Analogs of PD148903 –
Orally Active Anti-Parkinsonian Prodrugs

ABSTRACT

A series of analogs of the enone prodrug PD148903 (2.3), having different N-substituents, were synthesized and pharmacologically evaluated. The compounds were tested at different oral doses in the Ungerstedt rotation model for Parkinson’s disease in the rat. The newly synthesized analogs of 2.3 proved to be orally active and produced a pronounced and long lasting pharmacological effect. At 0.1 mg kg⁻¹ po, the N-methyl-N-propyl substituted analog (3.13) had a pharmacological profile similar to that of 2.3. The N-ethyl-N-propyl substituted analog (3.14) at that dose showed a significantly higher efficacy than 2.3 (p < 0.05). Analyses of rat brains after the administration of 2.3 and 3.14 indicated the presence of hydroxylated metabolites of both parent enones. It is hypothesized that these metabolites are involved as an intermediate in the bioactivation mechanism of these new prodrugs.
3.1 INTRODUCTION

The potential utility of the directly acting DA D$_2$ agonist 5-hydroxy-2-di-propylamino-1,2,3,4-tetrahydronaphthalene (5-OH-DPAT, 3.1) and the mixed DA D$_1$/D$_2$ agonist 5,6-dihydroxy-2-dipropylamino-1,2,3,4-tetrahydronaphthalene (5,6-di-OH-DPAT, 3.2) in treatment of Parkinson’s disease is generally recognized (Figure 3.1).$^{1-6}$ However upon oral administration, the phenol and the catechol moieties are rapidly metabolized to an extent that limits the therapeutic usefulness of these compounds.$^7$ For instance, the potential anti-Parkinson compound N-0437 (3.3) undergoes extensive first-pass metabolism (glucuronidation).$^8$ In the past it was attempted to circumvent this problem by developing prodrugs. Common prodrugs of phenols and catechols are esters, amides and carbamates.$^9-14$ These types of prodrugs generally do not improve the in vivo characteristics sufficiently to make oral administration feasible. For instance, the di-acetyl ester prodrug of the DA D$_1$ agonist ABT-431 (3.4) has a plasma half-life for both esters of 60 seconds and is only efficacious after intravenous (iv) administration.$^{15}$

![Figure 3.1](image)

Figure 3.1 Structures of some known hydroxylated 2-aminotetralin DA receptor agonists and prodrugs of dopaminergic catecholamines; 5-OH-DPAT (3.1), 5,6-di-OH-DPAT (3.2), N-0437 (3.3), ABT-431 (3.4), PD148903 (2.3), and its most active enantiomer, PD217015 (2.3a).
Chapter 2 described the synthesis and pharmacological evaluation of 2.3a (= (S)-2.3), as an orally active prodrug of (S)-5,6-di-OH-DPAT (3.2). This prodrug was found to be efficacious in vivo in models for Parkinson’s disease in rats. As an extension of this work, we synthesized a series of analogs of 2.3 in an attempt to investigate how general the bioactivation mechanism is and the structure activity relationship (SAR). Since 2.3 was subjected to enantioselective bioactivation, also the nature of the N-substituents could be of importance for the process of conversion to the active principle. In an attempt to investigate this, a series of analogs with different N-substituents was prepared.

Hydroxylated 2-aminotetralins are known to be pharmacologically active when having certain N-alkyl substituents. N,N-di-n-propyl substitution is considered optimal, with respect to DA D_2 binding affinity and CNS penetration. Substituents smaller than n-propyl make an aminotetralin less lipophilic and lowers the binding affinity for the DA D_2 receptor while retaining or increasing their potency at the DA D_1 receptor. Incorporation of one large substituent, like N-2-(phenyl)ethyl or N-2-(2-thienyl)ethyl (as in 3.3), increases the lipophilicity and improves the potency of the compounds. For 5,6-di-hydroxy-2-aminotetralins, only a few N-substitution patterns have been reported in literature. Instead, most of the work on N-substituents has been done using 5-hydroxy-2-aminotetralin as a scaffold. Once the in vivo disadvantages of catecholic 2-aminotetralins were discovered, researchers focussed their attention on their 5-hydroxylated, phenolic derivatives. Many N-alkylated 5,6-dihydroxy-aminotetralins and N-alkylated 5-hydroxy-2-aminotetralins have dopaminergic properties. For the phenolic 2-aminotetralins, DA D_2 agonism was retained but DA D_1 agonism was impaired, though they appear to be low affinity partial DA D_1 agonists. Double bonds and also heteroatoms like sulfur, oxygen and halogens have been incorporated into the N-substituents.

