

Improvement of the oral bioavailability of the selective dopamine agonist N-0437 in rats: the in vitro and in vivo activity of eight ester prodrugs

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Summary. The potent and selective D2-agonist N-0437 2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin undergoes considerable first-pass metabolism due to glucuronidation of the phenolic group after oral administration. In an attempt to improve the bioavailability, eight ester prodrugs of N-0437 were synthesized, i.e. the acetyl, isobutyryl, pivaloyl, benzoyl, 2-methylbenzoyl, 2-methoxybenzoyl, 2,4-dimethylbenzoyl and 2-aminobenzoyl analogues. To examine the hydrolysis rates of these compounds in vitro studies were performed in rat serum. The prodrugs showed a very diverse pattern of hydrolysis rates. The in vivo activities were determined by testing the prodrugs in rats with unilateral 6-OHDA lesions of the striatum. The resulting contralateral turning was used to measure the activity of the compounds. By calculating the area under the curve (AUC), of the time-effect curves of the prodrugs, a significantly improved duration of action was found for those prodrugs which have a slow in vitro hydrolysis rate. However no significant differences in total activity of these slowly hydrolysing prodrugs compared with N-0437 could be demonstrated, although the 2-aminobenzoyl and the 2,4-dimethylbenzoyl derivatives show interesting behavioural profiles. In contrast the isobutyryl ester, a prodrug with a relatively rapid hydrolysis rate, gave an improvement of turning behaviour over the whole time course in comparison with N-0437.

Key words: Prodrugs — 6-OHDA — N-0437 — Bioavailability — Dopamine agonist

Introduction

The dopamine (DA) agonist 2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin, N-0437, acts as a potent and D2 selective compound (Van der Weide et al. 1987).

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One of the structural requirements of a dopamine agonist is that it has a hydrogen-bonding group which can interact with the receptor. A hydroxyl group serves this function for many dopamine agonists (Seeman 1981). The disadvantage of a free hydroxyl group is an extensive first-pass metabolism after oral administration due to the enzyme UDP-glucuronosyltransferase (Siest et al. 1987). Gerding et al. (1989) have shown that the inactivation of N-0437 by glucuronidation occurs in the gut and liver in rat and monkey. Certain other compounds having a phenolic hydroxyl group such as the DA agonists 3-PPP (Thorberg 1988), PHNO (Vyas et al. 1988) and opiates such as nalbuphine (Hussain et al. 1985) also have a low bioavailability.

One way of approaching this problem is to use the concept of prodrugs (Sinkula and Yalkowsky 1975; Svensson 1987; Bundgaard 1984, 1985). In the last decade several ester prodrugs have been designed to reduce first-pass metabolism. Prodrug analogues have been made for apomorphine (Borgman et al. 1976; Baldessarini et al. 1977), ADTN (Horn et al. 1978, 1982), 3-PPP (Thorberg 1988), morphine (Broekkamp et al. 1988), naltrexone (Hussain et al. 1987), and estradiol (Hussain et al. 1988). One prodrug of the DA agonist epinine has been marketed as a cardiostimulant (Casagrande et al. 1984).

To avoid first-pass metabolism of N-0437 eight prodrugs (Fig. 1) were synthesized and then tested in an in vitro test system, by which the hydrolysis rate could be investigated, and in a behavioural system which reflects postsynaptic dopaminergic activity i.e. contralateral turning in the rat after a 6-hydroxy-dopamine (6-OHDA) lesion.

Materials and methods

Materials and animals. N-0437 and the ester prodrugs were synthesized in our laboratory and isolated as HCl salts. The compounds were dissolved in a solution of alcohol, water and polyethyleneglycol (3:3:4) for oral administration or in dimethylsulfoxide when needed for in vitro hydrolysis. 6-OHDA (Sigma, St. Louis, MO, USA) was dissolved in a solution of 0.9% sodium chloride

