activity of [S](-)PAI for inhibition of MAO-B.

Table 2

5 IC-50 VALUES (mg/kg) FOR INHIBITION OF MAO-A AND MAO-B
BY [R](+)PAI, [S](-)PAI OR RACEMIC-PAI, IN THE RAT
FOLLOWING INTRAPERITONEAL (I.P.) INJECTION OR ORAL
ADMINISTRATION (P.O.)

IC-50 (mg/kg)						
<u>MAO-A</u>			<u>MAO-B</u>			
10	Compound:	[S](-)PAI	[R](+)PAI	Rac	[S](-)PAI	[R](+)PAI
	I.P. BRAIN	>10	1.2	2.5	>10	0.07
	I.P. LIVER	>10	5	5	>10	0.06
	P.O. BRAIN	>10	>5	>5	>10	0.17
	P.O. LIVER	>10	>5	>5	>10	0.05
15	(Rac = Racemic PAI)					

EXAMPLE 24Inhibition of MAO activity in vivo: chronic treatmentExperimental protocol

20

Rats (specifications as in Example 23, 4 animals for each dose level) were treated with [R](+)PAI or the racemic mixture at three dose levels (0.05, 0.1 and 0.5mg/kg) by oral administration, one dose daily for 21 days, and
25 decapitated 2 hours after the last dose. The activities of MAO types A and B were determined in brain and liver as described in Example 23.

Results

30 A daily dose of 0.1mg/kg of compound [R](+)PAI produced a good degree of selective inhibition, with more than 80% inhibition of brain MAO-B and 20% or less inhibition of brain MAO-A. At the higher dose of 0.5mg/kg daily, MAO-A was still inhibited by less than 50% (Figures 12 and 13).
35 Hepatic MAO showed a similar degree of selective inhibition (Figures 14 and 15). Compound [R](+)PAI was again more

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potent than the racemic mixture by a factor of about twofold. In the case of brain MAO, [R] (+)PAI had a better degree of selectivity for inhibition of MAO-B than did the racemic mixture.

5

These results show that selectivity of MAO-B inhibition can be maintained following chronic treatment with the compounds. As with other irreversible inhibitors, the degree of enzyme inhibition is greater with chronic
10 treatments than that following a single dose of the drug. Compound [R] (+)PAI shows a better degree of selectivity for inhibition of brain MAO-B than the racemic mixture.

EXAMPLE 25

15 Irreversible nature of MAO inhibition Experimental protocol

A single dose of compound [R] (+)PAI (1 mg/kg) was administered by i.p. injection to groups of 4 rats, and the animals
20 killed 2, 6, 18, 24, 48 and 72 hours later. Activity of MAO-B was determined in whole brain tissues as described hereinabove.

Results

25 The results are shown in Figure 16. Maximal inhibition of MAO-B was attained at 6 hours after injection. MAO activity had only returned to 30% of control activity at 72 hours after injection. This experiment demonstrates the irreversible nature of the MAO inhibition by [R] (+)PAI.

30

EXAMPLE 26

Potentiation of tyramine pressor effect in conscious rats Experimental protocol

35 Rats were anesthetized with a mixture of pentobarbital

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(30mg/kg) and chloral hydrate (120mg/kg) by intraperitoneal injection. The left carotid artery and jugular vein were cannulated with fine polytene tubing (artery) or fine silicone rubber tubing connected to polyethylene tubing (vein), the distal end of which was brought under the skin to an anchor point behind the neck. The tubing was filled with heparinized saline solution, and plugged with a fine steel rod. The animals were treated with 20mg chloramphenicol by intramuscular injection and allowed to recover from the operation overnight. The following day, the rats were placed in a high-walled container permitting free movement. The arterial catheter was connected to a pressure transducer via a 100 cm length of saline-filled, fine-bore polyethylene tubing, and the venous catheter connected to a 1ml syringe via a similar length of tubing, which, together with the syringe, contained a solution of tyramine hydrochloride in saline (1 mg/ml). Following an equilibration period of 30 to 40 minutes, tyramine injections (50 or 100 μ g) were given, and blood pressure responses recorded. An interval of at least 15 minutes was maintained between injections after return of blood pressure to control values. Control pressor responses were established, then one of the drugs was injected intraperitoneally, and tyramine responses were repeated over the next 4 hours. The area under the blood pressure response curve was estimated, and the ratio of this area after treatment to before treatment and to 1 to 3 hours after injection of the compounds, was determined using the average of 3 to 4 values obtained in the control period.

30

Results

The results are shown in Table 3. Compound [R](+)PAI at a dose of 1mg/kg (which causes complete inhibition of MAO-B in brain and liver, and 40 to 50% inhibition of MAO-A in these tissues) caused no significant potentiation of tyramine

35

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pressor response. At the higher [R](+)PAI dose of 5mg/kg (which causes more extensive inhibition of MAO-A in brain and periphery), there was a significant potentiation of the tyramine pressor response, which was similar in extent to that produced by the same dose of deprenyl, and less than that produced by clorgyline (at a dose which inhibits hepatic MAO-A activity by over 85%).

Table 3

POTENTIATION OF TYRAMINE PRESSOR EFFECT IN CONSCIOUS RATS BY MAO INHIBITORS

Inhibitor	Dose (mg/kg)	No. of rats (n)	Ratio Area Under Pressor Response Curve; After/Before	SEM*
Saline		12	1.25	0.28
Clorgyline	2	6	10.39	2.13
(-)Deprenyl	1	2	1.15	
(+)Deprenyl	5	3	2.36	0.16
[R](+)PAI	1	3	1.38	0.7
[R](+)PAI	5	3	3.49	0.98

*SEM=standard error of the mean

From this experiment it can be concluded that compound [R](+)PAI causes no potentiation of the tyramine pressor effect at a dose which effectively inhibits MAO-B.

EXAMPLE 27Suppression of MPTP-induced dopaminergic toxicity by [R](+)PAI

35

1-Methyl-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin that damages nigrostriatal dopaminergic neurons in several mammalian species, including mice, and produces a Parkinsonian syndrome in humans and primates. A crucial initial step in the mechanism of its neurotoxicity involves

40

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conversion of MPTP to its toxic metabolite 1-methyl-4-phenyl pyridinium ion (MPP+). This reaction is catalyzed by the enzyme MAO-B and probably takes place outside of dopaminergic neurons, mainly in glia. It is known that MPTP is both a substrate and an irreversible inhibitor of MAO-B. Pretreatment of experimental animals with MAO-B inhibitors such as deprenyl or pargyline protects against and prevents the MPTP-induced damage to nigrostriatal neurons because the oxidative conversion of MPTP to MPP+ is blocked. The progressive nigrostriatal degeneration in Parkinson's may be due to exposure to environmentally-derived exogenous MPTP-like neurotoxins. In such cases, there is an additional strong indication of initiation of sustained treatment with an MAO-B inhibitor from the very early stages of Parkinson's disease in the hope that it will neutralize the damaging effects of such yet putative MPTP-toxins, and thus arrest or slow down the progression of the illness. A successful MAO-B inhibitor drug is currently judged by its ability to block MPTP-induced damage to nigrostriatal dopaminergic neurons in vivo. The (-) and (+) enantiomers of PAI were therefore tested for their potency in preventing or attenuating the MPTP-induced striatal dopamine depletions in mice.

Experimental Protocol

Male C57 black mice (20-25g weight) were (a) injected with MPTP·HCl (30 mg/kg dissolved in distilled water, s.c.), or vehicle alone, or one hour after pretreatment with the (-) or (+) isomers of PAI (2.5 mg/kg, i.p.), or with deprenyl (5 mg/kg, i.p.), and (b) decapitated 5 days later. Brains were removed and corpora striata dissected on an ice-cold glass plate and frozen on dry ice. Striatal tissues were homogenized in 0.1 M perchloric acid, and deproteinized aliquots containing dihydroxybenzylamine as an internal standard were assayed for dopamine and its major metabolite

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3,4-dihydroxy-phenylacetic acid (DOPAC) using HPLC with electrochemical detection.

Results

5 Table 4 shows the results of this experiment. Treatment with MPTP alone produced marked striatal dopamine (DA) and DOPAC depletions. Treatment with the (-) and (+) enantiomers of PAI or with (-) deprenyl did not affect striatal DA concentrations. Pretreatment with the (-)
10 isomer of PAI did not affect the MPTP-induced DA and DOPAC levels in the striatum. The (+)-isomer of PAI given before MPTP completely abolished the reduction in striatal DA and DOPAC levels produced by the toxin. At a dose of 2.5 mg/kg, (+)PAI was equipotent to (-) deprenyl (5 mg/kg) in its
15 protective effect.

Table 4

EFFECT OF PRETREATMENT WITH THE (-) AND (+) ENANTIOMERS OF THE MAO-B INHIBITOR PAI ON THE STRIATAL DA AND DOPAC
20 DEPLETIONS INDUCED BY MPTP IN MICE IN VIVO

	<u>DA</u>	<u>DOPAC</u>
	(ng/mg protein)	
Control	162.8±7.2	8.4±0.5
25 MPTP	53.1±6.2	3.2±0.3
(-) PAI	174.0±4.8	7.5±0.2
(-) PAI + MPTP	53.4±6.9	7.0±0.6
(+) PAI	185.0±6.9	3.3±0.3
(+) PAI + MPTP	177.8±14.4	6.0±0.3
30 (-) Deprenyl	170.6±7.1	5.6±0.3
(-) Deprenyl + MPTP	197.0±8.0	6.4±0.5

Above values for DA and DOPAC expressed as Mean±S.E.M. and number of rats. n = 7-11 in each group.

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These results indicate that the [R](+)PAI is an excellent MAO-B inhibitor in vivo, and is of especially great potential for the treatment of Parkinson's disease.

- 5 While the invention has been described with reference to the aforementioned Examples and the accompanying Tables and Figures, it is not restricted thereto. Various modifications and applications of the invention are possible. For example, [R](+)PAI may be combined, in a
10 synergistic way, with α -tocopherol (a vitamin E derivative) for the treatment of Parkinson's disease.

EXAMPLE 28

Effect of PAI enantiomers on amphetamine induced stereotype 15 behavior in senescent rats

- Amphetamine is known to induce stereotypic behavior (Sulser, F., and Sanders-Bush, E., Ann. Rev. Pharmacol., 11, 209-230 (1971)) by the mobilization of endogenous dopamine.
20 Amphetamine is not metabolized by MAO-B. Inhibition of MAO-B by an effective inhibitor and administration of amphetamine cause release of dopamine which will not undergo degradation by the inhibited MAO-B. Thus, an increase of synaptic dopamine is expected after administration of
25 amphetamine and effective MAO-B inhibitor leading to an increase in stereotype behavior-potentiation of the amphetamine effect. The extent of this behavior is rated in accordance with the number of lateral head movements over a period of 1 minute.

30

Experimental Protocol

- The test compound was administered at a dose of 0.5 mg/kg/day in drinking water, 24 hours before the infliction of hypoxia (92% nitrogen + 8% oxygen for 6 hours).
35 Following that, amphetamine was injected s.c. at a dose of

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0.5 mg/kg. 45 minutes later, lateral head movements were counted.

Results

5 The results of these experiments are shown in Table 5.

Table 5

10 EFFECT OF PAI ISOMERS ON AMPHETAMINE-INDUCED STEREOTYPE
BEHAVIOR IN SENESCENT RATS (CONTROL AND HYPOXIA LESIONED)

<u>Group</u>		<u>Stereotype Treatment Behavior Rating</u>
	Control (6) -	87±10
15	Control (5) (+)PAI	126±16*
	Control (4) (-)PAI	94±18
	Hypoxia lesioned (5) -	93±12
	Hypoxia lesioned (6) (+)PAI	143±6*

20 Numbers in parentheses are numbers of animals tested.
*P<0.001 with respect to untreated hypoxia group or
untreated control group correspondingly.

25 The results in Table 5 indicate that (+)PAI caused
significant potentiation of the amphetamine-induced
stereotype behavior in both hypoxia-lesioned and control
rats. (-)PAI was totally inactive in this respect. These
30 behavioral in vivo results corroborate previous biochemical
findings that (+)PAI is an active inhibitor of MAO-B in the
brain while (-)PAI is inactive in this respect.

EXAMPLE 29

35 Effect on [R] (+)PAI on the improvement or restoration of
memory.

Newborn rat pups subjected to a brief episode of anoxia and
then allowed to resume their growth in a normal way develop

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a long-lasting impairment of memory (Speiser, et al., Behav. Brain Res., 30, 89-94 (1988)). This memory impairment is expressed as an inferior performance in the passive avoidance test.

5

The effect of [R](+)PAI and [S](-)PAI on the improvement or restoration of memory was investigated in the passive avoidance test. If the drug is effective, it increases the latency of response to enter a dark compartment or chamber
10 where an electroshock has been experienced earlier by the rat being tested. The latency of the maximal response is 300 seconds.

Experimental Protocol

15 Young rats were subjected to post-natal anoxia as described in Example 27. [R](+)PAI or [S](-)PAI were administered according to one of the following protocols.

Protocol A - Nursing mothers were given a dose of either
20 isomer of 1-1.5 mg/kg/day in drinking water until weaning at 21 days. Following that, the weaned offsprings were directly treated with the same dose for 20 days. Treatment was terminated at 40 days and the test was performed at 60 days, that is 20 days after the last dose of the drug.

25

Protocol B - The dose was reduced to 0.5 mg/kg/day administered to the nursing mother until weaning at 21 days, then directly to the young rats to 60 days at which time the test was performed.

30

Passive Avoidance Test - The apparatus consisted of a lit chamber adjoining a dark chamber and a sliding door separating the two. At training, a rat was placed in the lit chamber for 30 seconds, and then the door was opened. The
35 rat moved to the dark chamber with a latency that was re-

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corded. Upon entry of the rat into the dark compartment, the door was closed and a 0.3 mA foot-shock was delivered for 3 seconds.

- 5 Retention (memory) after 48 hours was determined by repeating the test and recording the latency to step through from light to darkness to an arbitrary maximum of 300 seconds.

10 Results

The results of these experiments are shown in Table 6.

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Table 6

5 EFFECT OF PAI ISOMERS ON PASSIVE AVOIDANCE
RESPONSE IN YOUNG RATS (60-DAYS OLD)

PROTOCOL A

	<u>Group</u>	<u>Treatment</u>	<u>Before</u> <u>Electroshock</u>	<u>After</u> <u>Electroshock</u>
	Control	-	49 ± 13	201 ± 111
10	Control	(+) PAI	49 ± 19	220 ± 100 (+9%)*
	Control	(-) PAI	48 ± 13	192 ± 116
	Anoxia-lesioned	-	45 ± 11	183 ± 109
	Anoxia-lesioned	(+) PAI	49 ± 10	239 ± 99 (19%)*
15	Anoxia-lesioned	(-) PAI	55 ± 27	179 ± 123

PROTOCOL B

	<u>Group</u>	<u>Treatment</u>	<u>Before</u> <u>Electroshock</u>	<u>After</u> <u>Electroshock</u>
20	Control	-	53 ± 20	104 ± 101
	Control	(+) PAI	48 ± 11	128 ± 119 (+23%)*
	Anoxia-lesioned	-	45 ± 8	119 ± 105
	Anoxia-lesioned	(+) PAI	52 ± 12	137 ± 126 (+15%)*
25	Anoxia-lesioned	(-) PAI	48 ± 19	112 ± 112

Figures represent the latency in seconds for entering a dark compartment where an electroshock had been first experienced by the rat tested.

30

* The indicated percent increases are with respect to the corresponding anoxia or control groups.

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The experimental results indicated that (+)PAI but not (-) PAI is effective in improving the memory of anoxia-lesioned and control rats. Drugs active in this test are considered to be potentially useful for treatment of various memory impairment disorders, dementia and especially senile dementia of the Alzheimer's type.

EXAMPLE 30

Effect of [R](+)PAI on the anoxia-induced hyperactive syndrome in juvenile rats

Rats that had been exposed postnatally to anoxia and then left to grow under normal conditions show increased motor activity in the open field at the age of 10-42 days (Hertshkowitz, et al., Dev. Brain Res., 7, 145-155 (1983)).

The effect of [R](+)PAI and [S](-)PAI on such hyperactive syndrome was investigated.

Experimental Protocol

Anoxia was performed on rat pups on the first post-natal day. They were placed in a glass chamber and exposed to 100% nitrogen for 25 minutes. They were resuscitated by intermittent massage softly applied to the chest and then returned to their respective mothers. Control rats received the same treatment but with air instead of nitrogen.

The [R](+)PAI or [S](-)PAI (0.5 mg/kg/day) was administered to the nursing mothers in drinking water, thereby transferred to the sucklings through milk.

Locomotion was measured in 6 fully computerized cages (28 x 28 cm) by recording the number of crossings over a given period of time. Crossings of grid infrared beams at 4-cm intervals initiated electrical impulses which fed a counter.

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Recordings of motor activity were made at the ages of 15 and 20 days, over a period of 15 minutes.