To further investigate the bioactivation mechanism, prodrug equivalents of known pharmacologically active 5-hydroxy-2-(N,N-dialkylamino)tetralins were synthesized (Figure 3.2). Since 2.3 was inactive in vitro, the analogs of 2.3 were pharmacologically evaluated in vivo in the Ungerstedt model for Parkinson’s disease using unilaterally 6-OH-DA lesioned rats. To investigate the presence of metabolites, brain extracts of rats treated with enone prodrugs were analyzed. Doing this for 2.3 and one of the newly designed enones, could provide an analogy in metabolism leading to a deeper understanding of the bioactivation mechanism. (S)-5,6-di-OH-DPAT was the only pharmacologically active principle detected after administration of 2.3a, thus, 2.3a needs to undergo some form of aromatization in the
bioactivation process. We synthesized a ‘3,3-dimethyl’ analog of 2.3 to study the influence of geminal dimethyl substitution on the enone ring on the pharmacological effect in vivo.

![Figure 3.2](image1)

**Figure 3.2** Enone equivalents of pharmacologically active 5-hydroxy-2-dialkylaminotetralins.

The α- or β-rotameric conformation of 4-aryl-di-OH-DPATs may determine the activity or inactivity at the DA receptors. 4-aryl-di-OH-DPATs may also have other properties than dopaminergic since N,N-dimethyl substituted compounds of that type exhibit activity at the histamine H₁ and σ-like receptors.²⁸,²⁹ In this chapter, only the synthesis of these compounds and their effect in the Ungerstedt model for Parkinson’s disease were investigated.²⁵

![Figure 3.3](image2)

**Figure 3.3** Structures of an 8-Aryl substituted enone (3.5), the corresponding catecholamine, 4-Ph-5,6-di-OH-DPAT (3.6), its inactive congener 4-Ph-6,7-di-OH-DPAT (3.7), (R)-isoapomorphine (3.8) and (R)-apomorphine (3.9).
In Chapter 2, the synthesis of 2.3 was described using formaldehyde in the initial condensation reaction. Though the synthesis was very efficient, many by-products were formed during the first reaction step. By using other aldehydes in this reaction, we thought to investigate the extent of the role formaldehyde plays in the formation of the by-products. Benzaldehyde and 2-thienylcarboxaldehyde were selected because incorporation of an aromatic ring at the 8 position of 2.3 might affect the dopaminergic activity of the enone after bioactivation to its corresponding catecholamine (Figure 3.3).

It cannot be predicted whether bioactivation to the corresponding catecholamine takes place and whether these unknown 4-aryl-5,6-di-OH-DPATs (3.6) are active at the DA receptors. Literature reports both cis and trans 4-aryl-6,7-di-OH-DPAT (3.7) to be inactive at the DA receptors. Yet it is also known that (R)-isoapomorphine (3.8) has poor dopaminergic properties as compared to the potent mixed DA D1/D2 receptor agonist (R)-apomorphine (3.9).

### 3.2 CHEMISTRY

#### 3.2.1 Synthesis of N,N-dialkyl analogs of 2.3

The synthesis of the analogs of 2.3 is outlined in Schemes 3.1. The precursor 1,6-diketone (3.10) was prepared in three steps according to literature procedures. Reductive amination of 3.10 with primary amines in tetrahydrofuran, in the presence of 1 equivalent of acetic acid, gave excellent yields. The secondary amines formed were found to decompose upon standing and were used for further synthesis without purification.