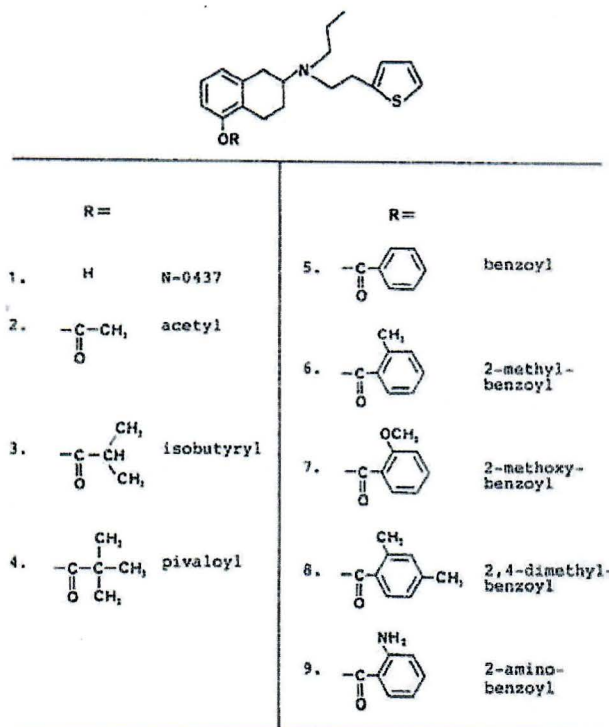


Fig. 1. Structures of N-0437 and eight ester prodrugs

containing 3 mg/ml of ascorbic acid. Apomorphine (Brocacef, Maarssen, The Netherlands) was dissolved in water. All solutions were freshly prepared before use.

Male albino Wistar rats (200–300 g) (C. D. L., Groningen) were used for the experiments. The rats were housed in plastic cages (55 × 35 × 20 cm) and had free access to food and water.

In vitro hydrolysis. The hydrolysis profiles of the ester prodrugs were determined in rat serum which was collected from the rats by puncture of the aorta abdominalis after ether anesthesia. The samples were kept frozen until the start of the experiments. Prodrug hydrolysis was performed in 3 ml of serum at 37°C with a prodrug concentration of 35 μM. At various incubation times, a 200 μl aliquot of serum was taken and added to 2 ml of acetonitrile to precipitate the proteins. The extraction procedure and the determination of N-0437 on the HPLC was performed as previously described (Den Daas et al. 1989). All ester prodrugs proved to be stable in the two solvents used for the experiments. After dissolving and storing at ambient temperature only 5% of the ester prodrugs was hydrolysed after 3 days.

Turning behaviour and surgery. The rats were anaesthetized with chloral hydrate (400 mg/kg) and placed in a stereotaxic instrument (Kopf). After removing skin and tissue a small hole was drilled in the skull and 6-OHDA (8 μg dissolved in 1 μl 0.9% NaCl containing 3 mg/ml of ascorbic acid) was injected in the left medial forebrain bundle at coordinates A 4.8, L 2.2 and V 2.4 (König and Klippel 1963) to destroy the dopaminergic input to the striatum. Successfully denervated animals were selected by testing them with 2.0 μmol/kg (0.61 mg/kg) apomorphine, starting 1 week after surgery. Rotational behaviour was automatically recorded in rotometers according to the method of Ungerstedt and Arbuthnott (1970). Only rats showing more than 100 contralateral turns per half hour were selected for the experiments. Animals which did not show good turning behaviour were tested 1 week after the first test. When they again showed no turning behaviour the rats were excluded from the experiments. Approximately 80% of the operated rats were used for

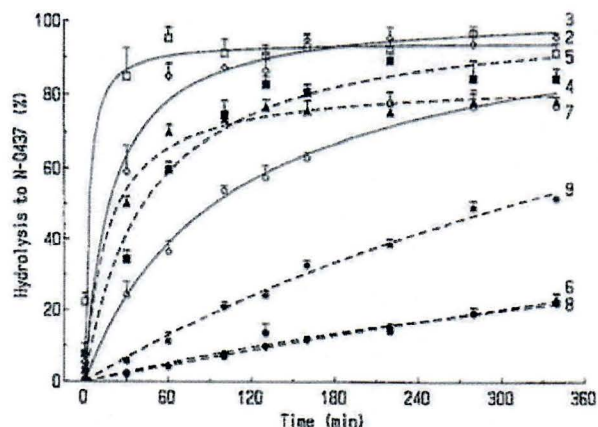


Fig. 2. The in vitro hydrolysis in rat serum of the prodrugs of N-0437; (error bars are SEM; $N = 4$)