Results

5 The experimental results are given in Table 7.

Table 7

EFFECT OF EACH OF THE TWO ENANTIOMERS ON THE
ANOXIA-INDUCED HYPERACTIVE SYNDROME

10

<u>Group</u>	<u>Treatment</u>	15-day old <u>rats</u>	20-day old <u>rats</u>
Control	-	414 ± 192 (11)	808 ± 212 (12)
Control	(+) PAI	254 ± 149 (11) c	719 ± 110 (13)
15 Anoxia- lesioned	-	482 ± 119 (7)	858 ± 96 (9)
Anoxia- lesioned	(+) PAI	276 ± 186 (15) a	737 ± 150 (16) c
20 Anoxia- lesioned	(-) PAI	334 ± 196 (5)	778 ± 232 (6)

Numbers in parenthesis are numbers of animals tested.
- The figures are the numbers of crossings of infrared
25 beam grid in the activity cage over a period of 15
minutes.

a P<0.001 compared to anoxia untreated group.

b P<0.05 compared to anoxia untreated group.

c P<0.05 compared to control group.

30

These results indicate that chronic oral treatment with
[R] (+) PAI at a dose of 0.5 mg/kg administered to the nursing
mother and reaching the milk-fed offspring significantly
35 improved the hyperactive syndrome. Consequently, [R] (+) PAI
is a potentially useful drug for the treatment of the
hyperactive syndrome in children.

EXAMPLE 31Stability differences among ten salts of PAI

Stability is an important factor in the selection of an
5 optimal salt as a therapeutic drug. Different salts may
alter the physicochemical and biological characteristics of
a drug and can have a dramatic influence on its overall
properties. (Berge, S.M., et al., J. Pharm. Sci. 66, 1
(1977); Gould, P.L., Int. J. Pharmaceutics, 33, 201 (1986)).

10

ExperimentalSynthesis of PAI salts

A solution of an appropriate acid (1 mol-eq.) in 2-propanol
was added to a solution of PAI (1 mol-eq.) while stirring in
15 2-propanol (Ar, BHT). The salt formed was filtered, washed
with 2-propanol and ether, and dried under low pressure.
Yields were between 70 to 90%. An exception in preparing
PAI acetate involved using ether as the solvent.

20 Analytical methods

The chromatographic separations were carried out using a
Lichrosphere 60 RP select B 5 μ 125x4 mm (Merck) column, an
HPLC (Jasco BIP-1) equipped with a L-4200 UV-Vis detector
(Merck-Hitachi) set to 210 nm, and a D-2500 chromato-
25 integrator (Merck-Hitachi). The eluent and diluent
consisted of 80% distilled water/20% acetonitrile (HPLC
grade), and 0.07 M perchloric acid adjusted to pH 2.5 with
aqueous ammonia. The flow rate used was 1 ml/min, the
appropriate PAI salt solution concentration was 250 μ g/ml,
30 and 20 μ l of the solution were injected onto the
chromatographic system.

The melting range was measured with an automatic apparatus
(Mettler FP 80) and thermo-gravimetric analysis was
35 performed on a Mettler TA 3000 system at a rate of 10°C/min

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in the applicable range. Solubility was determined by an appropriate dilution of the supernatant from a saturated PAI salt water solution and measured in a UVIKON 941 (Kontron) UV-Vis spectrophotometer. The salt form (mono- or di-salt) was obtained by elemental analysis using standard equipment for C, H, N and S determination. The pH was measured in a 1% aqueous solution of the PAI salts.

Results

The characterization of the various salts are summarized in Table 8.

Table 8

PHYSICOCHEMICAL PROPERTIES OF PAI SALTS

PAI-salt m.w.	pH	Solubility mg/ml	Melting range (°C)	%Wt. loss	Salt form
20 tartarate 492	5.5	33	176.2-177.3	LT 0.1	di
mesylate 267	4.3	635	156.8-157.6	0.1	mono
25 maleate 287	4.0	NLT 1000	87.2-87.8	0.1	mono
sulphate 440	3.9	485	159.4-161.1	3.2	di
30 chloride 207	4.2	238	177.0-180.0	LT 0.5	mono
tosylate 343	4.4	60-70	129.3-129.9	LT 0.1	mono
fumarate 287	3.5	95	125.4-126.2	0.2	mono
35 phosphate n.a.	7.0	NLT 720	109.5-110.4	n.a.	n.a.
esylate 279	2.4	NLT 300	n.a.	n.a.	mono
40 acetate 231	6.1	NLT 720	69.2-69.7	0.4	mono

n.a.= not available

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Comparative stability studies were carried out under sets of several accelerating conditions: I) heating at 80°C for 72, 96 or 144 hours; and II) reflux in isopropanol for 30 hours. The degradation products developed were measured by 5 HPLC and confirmed by TLC. The results are presented in Table 9 with the relative retention time (relative to the PAI peak; RRT) as an area percentage relative to total integrated peak area.

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Table 9

DEGRADATION PRODUCTS DEVELOPED IN PAI SALTS UNDER SHORT TERM CONDITIONS

5	Salt	80C/72h		80C/144h		Reflux in iPrOH/30h	
		RRT ^a	% ^b	RRT	%	RRT	%
	sulfate	ND ^c	ND	ND	ND	0.47	0.22
						0.60	0.72
10	phosphate	0.60	0.22	0.60	0.57	0.60	2.62
						0.74	0.21
						1.84	0.20
						1.98	0.73
15	chloride	ND	ND	ND	ND	2.23	0.71
	mesylate	ND	ND	ND	ND	0.60	0.08
20	maleate	0.60	0.41	n.a.		0.60	2.17
		1.27	0.50			0.65	1.35
		1.48	0.33			1.29	0.59
		1.81	0.10			1.42	1.30
		3.07	1.44			1.50	0.16
25		4.16	0.10			1.83	0.18
		4.84	7.76			1.98	0.23
						4.09	0.65
	acetate	0.44	0.10	n.a.		0.60	6.74
30		0.60	2.56			0.74	0.35
		0.73	0.13			1.76	0.33
		1.29	0.71			1.84	0.16
		1.55	1.06			1.99	4.17
		1.75	21.85			3.60	0.27
35		1.96	3.33				
		2.15	0.08				
		2.32	0.15				
		2.83	0.15				
		3.54	1.82				
40	esylate ^d	ND	ND	0.85	0.26	ND	ND
				1.96	0.31		

limit of quantitation=0.08%

45 n.a.=not available

^aRelative retention time (relative to the PAI peak).^bArea percentage relative to total integrated peak area.^cNo impurities detected.^dEthyl sulfate salt.

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The salts were submitted to visual inspection of color and form. The findings are shown in Table 10.

5

Table 10

APPEARANCE OF PAI SALTS UNDER DESTRUCTIVE CONDITIONS

	Salt	80°C/72h	80°C/96h	80°C/144h	reflux in iPrOH/30h
10	sulfate	off white powder	n.a.	off white powder	brown powder
15	phosphate	brownish powder	n.a.	brown powder	brown powder
20	chloride	white powder	n.a.	white powder	off white powder
	mesylate	white powder	n.a.	white powder	white powder
25	maleate	brown melted	brown	n.a.	brown melted
	esylate	brownish melted	n.a.	dark brown melted	dark brown melted
30	n.a.= not available				

These studies show that sulphate, esylate and mesylate possess significant advantages relative to the other salts due to good solubility and chemical stability. Of these three salts, mesylate is preferable due to its excellent stability even under destructive conditions.

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EXAMPLE 32**Reversal of haloperidol-induced catalepsy in mice**

Male, ICR mice 25-30g each, were pretreated with either of
5 the following drugs: Saline, [R](+)PAI mesylate, or
racemic-PAI mesylate. All drugs were administered i.p. in
a volume of 0.2 mL. Two hours later, haloperidol was
injected s.c. at a dose of 6mg/kg in a volume of 0.1-0.2 mL.
Motor coordination tests were made at 3 hours after giving
10 haloperidol, that is, 5 hours after administering the
presumed protective drugs.

Motor coordination tests and rigidity were quantified
according to three different parameters: (a) ability to
15 walk the length of a horizontal rod, 80 cm-long; (b) ability
to climb down, face down, a vertical rod, 80 cm-long; (c)
duration of immobility in an unnatural sitting posture
whereby the abdomen of the mouse is pressed against a
"wall." Full performance as in haloperidol-untreated mice
20 is given the score of 4 in each test, or a total of 12 in
all tests. Poor performance is given a score from 1 to 3.
A key to score ratings is given in Table 9A. The effects of
the various agents in antagonizing haloperidol-induced
catalepsy are given in Table 11. At three hours after
25 haloperidol, [R](+)PAI mesylate conferred protection against
haloperidol at 5-15 mg/kg, reaching a peak after effect at
7.5-10 mg/kg (activity score \approx 94% of saline control).
Racemic PAI mesylate conferred partial protection in the
range of 7.5-15 mg/kg, and was not active at 5 mg/kg. From
30 Figure 17, it can be seen that the dose-effect profile of
either [R](+)PAI mesylate or racemic PAI is such that an
increase in dose beyond 10 mg/kg entails a decrease in
effect, but that the racemic mixture is less potent
throughout. This means that racemic PAI mesylate at twice

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the dose of [R](+)PAI mesylate will always be less active than the (R) enantiomer.

Reversal of α -MpT-induced hypokinesia in rats

5 The drug α -MpT is assumed to inhibit the formation of L-DOPA from tyrosine, and consequently the formation of dopamine itself. Lack of CNS dopamine is expressed as hypoactivity. Six month-old male Wistar rats (from Harlan Orkack, UK) were pretreated with saline, [R](+)PAI Mesylate or Rac PAI
10 Mesylate, at the indicated doses. Two hours later they received i.p. α -MpT at a dose of 100 mg/kg in 0.3-0.5 mL. Controls received saline. Following this, motor activity was recorded in a computerized activity cage for the duration of 10 hours. The results are given in Table 12 and
15 Figure 18. At 2 mg/kg, [R](+)PAI Mesylate restored the level of activity to about 90% of the saline-treated rats, but Rac PAI Mesylate was not active. In either case, the profile of the dose-effect curve was bell-shaped, suggesting a decrease in effect with an increase in dose beyond a peak
20 of 2-5 mg/kg. At 5 mg/kg Rac PAI Mesylate could not elicit a level of activity comparable to that of [R](+)PAI Mesylate at 2 mg/kg.

From these measurements, [R](+)PAI Mesylate and Rac PAI
25 Mesylate do not share a similar pattern of activity in the restoration of normokinesia in haloperidol-treated mice and α -Mpt-treated rats. At all doses studied, [R](+)PAI Mesylate is always more potent than Rac PAI Mesylate at the corresponding dose. Also, peak activity of Rac PAI Mesylate
30 is always lower than peak activity of [R](+)PAI Mesylate. Thus, Rac PAI Mesylate at a given dose is always less effective than [R](+)PAI Mesylate at half the same dose. Doubling the dose of Rac PAI Mesylate with respect to
[R](+)PAI Mesylate does not produce an effect equivalent to
35 that of [R](+)PAI Mesylate.

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Pharmacologically, Rac PAI Mesylate cannot be considered as consisting of 50% active ingredient which is [R](+)PAI Mesylate and 50% inert material as diluent. The presence of [S](-)PAI in Rac PAI Mesylate has an adverse effect on the
5 activity of [R](+)PAI, resulting in a more than two-fold decrease in potency. The decrease may be due to a direct adverse effect of [S](-)PAI on behavioral parameters.

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Table 11

REVERSAL OF HALOPERIDOL-INDUCED CATALEPSY IN MICE
WITH [R] (+) PAI MESYLATE AND RACEMIC MESYLATE

5 Mice received each of the test drugs i.p. at the indicated doses. Two hours later they received haloperidol as described in the text. The doses shown are for the free base.

10

	Dose, mg/kg	<u>[R] (+) PAI Mesylate</u>			<u>Rac PAI Mesylate</u>		
		<u>Score+SE</u>	<u>n</u>	<u>% of control</u>	<u>Score+SE</u>	<u>n</u>	<u>% of control</u>
15	1.8	7.2±1	6	60	7.0±0.6	6	59
	3.0	6.4±0.5	6	60	5.9±0.7	6	49
	5.0	8.7±0.9*	6	73	6.4±0.4	6	53
	7.5	11.0±0.4***	5	92	9.4±0.8++	6	78
	10	11.3±0.3***	6	94	9.2±0.6***	6	77
20	15	10.8±0.5***	5	90	8.8±0.8*	6	73
<hr/>							
	Control						
	saline	12±0	12	100			
25	Haloperidol						
	alone	6.6±0.3	16	59			

Statistical significance with respect to haloperidol alone:
*p≤0.05; **p≤0.01; ***p≤0.001 by the Student's "t" test.

30 The scores for [R] (+) PAI are significantly different from those of racemic PAI at 5 mg/kg, p≤0.05; at 10 mg/kg, p≤0.01; and at 15 mg/kg, p≤0.05.

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Table 11A

5 KEY TO SCORE RATING OF HALOPERIDOL-INDUCED CATALEPSY
IN MICE AND ITS REVERSAL BY VARIOUS AGENTS

Vertical Rod:

	Unable to grasp rod with limbs	1
	Able to grasp but slips down	2
10	Able to grasp, partly slips, partly climbs down	3
	Able to grasp, climbs down using all limbs	4

Horizontal Rod:

	Unable to grasp, falls off rod	1
15	Able to grasp, unable to walk on rod more than 2 paces	2
	Able to grasp, walks half-length of rod	3
	Able to grasp, walks full length of rod	4

Immobility Sitting Against Wall:

20	Immobility > 5 min	1
	Immobility 3-5 min	2
	Immobility 1-3 min	3
	Immobility 0.1 min	4

- 25 Fractional scores are assigned, such as 2.5, when behavior falls between two categories, as between 2 and 3.

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Table 12

RESTORATION OF MOTOR ACTIVITY IN RATS TREATED WITH
 α -METHYL-p-TYROSINE (α -MpT) AT 100 mg/kg i.p.

5

Rats received the test drugs i.p. at the indicated doses. After two hours they received α -MpT and were immediately placed in activity cages. Total motor activity was automatically recorded for 10 hours, as described in the

10

	[R] (+) PAI Mesylate				Rac PAI Mesylate		
	Dose, mg/kg	Score+SE	n	% of control	Score+SE	n	% of control
15	2	14,132** ±1457	7	89	9,035 ±829	6	57
20	5	12,893* ±1,869	7	81	10,926* ±820	8	69
	7.5	6,679 ±414	4	42	9,698 ±557	4	61
25	Control saline	15,862 ±1,424	5	100			
30	α -Mpt alone	8,108*** ±810	6	51			

35 Statistical significance by the Student's "t", * $p \leq 0.01$;
 *** $p \leq 0.001$ for Test drugs+ α -Mpt versus α -MpT alone
 α -Mpt alone versus control saline

The scores of [R] (+) PAI versus racemic PAI are significantly
 different at 2 mg/kg, $p \leq 0.01$.

40

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EXAMPLE 33The effects of [R](+)PAI Mesylate following closed head injury in rats5 Methods1. Induction of trauma

Head trauma was induced in male rats under ether anesthesia by a well calibrated weight-drop device that falls over the exposed skull, covering the left cerebral hemisphere, 1-2 mm
10 lateral to the midline, in the midcoronal plane.

2. Evaluation of motor function

One hour after induction of trauma, the rats were tested by a set of criteria which evaluated their neurologic outcome
15 (the criteria described by Shohami, et al., J. Neurotrauma, 10, 113 (1993)). These criteria, referred to as the Neurological Severity Score (NSS), consist of a series of reflexes and motor functions. Points are given based on deficits in these criteria. At 24h the rats were re-
20 evaluated.

3. Evaluation of brain edema

The brains were removed after the second evaluation of motor function (24h). A piece of tissue (~20 mg) was weighed to
25 yield wet weight (WW). After drying in a desiccator oven for 24h at 95°C, it was reweighed to yield dry weight (DW). Water percentage in the tissue was calculated as $(WW-DW) \times 100/WW$.

30 4. Drug treatment

[R](+)PAI Mesylate was dissolved in water. The rats were injected intraperitoneally at a dose of 0.1 mg/kg, 0, 4, 8 and 12 h after induction of head trauma. Control rats were treated with water at the same times.

35

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Results

The NSS, which measures the "clinical" status of the rats, was almost identical in the treated and nontreated groups at 1 hour after the head injury, but significantly lower at 24 hours in the [R](+)PAI mesylate-treated rats (Table 13). These results indicate that PAI mesylate is effective in improving motor function recovery following closed head injury in rats.

At 24 hours after trauma, a major edema was found in the hemisphere (85.4% water in the brain of control rats vs. 78.5% in undamaged brain tissue). PAI mesylate was effective in reducing edema as verified by its effect on the percent of water.

15

In conclusion, the results reported herein demonstrate that [R](+)PAI mesylate has neuroprotective properties in a model intended to mimic human nerve injury and to induce trauma to a closed skull.