Enone 3.11 was synthesized by alkylation of the mono-n-propyl intermediate with 3-fluoropropyl-1-bromide. The reaction was carried out in acetonitrile with Cs2CO3 and KI at reflux temperatures and took prolonged heating for completion. The moderate yield of 3.11 is most likely due to the chemical instability of the intermediate, and the reaction time and temperatures required for the alkylation reaction. Reductive alkylation of the thienylethylamine intermediate with propionaldehyde gave 3.12 in good yield, and could be performed in the same pot. However, in order to have better control over the double bond selectivity in the second reduction step, it was preferred to work-up the reaction mixture after the first step.

Condensation of N-methyl-N-n-propylamine, N-ethyl-N-n-propylamine, or N-methyl-N-propargylamine with precursor 3.10 required heating in a sealed flask in the presence of powdered 4Å molecular sieves. In the preparation of these enamines, several side products were
formed that could not easily be separated from the desired product. Therefore, similar to the synthesis reported for 2.3, these enamine intermediates were reduced without purification, to their corresponding amines (3.13, 3.14 and 3.15). Yields were moderate since the selectivity for the desired double bond proved hard to control in the reduction step. Use of methanol or 1,2-dichloroethane increased reaction speed but decreased selectivity.\(^{31,33}\) In tetrahydrofuran the reaction and selectivity was controlled by the amount of acetic acid and maintaining a low temperature. Though both primary and secondary amines react with 3.10 to give an enamine, reduction of the primary enamine is far more selective\(^{34-36}\)

![Scheme 3.1 Synthesis of analogs of 2.3. Reagents and conditions: a) RNH\(_2\), NaBH\(_3\)CN, THF/AcOH, 0\(^\circ\)C; b) propionaldehyde, NaBH\(_3\)CN, THF/AcOH, 0\(^\circ\)C or F(CH\(_2\))\(_3\)Br, Cs\(_2\)CO\(_3\), acetonitrile, 80\(^\circ\)C; c) RR'NH, toluene, 60\(^\circ\)C; d) NaBH\(_3\)CN, THF/AcOH, 0\(^\circ\)C.](attachment:scheme_3.1.png)

### 3.2.2 Synthesis of an 3,3-dimethyl analog of 2.3

The 3,3-dimethyl analog (3.17) of 2.3 was synthesized analogously to the synthesis of 2.3 described in Chapter 2 (Scheme 3.2). Dimedone (3.16) was reacted with paraformaldehyde and dipropylamine in hot toluene in the presence of powdered 4Å molecular sieves followed by treatment with acetone. The presence of two methyl groups made the reaction proceed markedly slower than for 1,3-cyclohexadione. After 50h of heating, the reaction was incomplete but no further progress of the reaction was monitored and the heating was stopped. Analysis of the reaction mixture showed a similar amount of by-products had formed as in the preparation of
2.3. After work-up, the enamine intermediate was reduced to the corresponding amine. Like in the synthesis of 2.3 the overall yield of 3.17 was low.

Scheme 3.2 Synthesis of the 3,3-dimethyl analog of 2.3. Reagents and conditions: a) i) CH$_2$O, PrNH$_2$, toluene, 85°C; ii) acetone, toluene, 85°C; iii) NaBH$_3$CN, THF/AcOH, RT.

3.2.3 Synthesis of 8-aryl analogs of 2.3

Benzaldehyde and 2-thienylcarboxaldehyde were used analogously to the synthesis of 2.3 (Scheme 3.3). The corresponding aryl substituted enones, 3.19 and 3.20, were obtained with hardly any by-products. Obviously, aryllic aldehydes are able to stabilize the intermediates probably by conjugation of the ring electrons like in 3.21. The stabilizing effect of the aryllic aldehydes was also noticeable in the reaction time required for complete conversion to the initially formed enamine. The 8-Aryl substituted enones 3.19 and 3.20 were pharmacologically evaluated as diasteromeric mixtures.