experiments. Rats were used 4 times for a 20 h experiment with a weekly resting interval. Before the experiments the rats were fasted overnight. After an oral injection (2 ml/kg) the rats were placed in the rotometer. Registration of the turning behaviour started 10 min after injection. The time of every contralateral and ipsilateral turn of a maximum of 6 rats was registered on a microcomputer. To compare N-0437 and the prodrugs all doses were equimolar i.e. 50 μmol/kg. The order of compounds tested were randomly distributed during the experiments but no animal was used twice or more for one compound. After the experiments the rats were decapitated and the striata were removed for the determination of DA in the left and right striata by HPLC analysis. All rats exhibiting sufficient turning activity were found to have less than 5% of control DA levels in the lesioned striatum. Rats which exhibited no turning behaviour showed less than a 60% decrease of DA compared with contralateral DA levels.

Statistics. The data were analysed by use of a spreadsheet program (123-Lotus), which calculated the time-activity profile of the drug and the area under the curve (AUC) of 4 time intervals as a measure of the activity of the compound. The hydrolysis profiles were calculated by nonlinear curve fitting of the data points with the least squares method using the equation $y = bx/(a-x)$. The residuals of all the plotted curves were independent and no correlation of the residuals could be found. Statistical analysis was performed with the Dunnett's test of a control versus several treatments (Grimm 1973).

Results

The hydrolysis of the eight ester prodrugs

The calculated time-response curves of the hydrolysis of the prodrugs show a diverse pattern (Fig. 2). The acetyl prodrug is hydrolyzed very rapidly (half life ± 3 min) and has almost completely disappeared after 30 min. The total amount of prodrug conversion, calculated as the area under the curve (AUC), is estimated to be $\pm 91\%$ of the original amount of N-0437. The hydrolysis rate of the isobutyryl ester is slower. It has a half life time of ± 21 min and a AUC of $\pm 85\%$. The prodrug with the lowest rate of hydrolysis of the aliphatic group is the pivaloyl ester with a half life of ± 92 min and a AUC of $\pm 59\%$.

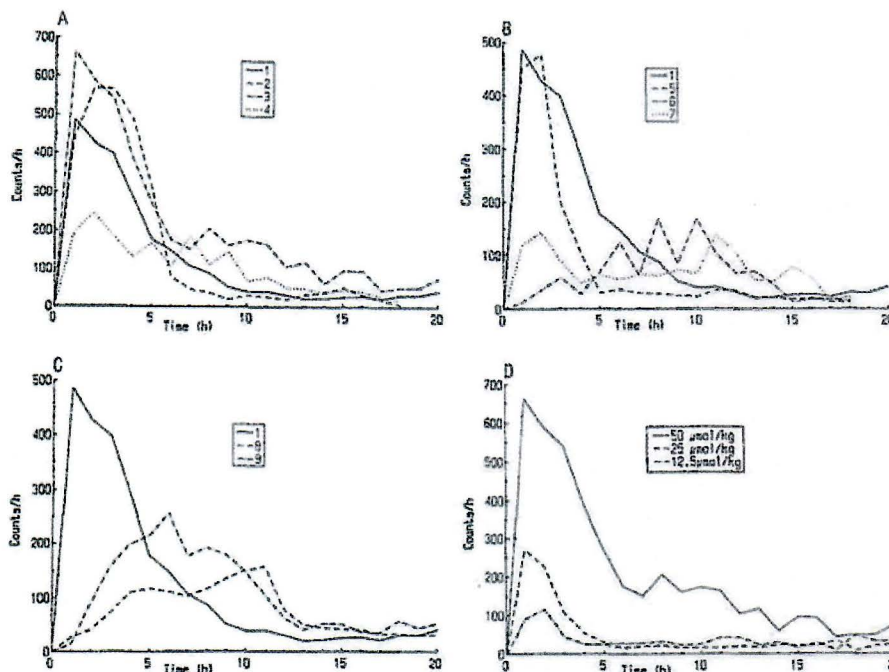


Fig. 3. Effect of p.o. administration (50 µmol/kg) of N-0437 and its prodrugs on contralateral turning behavior; (error bars are SEM; $N = 9$).
A N-0437 (1), acetyl (2), isobutyryl (3) and pivaloyl (4).
B N-0437 (1), benzoyl (5), 2-methylbenzoyl (6) and 2-methoxybenzoyl (7).
C N-0437 (1), 2,4-dimethylbenzoyl (8), 2-aminobenzoyl (9).
D Effect of three concentrations (50, 25, 12.5 µmol/kg) of the isobutyryl ester on contralateral turning.