20

Table 13

	NSS		Δ NSS (1h - 24h)	% H ₂ O in the brain
	1 h	24 h		
control (n = 6)	15.6	12.3	4.3 \pm 0.5	85.4 \pm 0.4
[R](+)PAI Mesylate (n = 6)	16.7	10.2	6.5 \pm 0.7*	82.1 \pm 0.6**

30

* P < 0.05 (Mann Whitney U-test)

** P < 0.005 (t-test)

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EXAMPLE 34Effects of PAI mesylate on prevention of NMDA induced cell death of cerebellum cell culturesResults of in vitro assays

5

Procedures: Cultures of mechanically dissociated neonatal rat cerebellum. The cerebella are dissected aseptically from 6 or 7-day-old rat pups and placed in a 15 ml sterile plastic conic tube containing 3 ml of enriched medium (the medium is made up of Dulbecco's modified Eagle's medium (DMEM) with high glucose concentration (1g/l), 2 mM (v/v) L-glutamine, antibiotic antimetabolic mixture, and enriched with 15% (v/v) heat-inactivated fetal calf serum). The cerebella are then dissociated after 20-25 passages through a sterile 13 gauge, 10 cm long stainless steel needle attached to a 5 ml syringe with an inserted 45 μ m pore size nylon sieve. The dissociated cells are centrifuged at 200g for 5 minutes, the supernatant discarded and the cells resuspended in enriched medium. The cell viability is determined by the trypan blue exclusion test. The cells are then plated at a density of 200/mm² on poly-L-lysine-coated surfaces (Poly-L-lysine-coated glass coverslips are prepared at least 1 hour before plating, by immersing in a sterile distilled water solution containing 15 μ g/ml poly-L-lysine, and just before use, washing with sterile water and drying), covered with enriched medium, and incubated at 37°C in an atmosphere of 5% CO₂ in air and 100% humidity. After 4 days in culture, the media are replaced with media containing the desired test compounds. Experiments are done in duplicate and repeated 2 or 3 times. After determining the test compound toxic dose-response, four groups are compared: (I) control (enriched medium alone), (II) test compound (one subgroup for each concentration (2 concentrations are tested)), (III) N-methyl-D-aspartate (NMDA, exposure to a concentration of

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1 mM for 3 h) as the cytotoxic challenge, (IV) test compound plus NMDA (one subgroup for each of the 2 concentrations of test compounds), (V) control group to test the effect of solvent (in which the test compound is dissolved), and (VI) 5 an additional "positive control" group of spermine (0.01 μ M dissolved in culture medium) plus NMDA. Nerve cell survival is evaluated by phase contrast microscopy and trypan blue staining after 24 h.

10 Results

It is well established that glutamic acid (Glu) possesses neurotoxic properties which are expressed in several neurological disorders including epilepsy and stroke, and most likely also in brain neurodegenerative diseases such as 15 Parkinson's disease, Alzheimer's disease and traumatic brain injury. The neurotoxic effects of Glu are mediated by membrane bound Glu receptors, such as N-methyl-D-aspartate (NMDA) receptors.

20 The results, as shown in Table 14, demonstrate that 10 μ M of [R](+)PAI mesylate increased the survival of cerebellum cells by 27 percent following 1 μ M NMDA exposure. These in vitro results support the in vivo effects of [R](+)PAI mesylate presented in Examples 33 and 35, indicating that 25 this drug has neuroprotective properties against neurotoxic concentration of NMDA.

Table 14

5 NEUROPROTECTIVE EFFECT OF [R] (+) PAI MESYLATE ON
PREVENTION OF NMDA-INDUCED CELL DEATH OF CEREBELLUM
CELLS

10	Experimental Group	Surviving Cells	Percent Protection
	<u>Cerebellar Cultures</u>		
	(Toxicity TD_{25} = 30 μ M; TD_{50} = 85 μ M; TD_{100} = 320 μ M)		
	Control	100	
15	Solvent	97	
	NMDA	10	
	Solvent + NMDA	10	0
	Compound + NMDA:		
	1) 0.01 μ M + "	12	2
20	2) 1.00 μ M + "	22	12
	3) 10.00 μ M + "	37	27
	Spermine + NMDA	75	65

25 Values, expressed as the percent of untreated controls, represent the average of 2 experiments run in duplicate for culture experiments, and the mean \pm SEM of 4 animals for ischemia. The percent protection value is the effect of the test compound after subtraction of the solvent effect.

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EXAMPLE 35Effects of [R] (+)PAI Mesylate after graded crush of the rat optic nerve

5 Neuroprotective effects of [R] (+)PAI Mesylate were determined for application immediately after crush injury of the optic nerve in the adult rat. Short-term effects were measured metabolically, and long-term effects electrophysiologically.

10

METHODS1. Metabolic measurements

15 a) General. The method is described by Yoles, et al., Investigative Ophthalmology & Visual Science, 33, 3586-91 (1992). At short terms, metabolic measurements were monitored in terms of the mitochondrial NADH/NAD ratio, which depends on the activity of the electron transport
20 system, and thus indicate levels of energy production. Changes in ability of the nerve to produce energy as a consequence of injury were determined by comparing NADH levels in response to artificial transient anoxic insult before and after the injury.

25

b) Surface fluorometry - reflectometry. Monitoring of the intramitochondrial NADH redox state is based on the fact that NADH, unlike the oxidized form NAD⁺, fluoresces when illuminated at 450 nm. A flexible Y-shaped bundle of optic
30 fibers (light guide) was used to transmit the light to and from the optic nerve. The light emitted from the nerve was measured at two wavelengths: 366 nm (reflected light) and 450 nm (fluorescent light). Changes in the reflected light were correlated with changes in tissue absorption caused by

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hemodynamic effects and with movements of the optic nerve secondary to alterations in arterial blood pressure and nerve volume. The fluorescence measurements were found to be adequately corrected for NADH redox state measurements by subtraction of the reflected light (366 nm) from the fluorescent light (1:1 ratio) to obtain the corrected fluorescence signal.

c) Animal preparation. Animal utilization was in accord with the ARVO Resolution on the use of animals in research. Male Sprague-Dawley (SPD) rats weighing 300-400g were anesthetized with sodium pentobarbitone (50 mg/kg intraperitoneally). With the animal's head held in place by a head holder, a lateral canthotomy was performed under a binocular operating microscope and the conjunctiva was incised lateral to the cornea. After separation of the retractor bulbi muscles, the optic nerve was identified and a length of 3-3.5 mm was exposed near the eyeball by blunt dissection. The dura was left intact and care was taken not to injure the nerve. A special light-guide holder was implanted around the optic nerve in such a way that the light guide was located on the surface of the optic nerve 1 mm distal to the injury site. Animals, while still anesthetized, were allowed to recover for 30 minutes from the surgical procedures and were then exposed to anoxic conditions. An anoxic state was achieved by having the rat breathe in an atmosphere of 100% nitrogen for 2 minutes, after which time it was returned to air. In order to evaluate the metabolic activity of the optic nerve, the relative changes in reflected and fluorescent light intensities in response to anoxia were measured before and after crush injury.

d) Experimental protocol for crush injury and metabolic measurements. With the aid of calibrated cross-section

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forceps, a moderate crush injury was inflicted on the nerve between the eye and the light guide holder at a pressure corresponding to 120g for 30 sec. Immediately after injury, animals received intraperitoneal injections of water with and without [R](+)PAI Mesylate (2 mg/kg). To assess the activity of the energy production system, NADH response to 2 minutes of anoxia was measured in all animals prior to injury, 30 minutes after injury, and thereafter at hourly intervals up to 4 hours (see Figure 19).

10

2. Electrophysiological Measurements. This method is described by Assia, et al., Brain Res., 476, 205-212 (1989). Animal preparation and optic nerve injury were preferred as in the metabolic studies. Immediately after injury, animals received a single injection of water with or without [R](+)PAI Mesylate (0.5 mg/kg). Fourteen days after injury and treatment, the optic nerves were excised and measured electrophysiologically. Prior to removal of optic nerves for electrophysiological measurement, the rats were deeply anesthetized with 70 mg/kg pentobarbitone. The skin was removed from the skull and the optic nerves were detached from the eyeballs. Subtotal decapitation was performed and the skull was opened with a rongeur. The cerebrum was displaced laterally, exposing the intracranial portion of the optic nerve. Dissection was at the level of the nerve, which was transferred to vials containing fresh salt solution consisting of NaCl (126 mM), KCl (3 mM), NaH_2PO_4 (1.25 mM), NaHCO_3 (26 mM), MgSO_4 (2mM), CaCl_2 (2 mM), and D-glucose (10 mM), and aerated with 95% O_2 and 5% CO_2 at room temperature. The nerves were kept in this solution, in which electrical activity remained stable for at least 3-4 hours. After 0.5 hours of recovery at room temperature, electrophysiological recordings were obtained from the nerve distal to the crush lesion. The nerve ends were then

30

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connected to two suction Ag-AgCl electrodes immersed in a bathing solution at 37°C. A stimulating pulse was applied through the electrode at the proximal end and the action potential was recorded by the distal electrode. A Grass SD9
5 stimulator was used for supramaximal electrical stimulation (0.5 pps). The measured signal was transmitted to a Medelec PA36 preamplifier and then to an electromyograph (Medelec MS7, AA7T amplifier). The solution, stimulator and amplifier had a common ground. The maximum amplitude of
10 eight averaged compound action potentials (CAPs) was recorded and photographed with a Polaroid camera. The CAP values measured in contralateral uninjured nerves served as a reference.

15 Results

The results demonstrate that [R](+)PAI Mesylate applied immediately after optic nerve injury blocked the injury-induced reduction in energy production. [R](+)PAI Mesylate also has a long-term effect measured by electrophysiological
20 monitoring.

The CAP (compound action potentials) amplitude is directly correlated with the number of conducting fibers in the tested segment of the nerve.

25

[R](+)PAI Mesylate significantly attenuated the injury-induced loss of activity in the distal segment of the injured nerve, indicating that [R](+)PAI Mesylate is a neuroprotective agent or at least slows down degeneration.

30

Table 15
Electrophysiological Measurements

Group	CAP amplitude (μ V) (Mean \pm Std. Error.)
Vehicle N = 13	441 \pm 95
[R] (+) PAI Mesylate N = 7	2104 \pm 313*

EXAMPLE 36

Comparison of Anticonvulsive Properties of [R] (+) PAI and [S] (-) PAI Salts

Both [R] (+) PAI and [S] (-) PAI HCl salts have significant anticonvulsant activities. In mice (i.p. administration) in the maximal electroshock test (MES test), [S] (-) PAI HCl has greater anticonvulsant activity (ED_{50} =57 mg/kg) than [R] (+) PAI HCl (ED_{50} =79 mg/kg). Analogous results were observed in rats (p.o. administration). Four out of four rats were protected from seizures in the MES test when 50 mg/kg of [S] (-) PAI HCl was administered, whereas three out of four mice were protected after the same dose of [R] (+) PAI HCl. With respect to efficacy for Parkinson's disease, the enhanced anticonvulsant activity is a detrimental side effect. The same trend occurs with the mesylate salts. [S] (-) PAI Mesylate has greater anticonvulsant activity than [R] (+) PAI Mesylate in the MES test. At doses of 100 mg/kg, [S] (-) PAI Mesylate protected three out of three mice,

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whereas only one out of three mice was protected with [R] (+)PAI Mesylate.

The MES test is a classical model to indicate efficacy for partial and generalized seizures in humans. The agents' mechanism of action is via their ability to prevent the spread of seizures. Some agents, however, that prevent seizure spread have the side effect of lowering seizure threshold. These agents therefore have both proconvulsive and anticonvulsive side effects.

Results herein show that [S] (-)PAI Mesylate has proconvulsive activity. In the Timed Intravenous Infusion of Metrazol test, 141 mg/kg of [S] (-)PAI Mesylate reduces the time, and therefore the amount of Metrazol, required to induce the appearance of both the first focal seizure and the onset of clonus. Other agents that are classically used for partial and generalized seizures, such as phenytoin and carbamazepine, do not show this effect. (H.J. Kupferberg, Epilepsia, 30, s51-s56 (1989)). Likewise, [S] (-)PAI Mesylate showed a significantly higher acute neurotoxicity than [R] (+)PAI Mesylate. At 300 mg/kg, [R] (+)PAI Mesylate did not show any neurotoxicity with mice in the rotorod ataxia test. With [S] (-)PAI Mesylate, four out of four mice showed neurotoxicity and spasticity.

Methods

TD₅₀ (median toxic dose). This test measures neurological deficits by the rotorod ataxia test. A mouse is placed on a knurled rod rotating at 6 rpm. It is then determined whether a mouse has the ability to maintain its equilibrium and stay on the rod for one minute in each of three trials.

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Timed Intravenous Infusion of Metrazol Test. This test measures the minimal seizure threshold of each animal. Metrazol is infused at 0.185 mg/ml into the tail veins of mice. The time is then recorded (sec) from the start of
5 infusion until the appearance of the first twitch (first focal seizure) and onset of clonus (clonic seizure). Proconvulsants require less Metrazol to produce these symptoms and therefore show endpoints at a shorter period of time.

10

EXAMPLE 37Peripheral effects of [R](+)PAI and [S](-)PAI on the contractility of intestinal smooth muscle preparations

15 Peripheral effects of the hydrochloride salts of the enantiomers of PAI were determined in isolated rabbit or guinea-pig small intestine. These observations provide useful information on their relative peripheral side effects in humans. The first point of contact of the subject with
20 an orally administered drug is the gastrointestinal tract where concentrations of the drug are much higher than after absorption and distribution. In the case of PAI hydrochloride (MW = 208), a 10 mg oral dose contained in a liquid volume of about 100 ml would be equivalent to a
25 concentration of about 0.5 mM. In contrast, the therapeutic plasma concentration of [R](+)PAI hydrochloride is in the nanomolar range.

The effect of the enantiomers of PAI in the isolated rabbit
30 jejunum and the guinea-pig ileum were determined so as to find out whether the intake of [S](-)PAI together with [R](+)PAI (as found in racemic PAI) would produce side effects absent in the administration of pure [R](+)PAI. [R](+)PAI is the preferred enantiomer for the inhibition of
35 MAO-B in the brain, in view of its potency and high

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selectivity towards this form of the enzyme. [S](-)PAI is much less potent than [R](+)PAI in this respect and is also not selective toward MAO-B. In principle, its presence in PAI racemate might be tolerated or overlooked provided [S](-)PAI is inert at the recommended doses of [R](+)PAI. The results provided in Tables 16-19 show that [S](-)PAI is not an inert substance. On the contrary, in the guinea-pig ileum, it is a more potent relaxant than [R](+)PAI. Hence its peripheral effects cannot be discounted as negligible.

10 These data show that there would be fewer peripheral side effects in the administration of pure [R](+)PAI than in the administration of racemic PAI containing an equivalent dose of [R](+)PAI.

Table 16

5 TYRAMINE POTENTIATION BY EACH OF THE TWO ENANTIOMERS
OF PAI HCl IN RATE JEJUNUM PREPARATION

A stretch of rabbit jejunum, mounted in an organ bath, displays rhythmic contractions that are inhibited by norepinephrine but not by tyramine. If however the jejunum
10 is pretreated with a monoamine oxidase inhibitor such as PAI, then tyramine causes relaxation of the spontaneous contractions. The extent of relaxation can be correlated with the relative potency of the inhibitor.

15	<u>Drug and concentration (μM)</u>		<u>P e r c e n t</u> <u>relaxation</u>
	Tyramine alone	40	0
	Norepiniphrine	0.002	100
	[R] (+) PAI alone	0.2-4.0	0
20	[S] (-) PAI alone	0.2-4.0	0
	Tyramine	40	
	after R(+) PAI	0.2	67
		2	88
		40	85-90
25	after (S) PAI	0.2	0
		2	35
		40	33-50

30 Results

[S] (-) PAI is much less potent than [R] (+) PAI as an inhibitor of brain MAO-B. Therefore, [S] (-) PAI is not a useful agent for the prevention of brain dopamine degradation, but can potentiate the tyramine-evoked release of norepinephrine in
35 the small intestine. Its activity in the small intestine is

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an undesirable side effect as it is expected to increase the absorption and action of undegraded tyramine. Thus, [S](-)PAI is not an inert substance when used together with [R](+)PAI as found in racemic PAI.

5

Table 17

ANTAGONISM OF BETHANECHOL-INDUCED CONTRACTIONS OF THE GUINEA PIG ILEUM PREPARATION IN THE PRESENCE OF 400 μ M OF EACH OF THE TWO ENANTIOMERS OF PAI HCl

10

A stretch of guinea-pig ileum mounted in a physiological solution in an organ bath contracts dose-dependently when treated with bethanechol which is an enzymatically stable analog of the natural gastrointestinal neurotransmitter acetylcholine. These contractions are attenuated in the presence of PAI. The data are expressed in gram-tension.