Table 1. Half life times ($t_{1/2}$), area under the curve (AUC), correlation coefficient of the curves (r_{curve}) and the residual standard error (RSD) of the curves of the prodrugs of N-0437. AUC was calculated as a percentage of the total amount of N-0437 released from the prodrug

Compound	$t_{1/2}$	AUC (%)	r_{curve}	RSD
2	3 min	91	0.936	10.5
3	21 min	85	0.991	7.7
7	24 min	72	0.993	5.5
5	42 min	74	0.981	7.8
4	92 min	59	0.992	6.4
9	5 h	30	0.995	6.7
6	16 h	12	0.999	2.0
8	31 h	12	0.982	4.0

The most labile aromatic prodrug is the benzoyl ester ($t_{1/2} = 42$ min; AUC = 74%) and the most stable ester is the 2,4-dimethylbenzoyl compound. However, the lability of the 2-methoxybenzoyl ester is striking. This ester has a relatively short half life of 24 min and, for the aromatic esters, a high, AUC of $\pm 72\%$.

The activity of N-0437 and the ester prodrugs in the 6-OHDA turning model

The time response profile of N-0437 is typical for a lipophilic drug with a high first pass metabolism (Fig. 3). Almost immediately after p.o. administration there is a period of rapid contralateral turning which reaches its maximum in the first hours and then decreases rapidly until at 5 h the rats are turning at approximately 175 turns/h. After 5 h the decline is smaller until, at approxi-

mately 7 h, it reaches a point when there is no significant turning, compared with vehicle administration.

The aliphatic prodrugs have the same profile compared with the N-0437 time response (Fig. 3A) with first a peak at 1 h and a decline thereafter. The acetyl ester shows a higher potency in the first 5 h but this is not statistically significant (Fig. 4A). The isobutyryl ester shows a significantly higher potency compared with N-0437 at all times measured. The pivaloyl ester does not produce high activity after administration but it gives a more plateau-like profile. The total activity of all the aliphatic prodrugs is not significantly different compared with N-0437, except for the isobutyryl ester (Fig. 4D).

The benzoyl ester is the only aromatic ester with a profile that has some resemblance with N-0437, although the duration of peak activity is very short (Fig. 3B). The 2-methylbenzoyl and 2-methoxybenzoyl esters have a more plateau-like curve, which stays stable until ± 11 h after administration. In the third time interval (11–15 h, Fig. 4B) the 2-methylbenzoyl and the 2-methoxybenzoyl ester have a significantly greater effect than N-0437.

The profiles of the 2-aminobenzoyl and 2,4-dimethylbenzoyl esters show a different time-response activity (Fig. 3C). Very little effect can be seen immediately after administration. A very large increase of turning activity starts after 1 h and reaches a maximum at 6 h for the 2-aminobenzoyl ester and at 11 h for the 2,4-dimethylbenzoyl compound. After reaching its peak effect the 2-aminobenzoyl ester decreases slowly for ± 7 h. After 11 h the action of the 2,4-dimethylbenzoyl analogue decreases rapidly within 2 h.

The prodrugs with a significantly higher potency than N-0437 in a certain interval are the following (Fig. 4A, B, C, D): isobutyryl from 1–5 h; isobutyryl and 2-