<u>Bathenechol (μM)</u>		<u>gram-tension</u>		
	<u>control</u>	<u>[R](+)PAI</u>	<u>control</u>	<u>[S](-)PAI</u>
0.8	0.5	0.2	0.6	0
2	1.5	0.3	2.0	0
4	2.2	0.7	3.0	0
8	4.0	1.0	3.8	0.6
25 20	5.6	2.0	3.8	1.2
40	6.2	2.8	3.8	1.7
80	6.2	3.1	3.8	2.6
200	6.2	4.3	3.8	2.6

30

Results

[S](-)PAI is almost inactive as a MAO-B inhibitor with respect to [R](+)PAI, and hence is not effective in preventing the degradation of brain dopamine. However, it is more effective than [R](+)PAI in the prevention of the

35

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bethanechol-induced contraction of the small intestine. Thus [S](-)PAI is not an inert substance when used with [R](+)PAI as found in racemic PAI.

5

Table 18

ANTAGONISM OF THE HISTAMINE-INDUCED CONTRACTIONS OF THE GUINEA-PIG ILEUM PREPARATION BY EACH OF THE TWO ENANTIOMERS OF PAI HCl

10 A fixed dose of histamine (40 nM) causes a sustained contraction of a stretch of guinea-pig ileum mounted in physiological solution in an organ bath. Incremental addition of each of the two enantiomers of PAI HCl causes a dose-dependent relaxation of the muscle. Results are
15 expressed as percent relaxation with respect to the baseline before addition of histamine, which is taken as 100% relaxation.

PAI concentration		Percent relaxation	
	<u>μM</u>	<u>[R] (+) PAI</u>	<u>[S] (-) PAI</u>
20	2	0	11
	4	0	15
	10	0	30
	20		20
	30	31	33
25	40	37	36
	100	81	71
	200		90
	300	92	
30	400	100	98
	700	100	
	1000		100

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Results

[S](-)PAI is inactive with respect to [R](+)PAI as a MAO-B inhibitor in the brain, and hence useless for preventing the degradation of brain dopamine, but is more active than the (R) isomer in causing relaxation of intestinal smooth muscle. Thus, [S](-)PAI is not an inert substance when taken together with the (R)isomer as found in racemic PAI.

Table 19

ANTAGONISM OF THE BETHANECHOL-INDUCED CONTRACTIONS OF THE GUINEA-PIG ILEUM PREPARATION BY EACH OF THE TWO ENANTIOMERS OF PAI HCl

A fixed dose of bethanechol (0.8 μ M) causes a sustained contraction of a stretch of guinea-pig ileum mounted in physiological solution in an organ bath. Incremental addition of each of the two enantiomers of PAI HCl causes a dose-dependent relaxation of the preparation. Results are expressed as percent relaxation with respect to the baseline before addition of histamine, which is taken as 100% relaxation.

PAI concentration μ M	Percent relaxation	
	<u>[R](+)PAI</u>	<u>[S](-)PAI</u>
20	25	40-50
60	25-50	60-70
100	50-70	100
300	100	100

Results

[S](-)PAI is inactive with respect to [R](+)PAI as a MAO-B inhibitor in the brain, and hence useless for the prevention of the degradation of brain dopamine, but is more active than the (R) isomer in causing relaxation of intestinal

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smooth muscle. Thus, [S](-)PAI is not an inert substance when taken together with the (R) isomer as found in racemic PAI.

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What is claimed is:

1. Use of R(+)-N-propargyl-1-aminoindan or a
5 pharmaceutically acceptable salt thereof for the
manufacture of a composition for the treatment of a
neurodegenerative disease.
2. Use of R(+)-N-propargyl-1-aminoindan or a
10 pharmaceutically acceptable salt thereof for the
manufacture of a composition for the treatment of a
neurotoxic injury.
3. Use of R(+)-N-propargyl-1-aminoindan or a
15 pharmaceutically acceptable salt thereof for the
manufacture of a composition for the treatment of brain
ischemia.
4. Use of R(+)-N-propargyl-1-aminoindan or a
20 pharmaceutically acceptable salt thereof for the
manufacture of a composition for the treatment of head
trauma injury or spinal trauma injury.
5. Use of R(+)-N-propargyl-1-aminoindan or a
25 pharmaceutically acceptable salt thereof for the
manufacture of a composition for the treatment of
schizophrenia.
6. Use of R(+)-N-propargyl-1-aminoindan or a
30 pharmaceutically acceptable salt thereof for the
manufacture of a composition for the treatment of
attention deficit disorder.
7. Use of (+)-N-propargyl-1-aminoindan or a
35 pharmaceutically acceptable salt thereof for the

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manufacture of a composition for the treatment of multiple sclerosis.

- 5 8. Use of R(+)-N-propargyl-1-aminoindan or a
 pharmaceutically acceptable salt thereof for the
 manufacture of a composition for the prevention of
 nerve damage.
- 10 9. The use of claim 8, wherein the nerve damage is
 structural nerve damage.
10. The use of claim 8, wherein the structural nerve damage
 is optic nerve damage.
- 15 11. Use of R(+)-N-propargyl-1-aminoindan or a
 pharmaceutically acceptable salt thereof for the
 manufacture of a composition for the treatment of an
 affective illness.
- 20 12. Use of R(+)-N-propargyl-1-aminoindan or a
 pharmaceutically acceptable salt thereof for the
 treatment of symptoms of withdrawal from an addictive
 substance.
- 25 13. The use of claim 12, wherein the addictive substance is
 cocaine or alcohol.
14. A pharmaceutically acceptable salt of R(+)-N-propargyl-
 1-aminoindan, selected from the mesylate salt of R(+)-
30 N-propargyl-1-aminoindan, the esylate salt of R(+)-N-
 propargyl-1-aminoindan, and the sulfate salt of R(+)-N-
 propargyl-1-aminoindan.

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15. A pharmaceutical composition which comprises a therapeutically effective amount of the salt of claim 14 and a pharmaceutically acceptable carrier.
- 5 16. The pharmaceutical composition of claim 15, wherein the pharmaceutically acceptable carrier is a solid and the pharmaceutical composition is a tablet.
- 10 17. The pharmaceutical composition of claim 16, wherein the therapeutically effective amount is an amount from about 0.1 mg to about 100 mg.
- 15 18. The pharmaceutical composition of claim 17, wherein the therapeutically effective amount is an amount from about 1 mg to about 10 mg.
- 20 19. The pharmaceutical composition of claim 15, wherein the pharmaceutically acceptable carrier is a liquid and the pharmaceutical composition is an injectable solution.
- 25 20. The pharmaceutical composition of claim 19, wherein the therapeutically effective amount is an amount from about 0.1 mg/ml to about 100 mg/ml.
- 30 21. The pharmaceutical composition of claim 20, wherein the therapeutically effective amount is an amount from about 1 mg/ml to about 10 mg/ml.
22. The pharmaceutical composition of claim 15, wherein the carrier is a gel and the pharmaceutical composition is a suppository.
- 35 23. The pharmaceutical composition of claim 15, wherein the salt is the mesylate salt of R(+)-N-propargyl-1-aminoindan.

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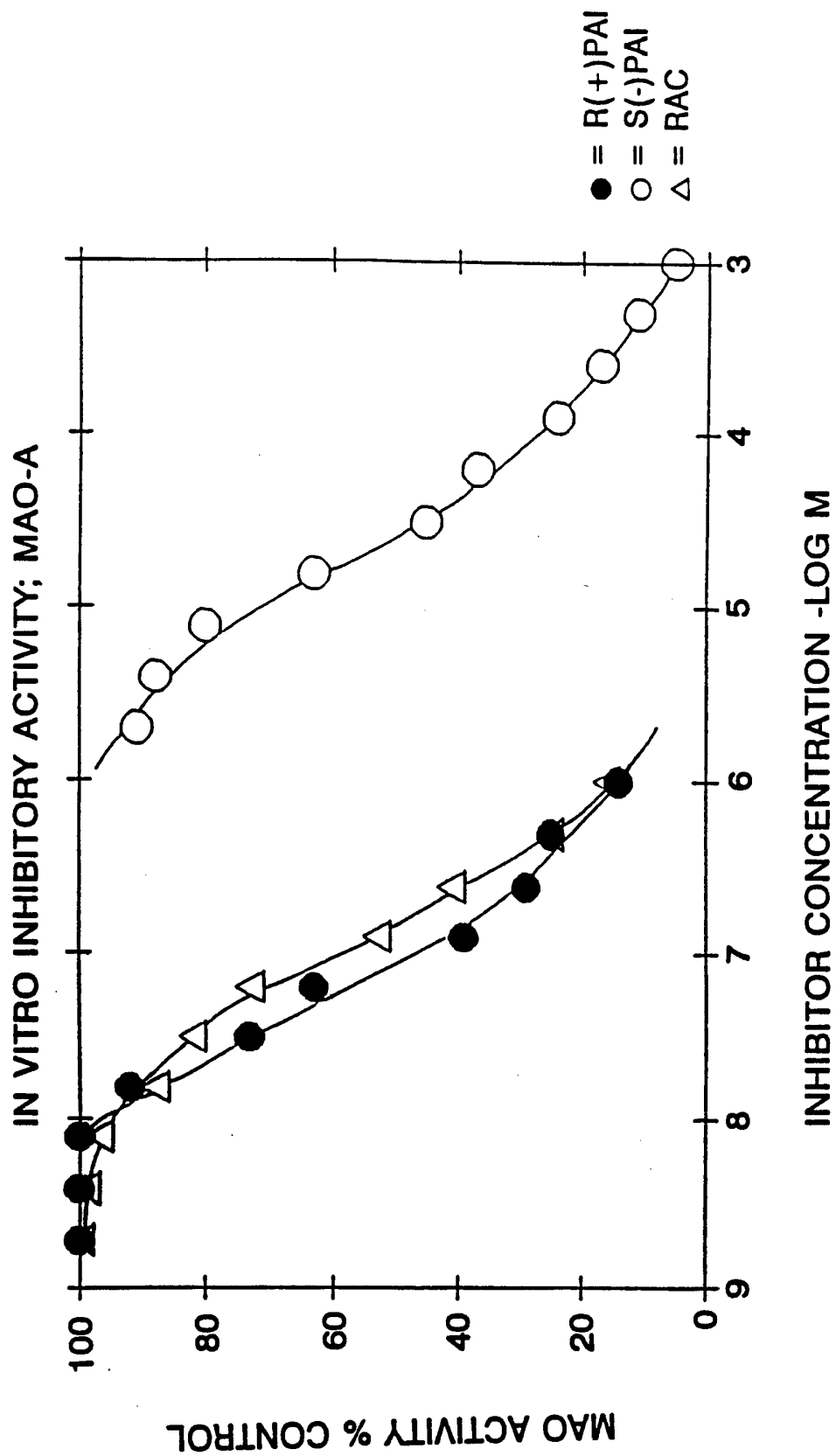
24. The pharmaceutical composition of claim 15 further comprising a therapeutically effective amount of Levodopa.
- 5 25. The pharmaceutical composition of claim 24 further comprising an effective amount of a decarboxylase inhibitor.
- 10 26. The pharmaceutical composition of claim 25, wherein the decarboxylase inhibitor is L-Carbidopa.
- 15 27. The pharmaceutical composition of claim 26, wherein the therapeutically effective amount of the salt of R(+)-N-propargyl-1-aminoindan is about 0.1 mg to about 100 mg, the therapeutically effective amount of Levodopa is about 50 mg to about 250 mg, and the effective amount of L-Carbidopa is about 10 mg to about 25 mg.
- 20 28. The pharmaceutical composition of claim 25, wherein the decarboxylase inhibitor is benserazide.
- 25 29. The pharmaceutical composition of claim 26, wherein the therapeutically effective amount of the salt of R(+)-N-propargyl-1-aminoindan is about 0.1 mg to about 100 mg, the therapeutically effective amount of Levodopa is about 50 mg to about 200 mg, and the effective amount of benserazide is about 12.5 mg to about 50 mg.
- 30 30. Use of the salt of claim 14 for the manufacture of a composition for the treatment of Parkinson's disease.
31. The method of claim 30, wherein the salt is the mesylate salt of R(+)-N-propargyl-1-aminoindan.

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32. The method of claim 30, wherein the salt is administered orally, rectally, transdermally, or parenterally.
- 5 33. The method of claim 30, which further comprises administering to the subject a therapeutically effective amount of Levodopa.
- 10 34. The method of claim 30, which further comprises administering to the subject an effective amount of a decarboxylase inhibitor.
- 15 35. The method of claim 34, wherein the decarboxylase inhibitor is L-Carbidopa.
36. The method of claim 34, wherein the decarboxylase inhibitor is benserazide.
- 20 37. Use of the salt of claim 14 for the manufacture of a composition for the treatment of a memory disorder.
38. Use of the salt of claim 14 for the manufacture of a composition for the treatment of dementia.
- 25 39. The method of claim 38, wherein the dementia is of the Alzheimer type.
40. Use of the salt of claim 14 for the manufacture of a composition for the treatment of depression.
- 30 41. Use of the salt of claim 14 for the manufacture of a composition for the treatment of hyperactive syndrome.
- 35 42. The method of claim 37, 38, 40, or 41, wherein the salt is administered orally, rectally, or parenterally.

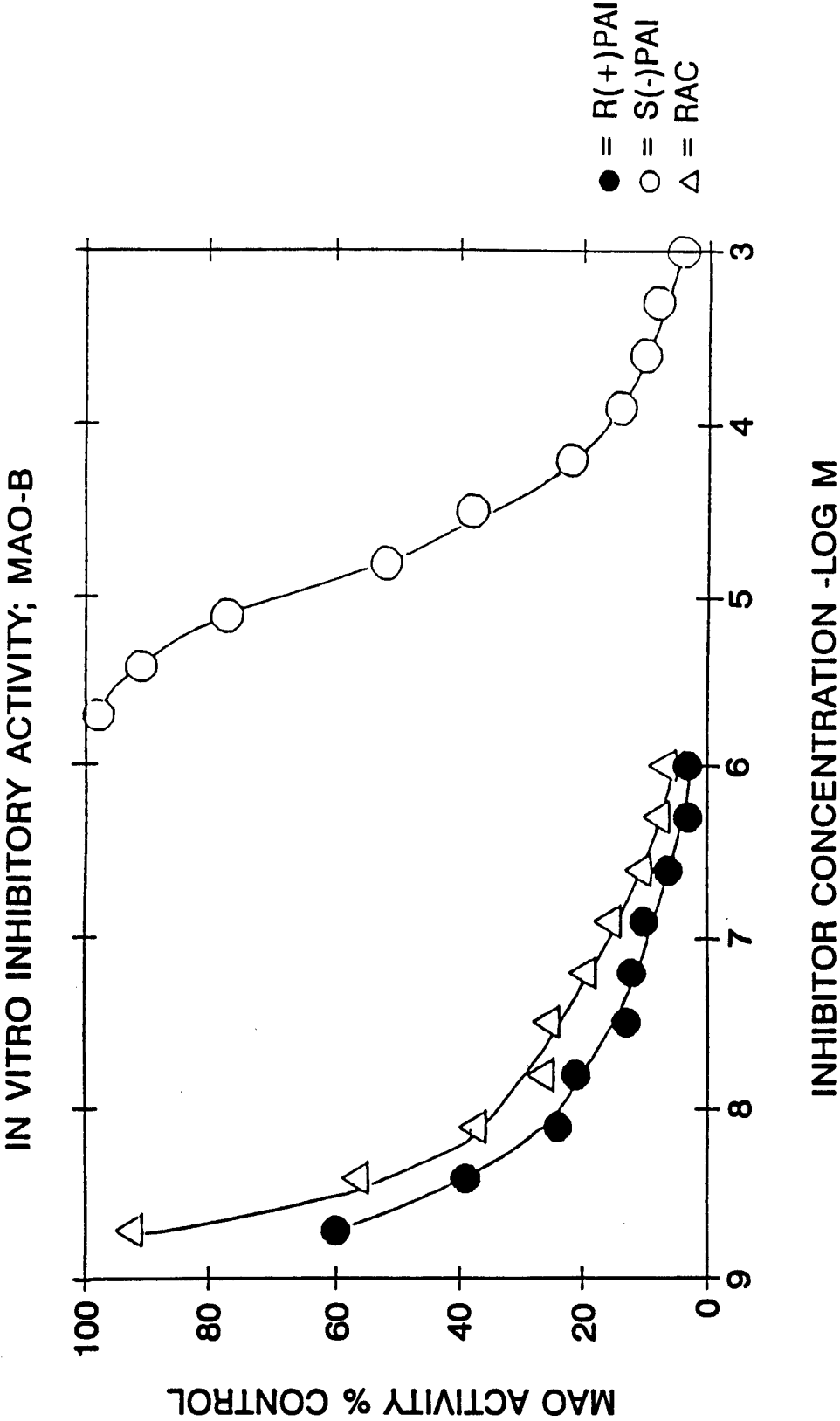
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FIGURE 1



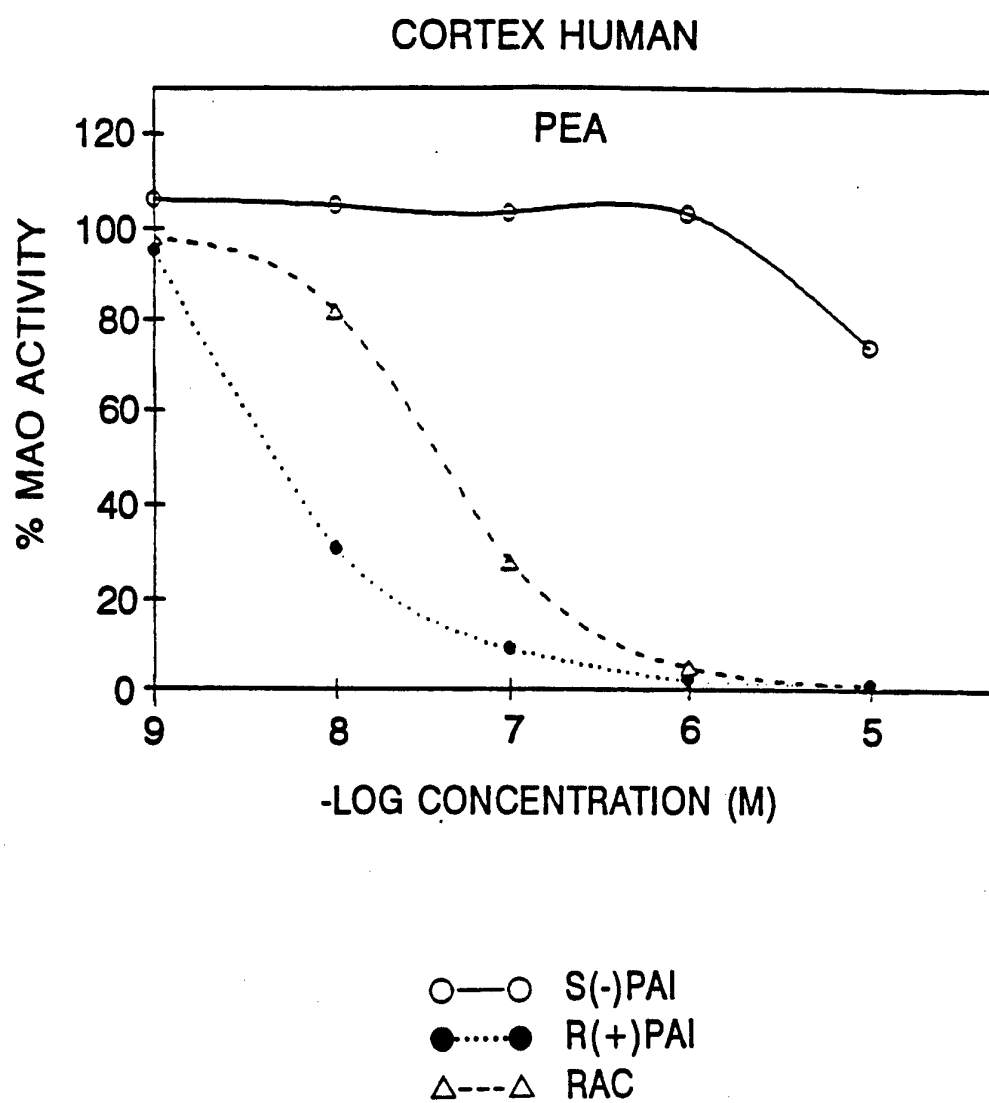
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FIGURE 2



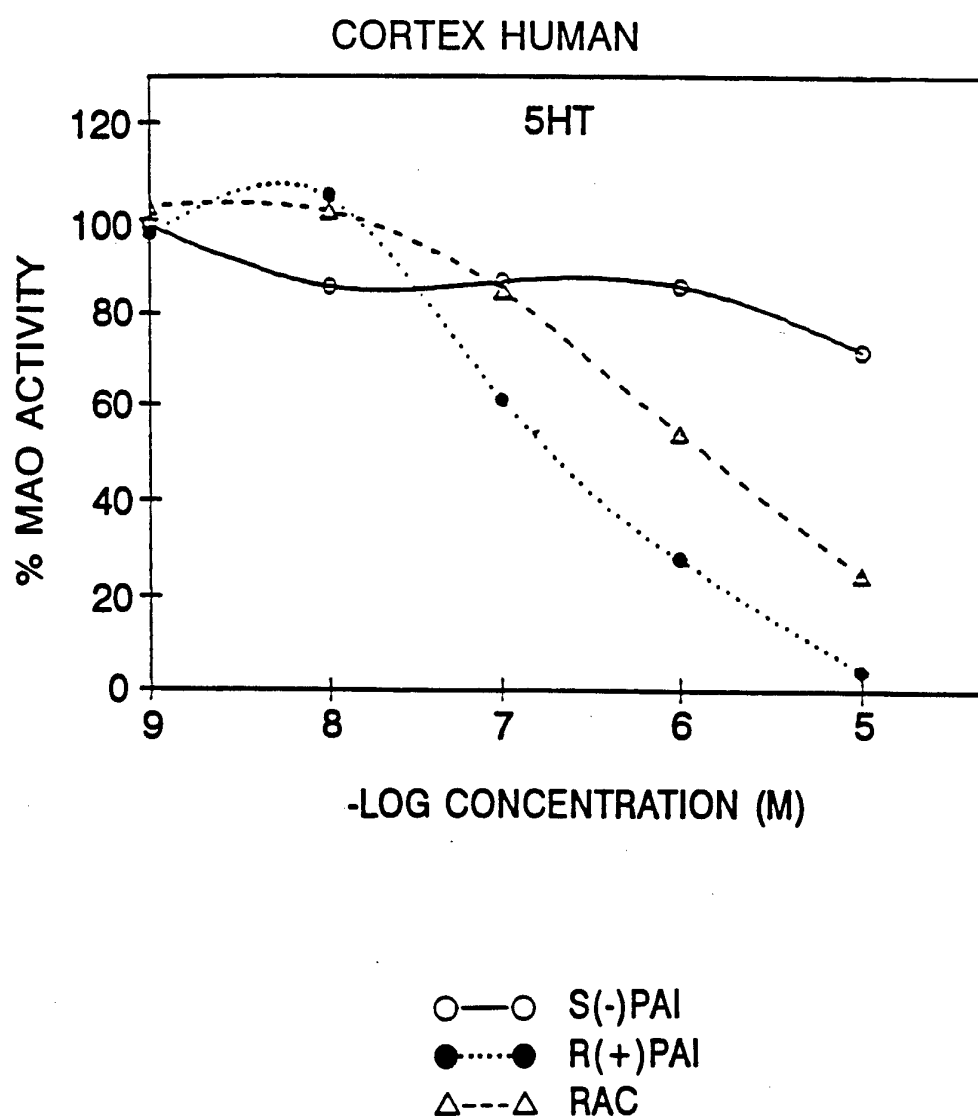
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FIGURE 3A



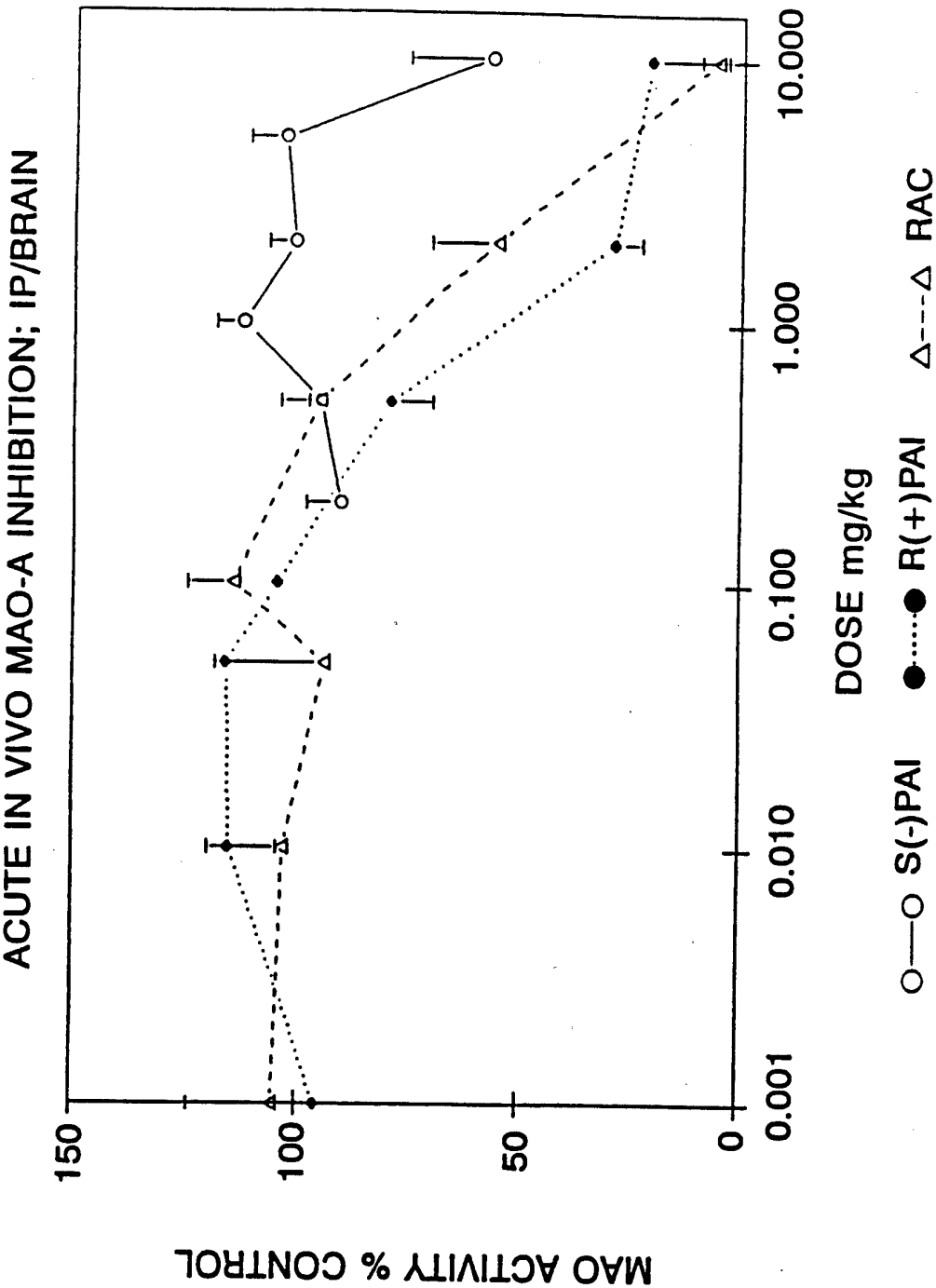
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FIGURE 3B



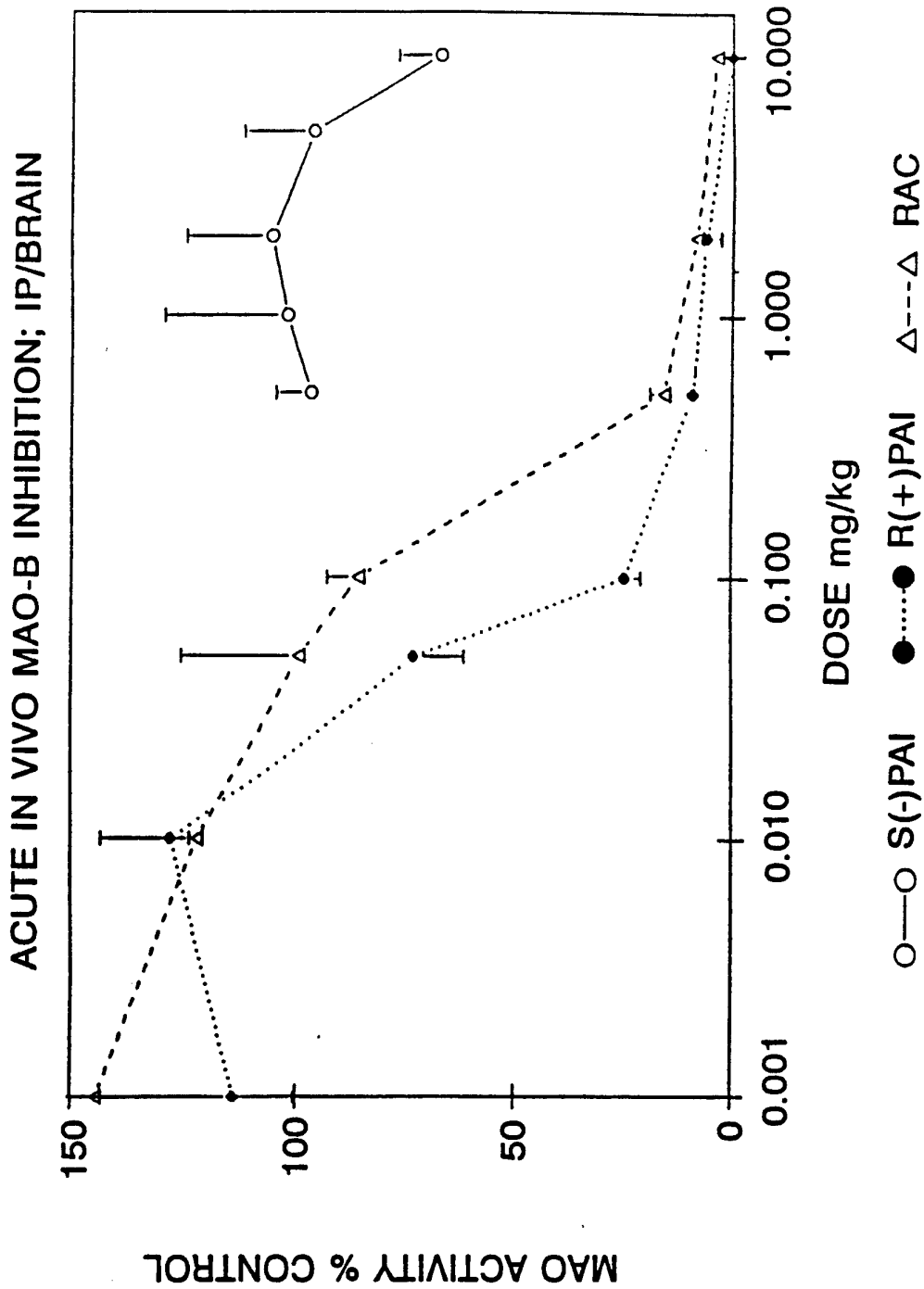
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FIGURE 4



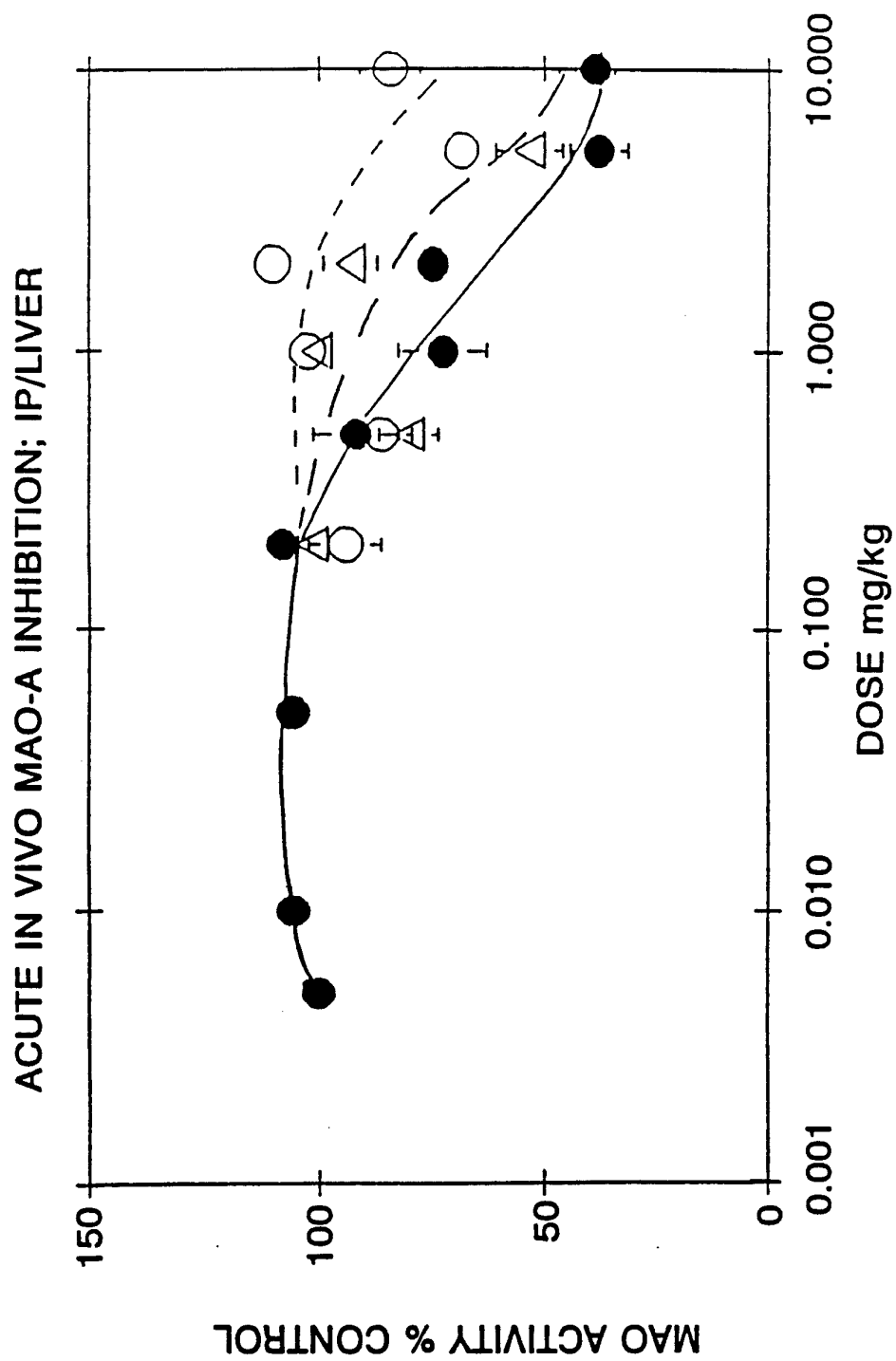
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FIGURE 5