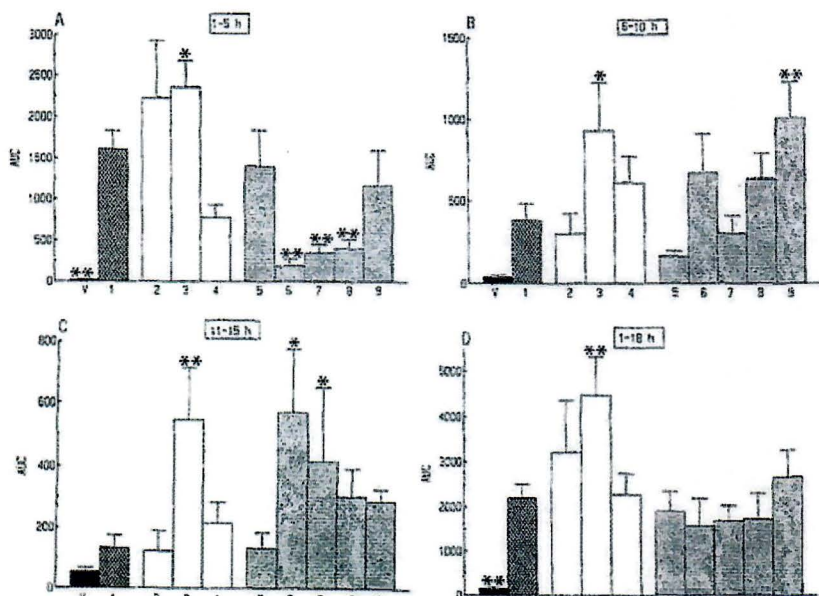


Fig. 4. The AUC from the time-response profile of N-0437 (I), vehicle (V) and prodrugs (2-9) after p.o. administration (50 µmol/kg) calculated from the time intervals 1-5 h (A), 6-10 h (B), 11-15 h (C) and 1-18 h (D) (Error bars are SEM, * $p < 0.05$, ** $p < 0.01$ vs. N-0437, Dunnett's test)

aminobenzoyl from 6-10 h; isobutyryl, 2-methylbenzoyl, 2-methoxybenzoyl and 2-aminobenzoyl from 11-15 h; isobutyryl for the whole measured time interval 1-18 h.

In the time intervals: 1-5 and 1-18 h, N-0437 was significantly more active than vehicle.

The dose response activity of the isobutyryl ester in the 6-OHDA turning model

Three doses of the isobutyryl ester were examined in the turning model, i.e. 50, 25 and 12.5 µmol/kg. The time-activity profile (Fig. 3D) shows a decrease in activity after lowering the dose. The turning activity after administration decreases almost proportionally with the lowering of dose but the long term effect after the peak vanishes quickly when the dose decreases. At all intervals of the total activity (Fig. 5) there is a significant difference between 50 µmol/kg and vehicle. For the interval 1-5 h a significant difference also can be found between 25 µmol/kg and vehicle. All other comparisons between vehicle and dose are not statistically significant.

Discussion

The activities of eight ester prodrugs of N-0437 have been examined in vitro, in which the hydrolysis of the prodrugs was determined, and in vivo, in which the effects on turning behaviour after a 6-OHDA lesion was studied. The AUC of the turning behaviour profiles was taken as a measure of the dopaminergic activity of the prodrug and also gives information about the time course of formation of the active compound. Hydrolysis experiments were performed to examine the strength of the ester bond and to examine the relation between the structure of the

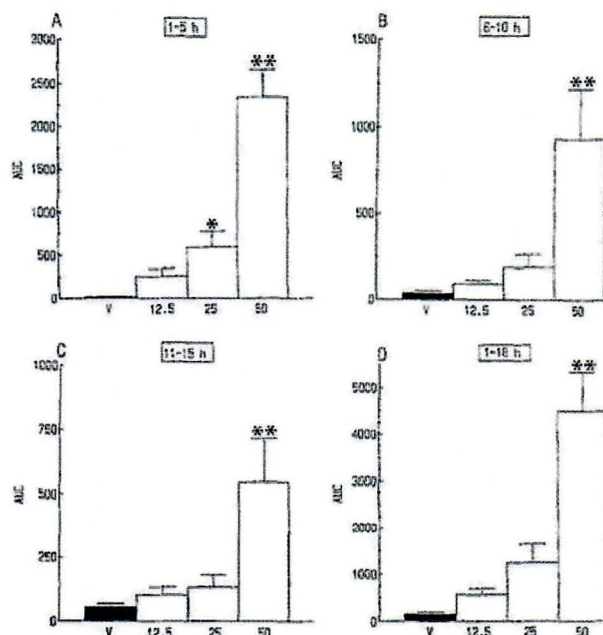


Fig. 5. The AUC from the time-response profile for vehicle (V) and three concentrations of isobutyryl ester (12.5, 25, 50 µmol/kg) after p.o. administration calculated from the time intervals 1-5 h (A), 6-10 h (B), 11-15 h (C) and 1-18 h (D). (Error bars are SEM, * $p < 0.05$, ** $p < 0.01$ vs. vehicle, Dunnett's test)

ester group and velocity of hydrolysis. Concerning the hydrolysis rates of the compounds we can divide them into two groups namely the aliphatic and aromatic esters. The former group displayed the effects one would expect i.e. increasing substitution on the carbon atom α to the carbonyl group led to a decline in the hydrolysis rate. This is due to an increase in steric crowding around the carbonyl function (Gould 1959).