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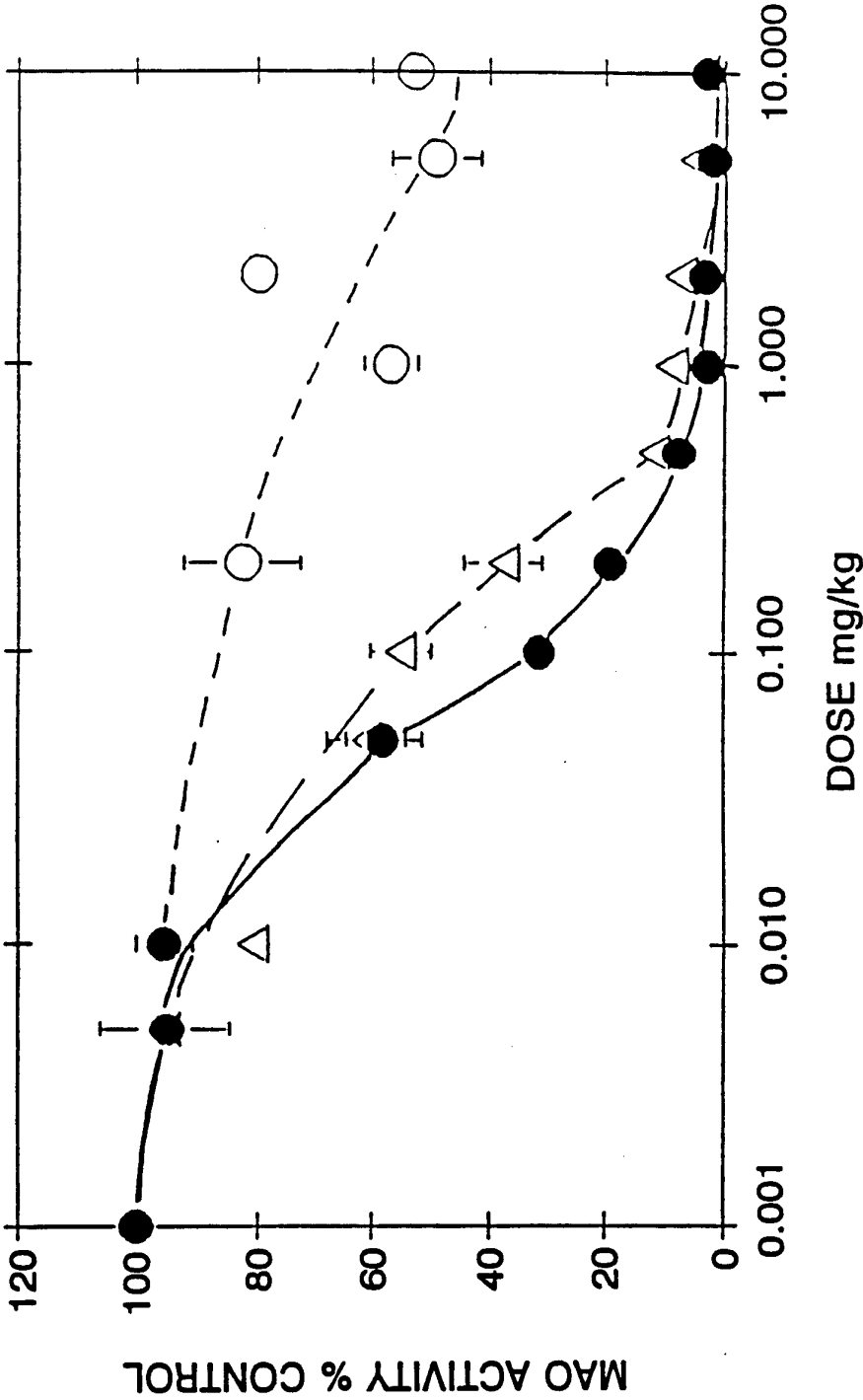
FIGURE 6



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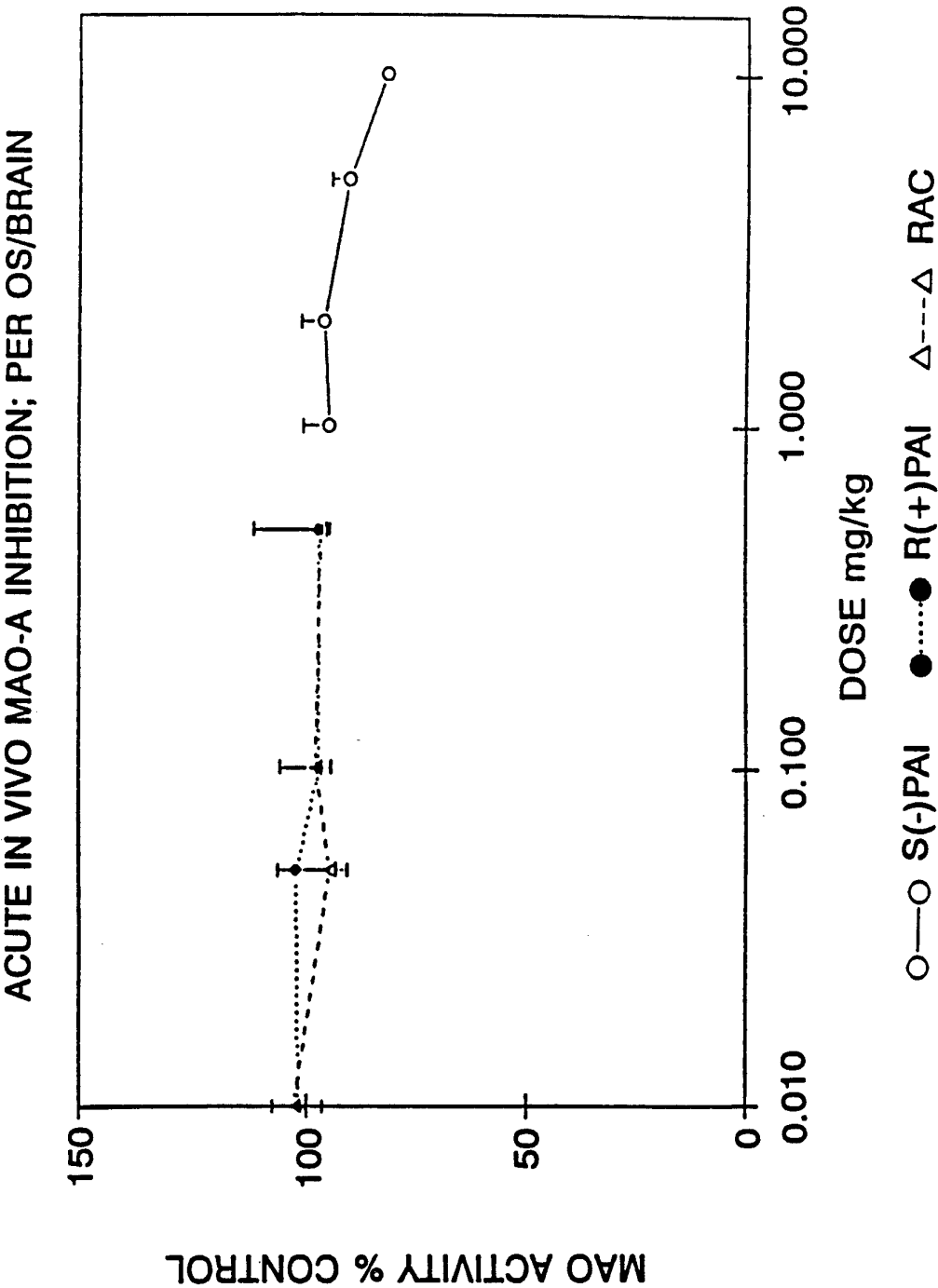
FIGURE 7

ACUTE IN VIVO MAO-B INHIBITION; IP/LIVER



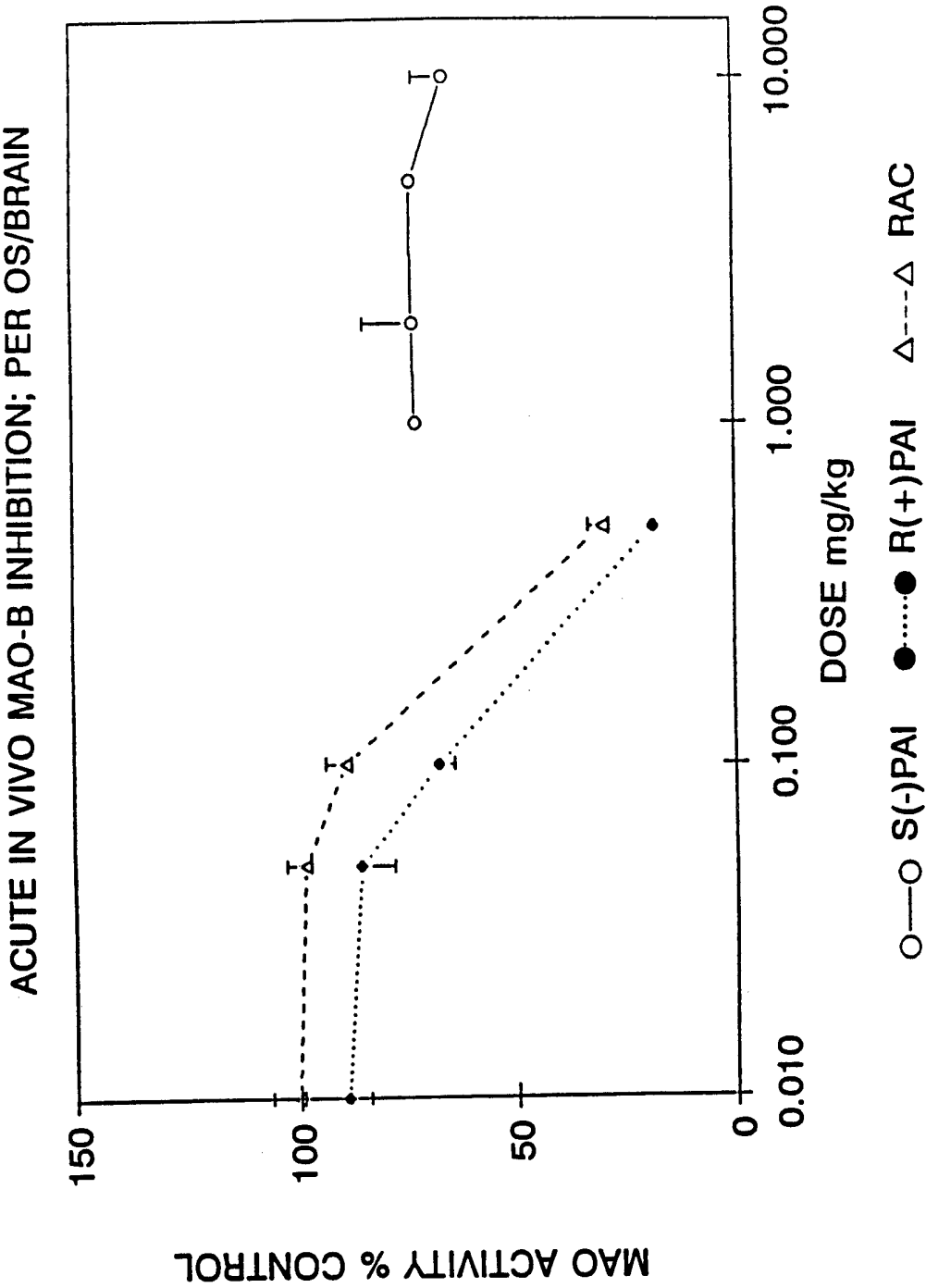
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FIGURE 8



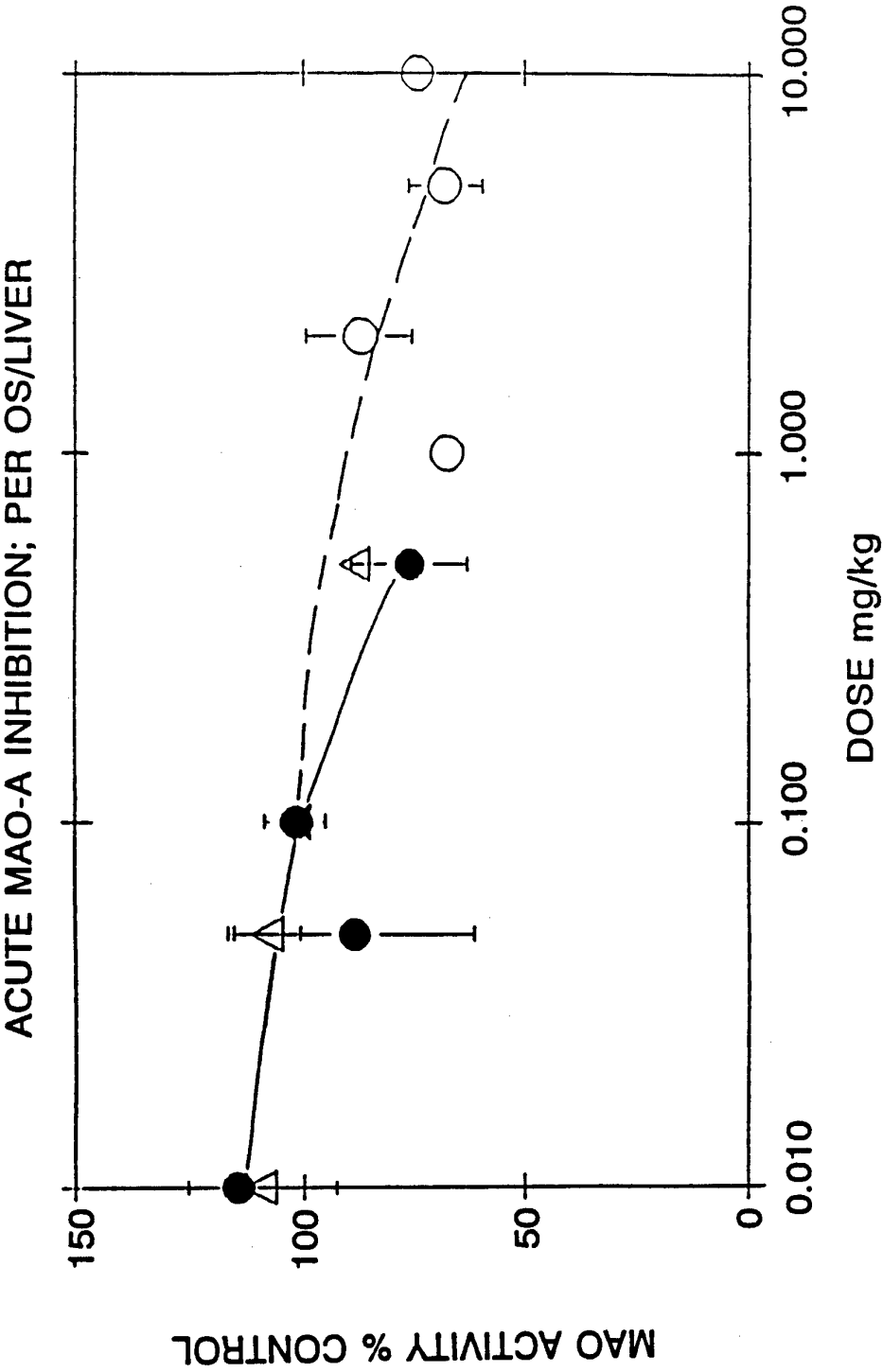
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FIGURE 9



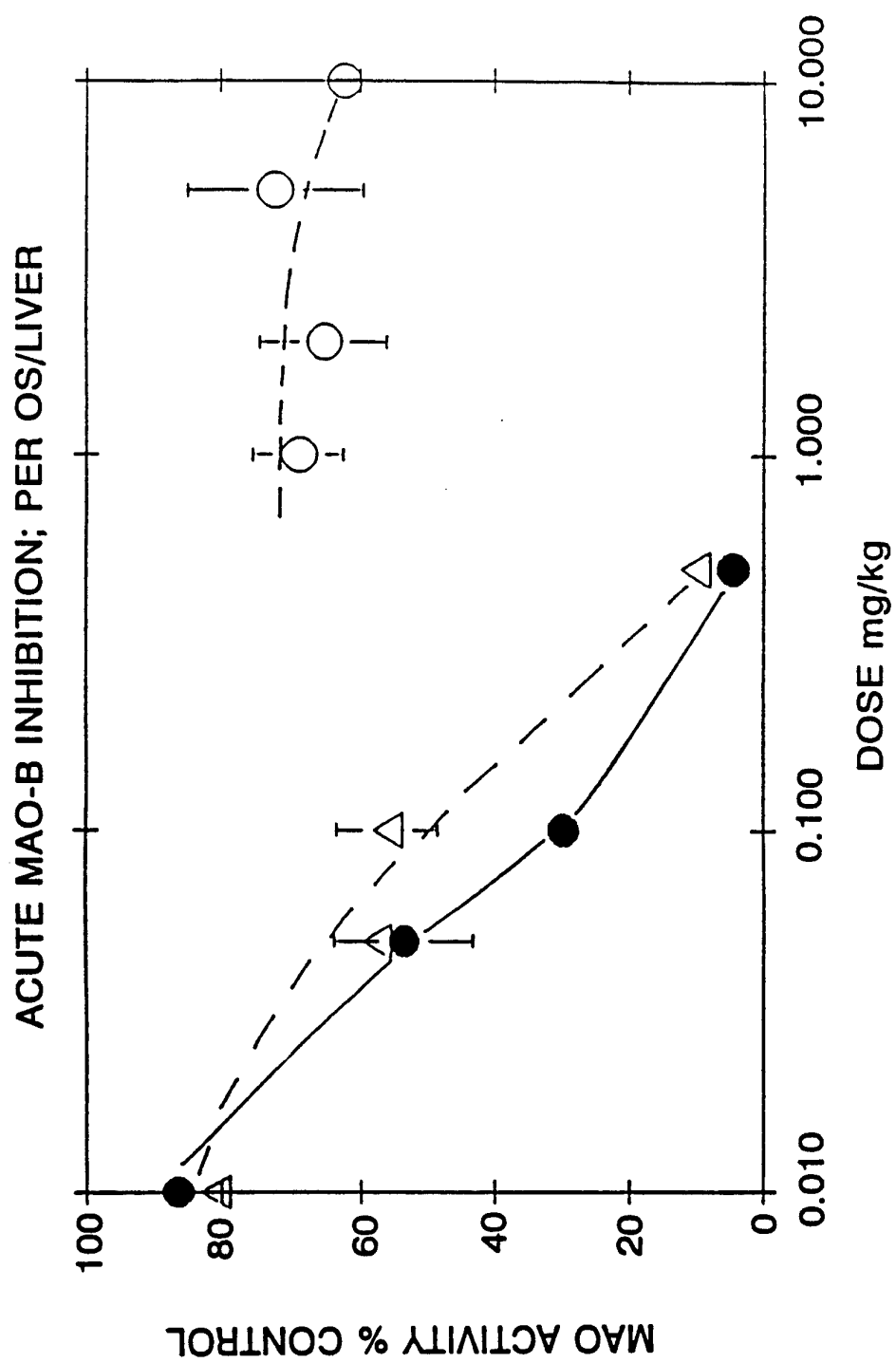
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FIGURE 10



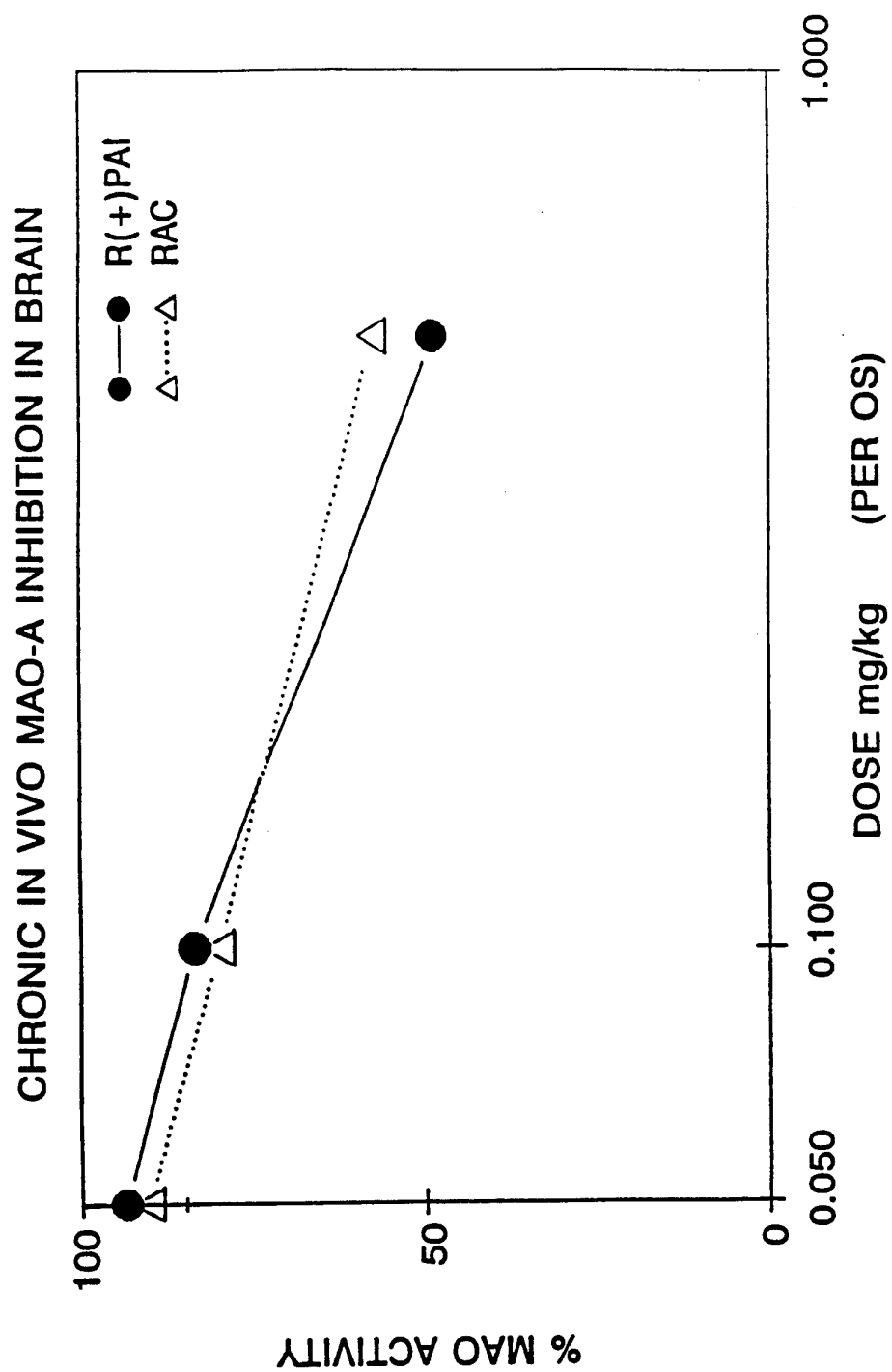
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FIGURE 11



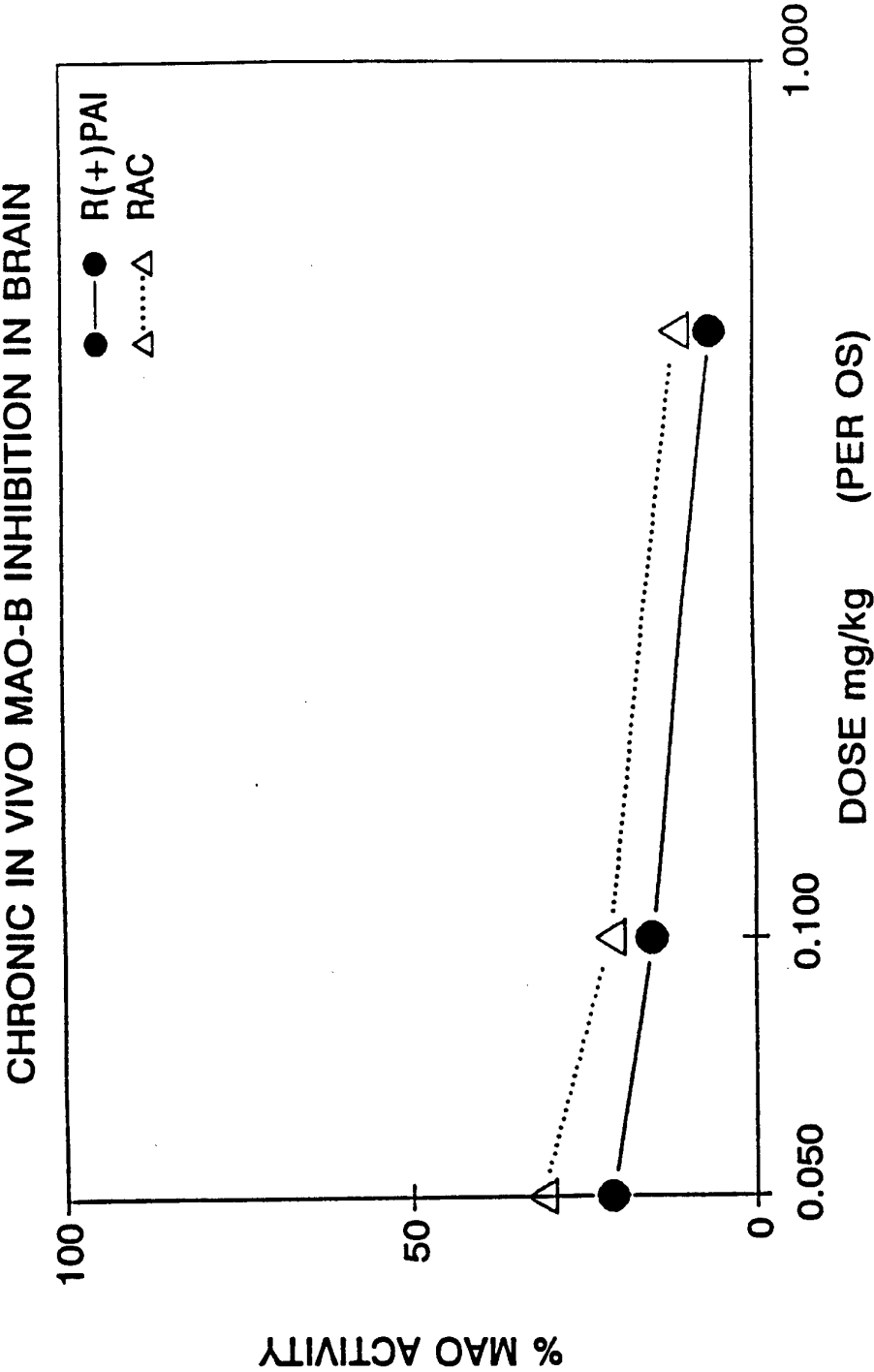
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FIGURE 12



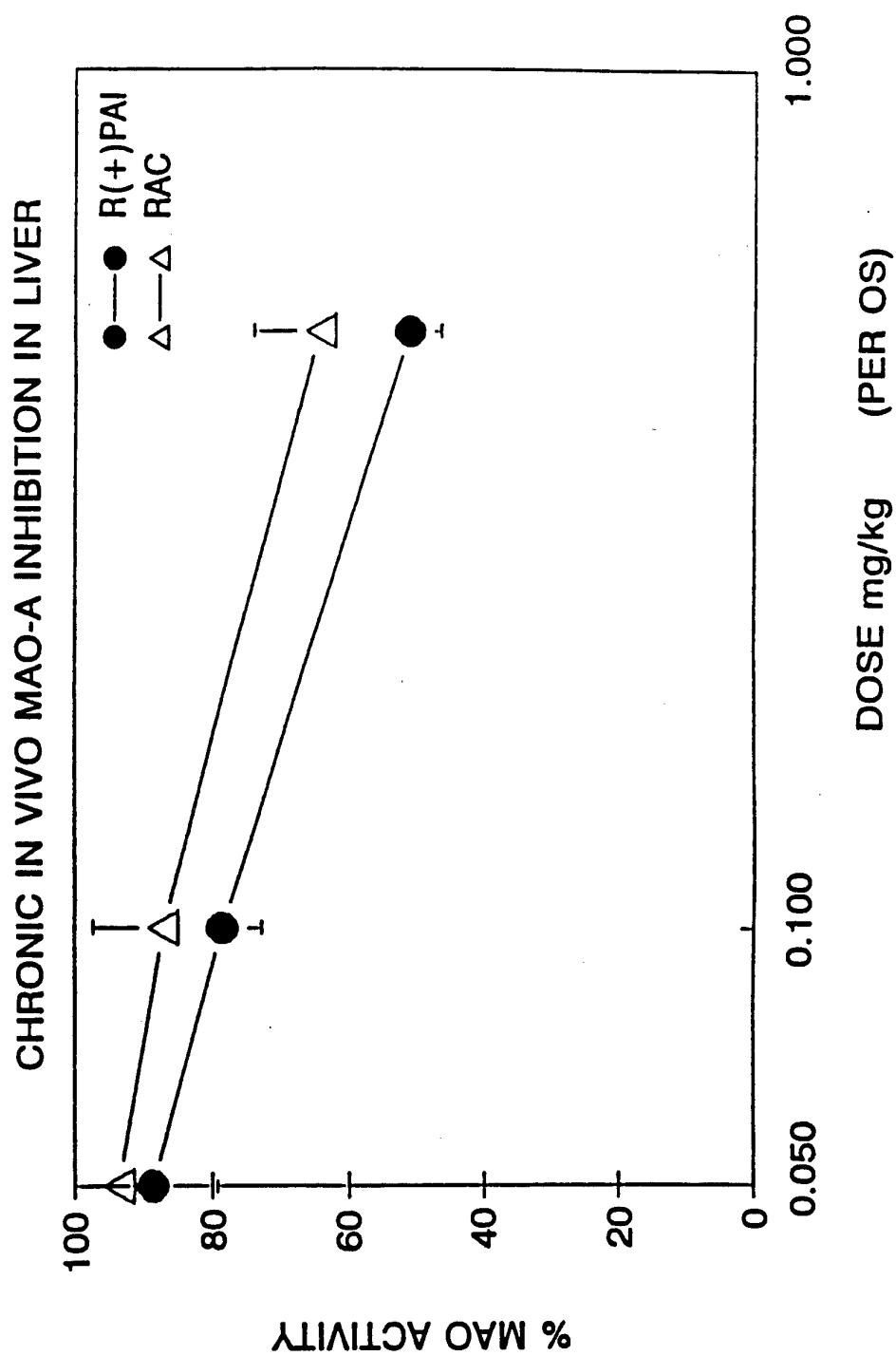
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FIGURE 13