In the aromatic group the pattern of activity is again what one would predict (Gould 1959), except for the 2-methoxybenzoyl analogue. Thus the steric bulk and electron donating character of the aromatic ring and its methyl and amino substituents produces a stabilization of the carbonyl bond leading to long half lives. The 2-methylbenzoyl and 2-methoxybenzoyl compounds display a very large difference in in-vitro stability. Although both the methoxy and methyl groups are electron donating the steric bulk of the former is smaller (Shorter 1973). It is therefore possible that the lability of the 2-methoxybenzoyl compound is due to a contribution of the oxygen atom which may enhance hydrolysis via complexation.

The first question that arises in this type of research is: what kind of prodrug is needed for an improved bioavailability, a rapidly hydrolysing prodrug or a slow conversion? Notari (1985) suggests that prodrug conversion to drug must be completed no later than their arrival in the blood, which means that a fairly rapid hydrolysis could be what is required to avoid first-pass metabolism. When we compare the in vitro experiments with the in vivo experiments there is evidence for this hypothesis, especially with the aliphatic esters. The acetyl ester converts too rapidly, probably already in the gastro-intestinal system. The pivaloyl ester hydrolyses too slowly and is probably excreted before it can be converted. The timing of the hydrolysis of the isobutyryl is apparently just right to increase the concentration of N-0437 in the rat and thereby increase the pharmacological effect by $\pm 100\%$ over the range 1–18 h and in the time interval 11–15 h by as much as $\pm 250\%$. The fact that the isobutyryl analogue shows such a favourable pharmacological profile is of interest bearing in mind the fact that another dopaminergic prodrug, ibopamine also contains this ester function. It has recently been marketed as an orally effective prodrug which is active at peripheral DA receptors (Casagrande et al. 1985).

With the aromatic esters a discrepancy is found between rapid conversion of the 2-methoxybenzoyl compound and the in vivo activity which at the moment cannot be explained.

The relatively stable prodrugs show an interesting phenomena. The 2-methylbenzoyl, 2,4-dimethylbenzoyl and 2-aminobenzoyl esters do not produce an N-0437-like profile, i.e. a very high initial activity which then declines. In contrast they display a plateau-like activity. This effect may be caused by the increase of lipophilicity due to the large ester group. Theoretical estimations of the logP values (Martin 1978) of the prodrugs reveal an approximate 50-, 75-, 100-fold increase of the lipophilicity of the 2-aminobenzoyl, 2-methylbenzoyl and 2,4-dimethylbenzoyl analogues compared with N-0437. This increase of lipophilicity could cause an accumulation of prodrug in the fatty tissues of the organism after administration, with a resulting slow release into the circulation when the prodrug hydrolyses. Several authors (Notari 1985; Hussain et al. 1987) have found no relation between prodrug bioavailability and in vitro conversion and although these studies examined mainly pharmacokinetic parameters like plasma concentrations, care has

to be taken in comparing in vitro experiments with a pharmacological effect.

The treatment of patients suffering from Parkinson's disease in the last decades is based on the precursor of dopamine, levodopa. One of the undesirable side effects of this treatment are fluctuations in response, called on-off effects which could be caused by peripheral pharmacodynamic mechanisms (Wooten 1988). A plateau-like activity profile, which has been found with some of the aromatic esters, could be a possible solution for the problems of on-off fluctuations. Other strategies of interest, to avoid first-pass metabolism and ensure a well balanced administration of drug to the central nervous system, are transdermal administration of N-0437 (Timmerman et al. 1989) or of its prodrugs (for a review see Sloan 1989). These possibilities are under investigation in our laboratory.

The promising results of this functional study regarding the possibilities of improving bioavailability need to be tested in other systems, especially because of the difference of esterase activity in different species. Testing in monkeys, which have been treated with MPTP, is currently under investigation. Pharmacokinetic studies of the most promising compounds could answer some questions raised by this findings and are under consideration.

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