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FIGURE 14



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FIGURE 15

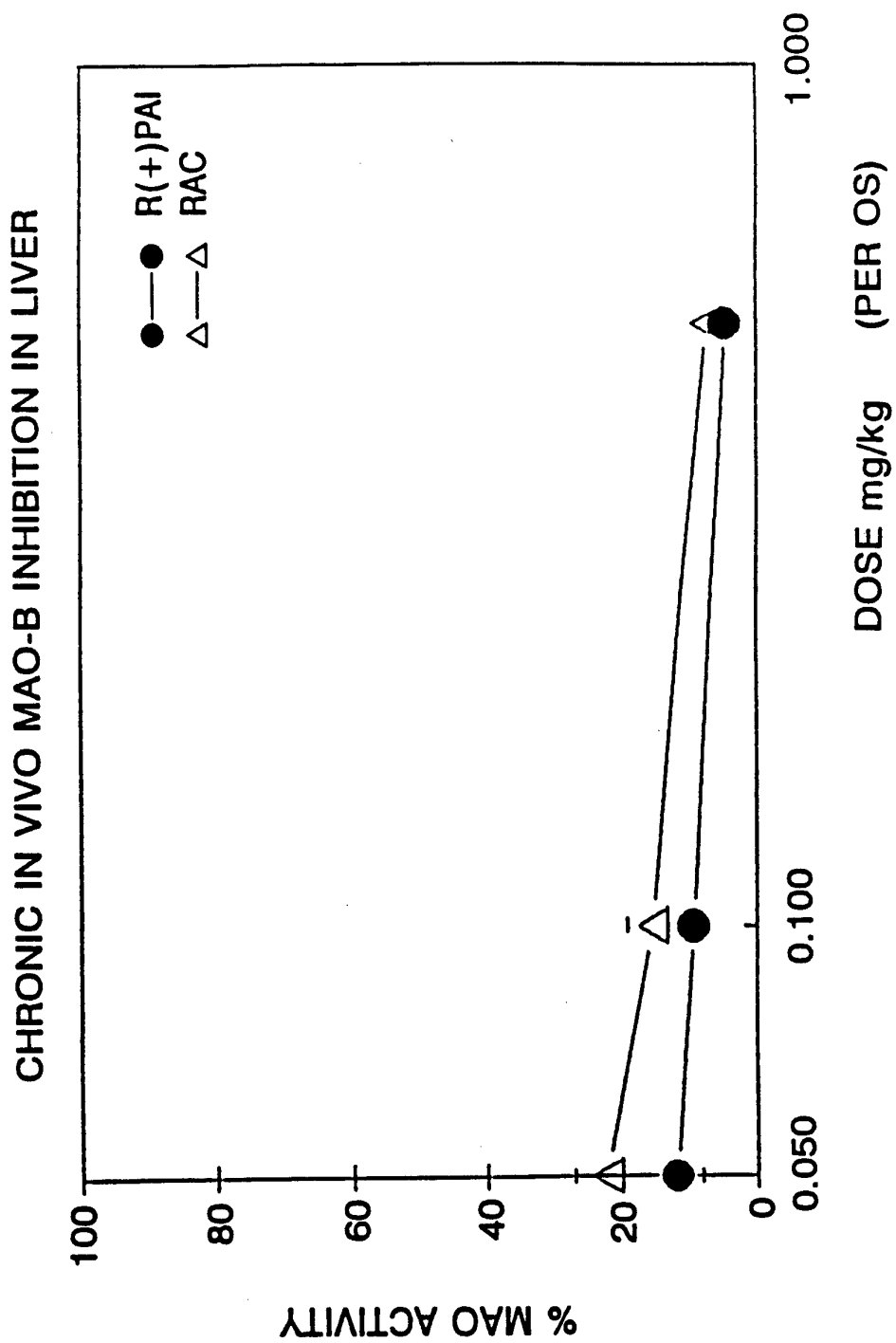
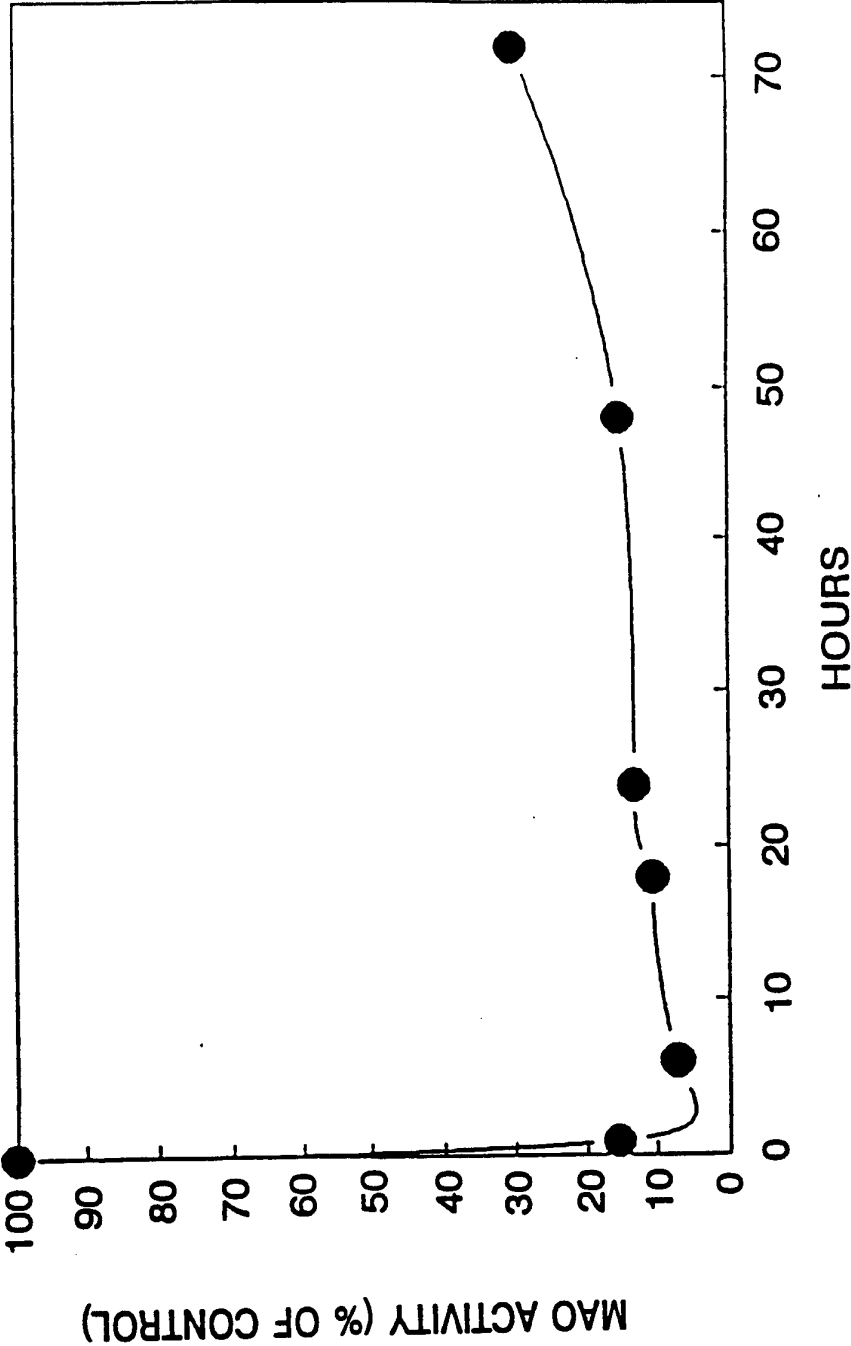


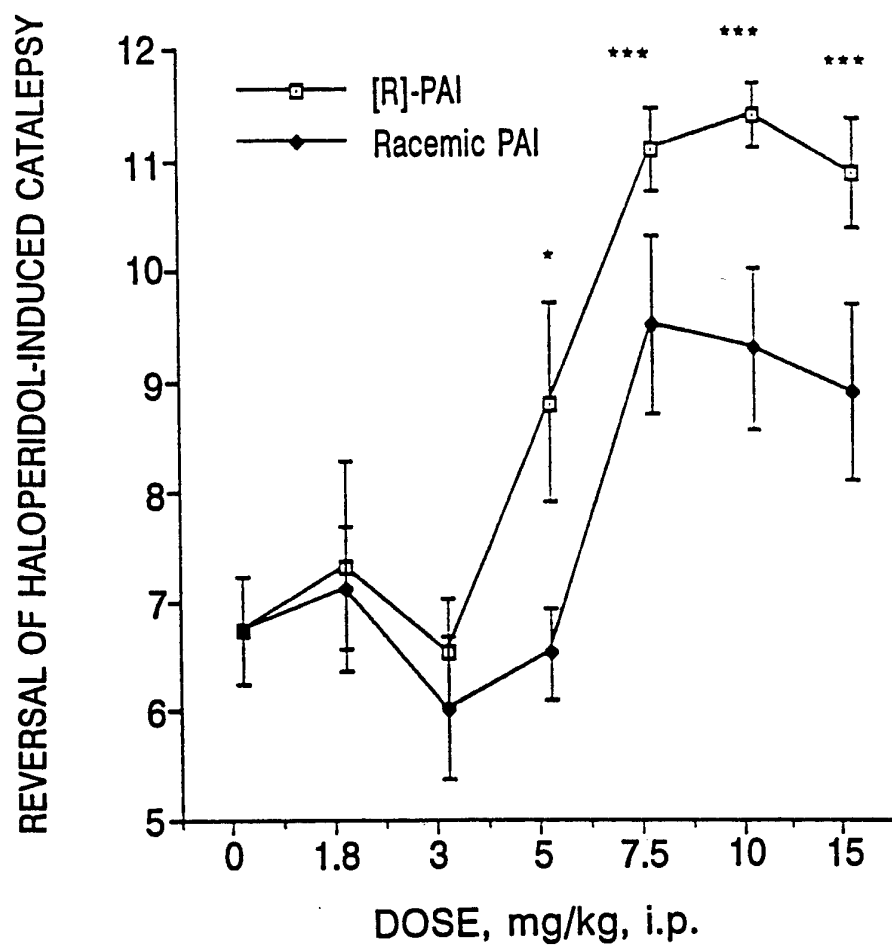
FIGURE 16

MAO-B ACTIVITY IN RAT BRAIN AT DIFFERENT TIMES
FOLLOWING I.P. INJECTION OF R(+)-PAI (1mg/kg)



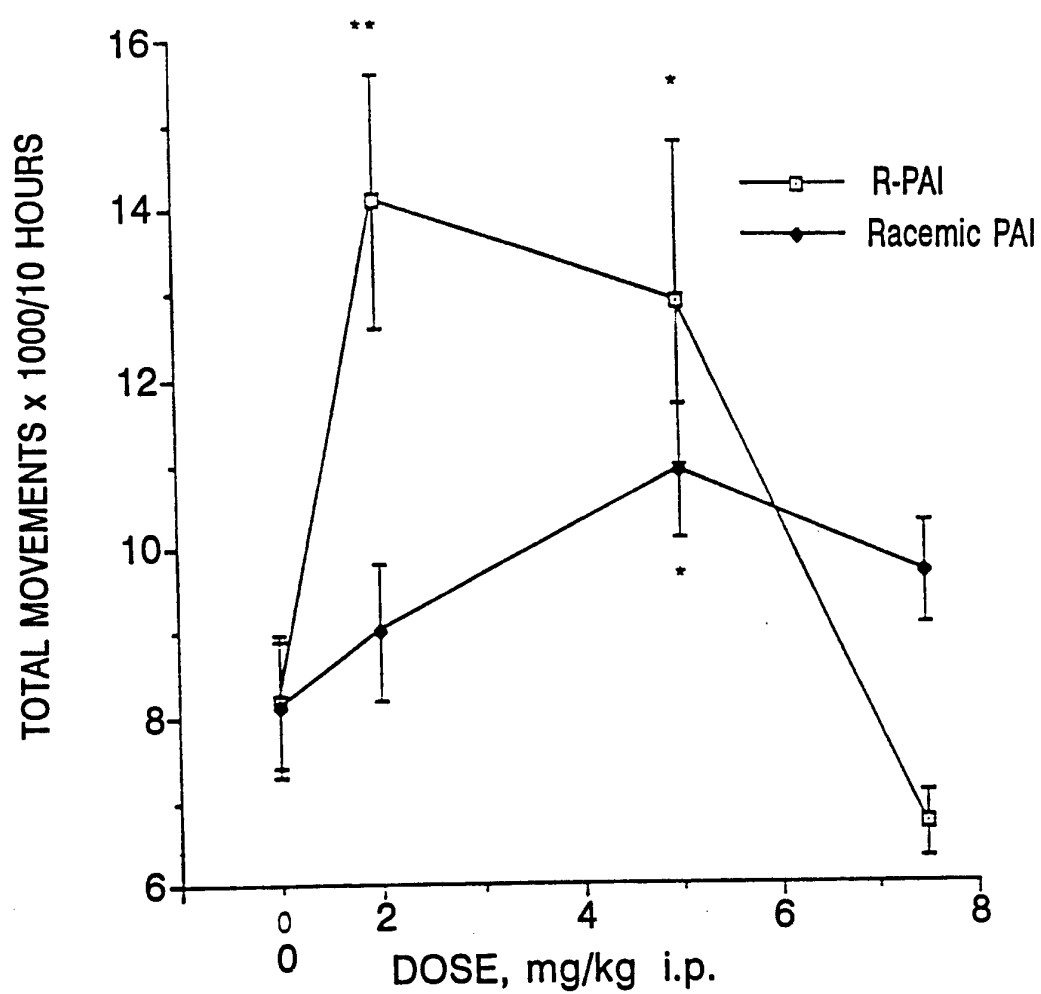
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FIGURE 17



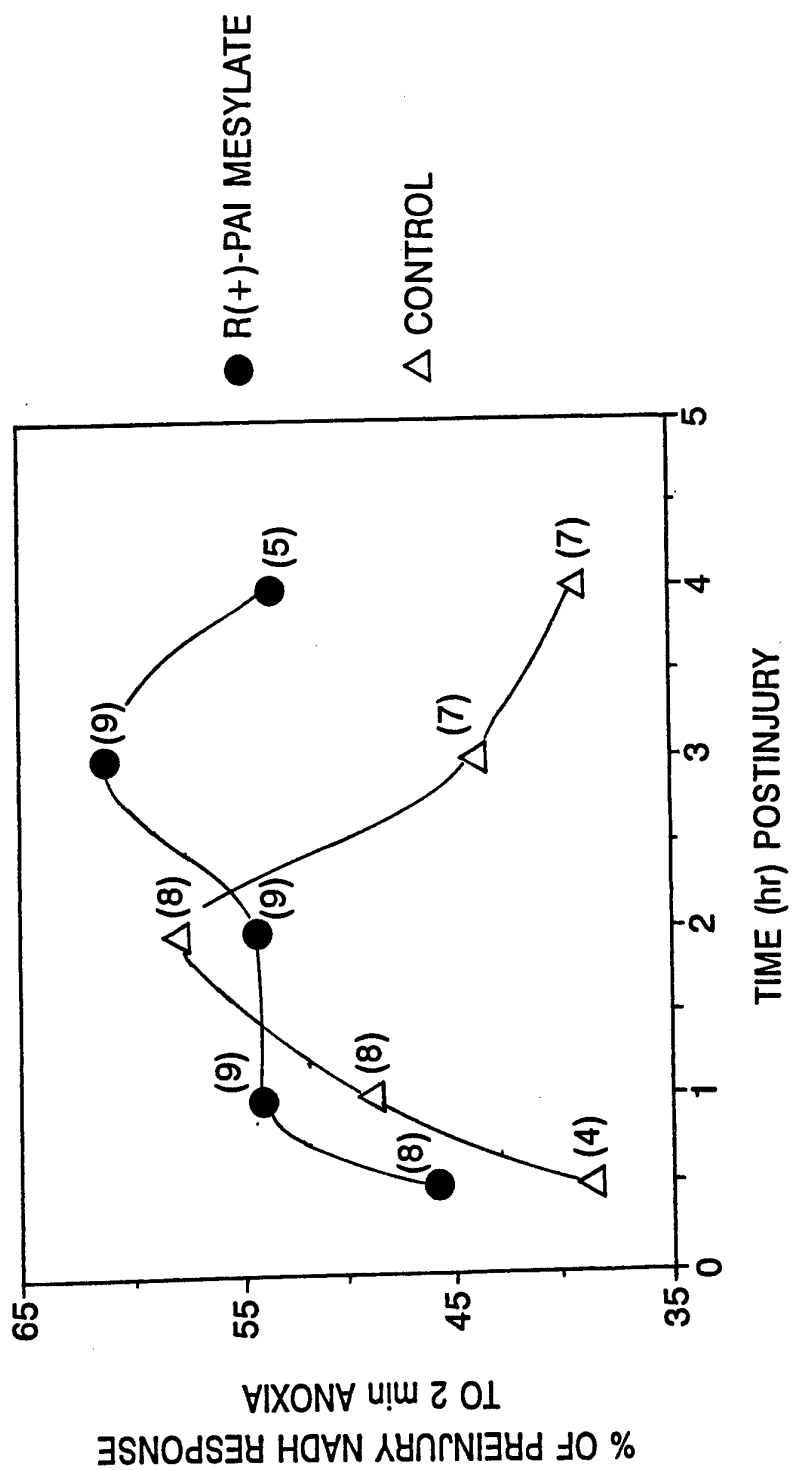
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FIGURE 18



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FIGURE 19



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/11567

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/135; C07C 211/19, 211/30

US CL : 514/647, 811, 812; 564/308

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/647, 811, 812; 564/308

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,826,875 (Chiesi) 02 May 1989, column 1, lines 45-55.	1-42
Y	US, A, 3,513,244 (Gittos et al.) 19 May 1970, column 1, line 70.	1-42
Y	British Journal of Pharmacology, Volume 85, No. 2, issued June 1985, Finberg et al., "Modification of Blood Pressure and Nictitating Membrane Response to Sympathetic Amines by Selective Monoamine Oxidase Inhibitors, Types A and B, in the Cat", pages 541-546, especially page 541.	1-42

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 28 FEBRUARY 1995	Date of mailing of the international search report 09 MAR 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BRIAN M. BURN Telephone No. (703) 305-1235