Scheme 3.3 Synthesis of 3.19 and 3.20. The reaction may profit from the stabilizing effect of the aryl group in an intermediate like 3.24. Reagents and conditions: a) i) CH$_2$O, PrNH$_2$, toluene, 85°C; ii) acetone, toluene, 85°C; iii) NaBH$_3$CN, THF/AcOH, RT.
3.3 PHARMACOLOGY

3.3.1 In vivo pharmacology

All new compounds were tested on in vivo activity in the Ungerstedt model for Parkinson’s disease, using unilaterally 6-hydroxy-DA lesioned rats. In this model the DA receptors in the striatum on the lesioned side become hypersensitive. Administration of centrally acting DA agonists causes disproportionate stimulation of locomotor activity, making the rats move in circles. The more rotations, the more efficient a compound is considered to be against Parkinson’s disease. Rotations were recorded for 12 h after oral administration at several doses of the test compounds. A detailed description of this model is given in Chapter 2.

3.3.2 Brain extraction experiments

In order to gain further insight into the bioactivation mechanism of enone prodrugs, whole rat brain tissues were homogenized, extracted and analyzed by GC-MS to identify possible metabolites.

3.4 RESULTS AND DISCUSSION

3.4.1 The Ungerstedt model for Parkinson’s disease

In Table 3.1 the total cumulative full contralateral rotations for the tested analogs of 2.3 are presented. At a dose of 0.1 mg kg$^{-1}$ po compounds 3.13 and 3.14 produced potent effects. Compound 3.13 produced a similar effect to 2.3, however, 3.14 showed a significantly more potent effect ($p < 0.01$, t-test, power: 0.77). A significant difference between the effects of compounds 3.13 and 3.14 was not found. N-0437 analogous enone prodrug 3.11, and N-methyl-N-propargyl substituted 3.15, only induced a weak effect. 3,3-Dimethyl substituted analog 3.17 and the 8-aryl analogs, 3.19 and 3.20, induced such a weak effect at 1.0 mg kg$^{-1}$ po that they are considered inactive.

At 0.3 mg kg$^{-1}$ po, compound 3.13 produces similar results relative to 2.3. Analog 3.14 reaches maximum rotations possible and the effect is significantly larger than that of 2.3 ($p < 0.05$, Mann-Whitney rank sum test). A higher dose of 3.15 did not change the pharmacological effect significantly. The effect of 3.15 may be partially attributed to its possible MAO-inhibiting
properties. N-methyl-N-propargyl substituted amines, like deprenyl, pargyline and also aminotetralin based structures, are known to irreversibly bind the MAO enzyme.\textsuperscript{38} Compound 3.15 was not tested in a MAO-inhibitory essay.

Table 3.1 The pharmacological effect of 2.3 and some of its analogs at three doses \textit{po} in the Ungerstedt model for Parkinson’s disease.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total full contralateral turns (X ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 mg kg\textsuperscript{-1}</td>
</tr>
<tr>
<td>2.3</td>
<td>2312±440 (10)</td>
</tr>
<tr>
<td>3.11</td>
<td>262±75 (7)</td>
</tr>
<tr>
<td>3.12</td>
<td>-</td>
</tr>
<tr>
<td>3.13</td>
<td>2928±961 (4)</td>
</tr>
<tr>
<td>3.14</td>
<td>4840±757 (8)</td>
</tr>
<tr>
<td>3.15</td>
<td>713±237 (8)</td>
</tr>
<tr>
<td>3.17</td>
<td>-</td>
</tr>
<tr>
<td>3.19</td>
<td>-</td>
</tr>
<tr>
<td>3.20</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Numbers noted are the cumulative full contralateral turns in 12h. \textsuperscript{b}n, number of rats tested; \textsuperscript{c}-, not tested; \textsuperscript{d}3 hour totals.

A 3-fluoro substituent in one of the n-propyl side chains gives 3.12, in which the pharmacological effect of 2.3 is retained. The fluoro atom evidently can be considered as metabolically rather stable and not interfering with the bioactivation mechanism. The same is observed when administered at 1.0 mg kg\textsuperscript{-1} \textit{po}; 2.3 and 3.12 do not differ significantly. However, it must be noted that the effect at 1.0 mg kg\textsuperscript{-1} \textit{po} does not significantly differ from its effect at 0.3 mg kg\textsuperscript{-1} \textit{po}. Surprisingly the N-0437 analogous enone prodrug 3.11 had only a weak effect at 1.0 mg kg\textsuperscript{-1}. Little increased lipophilicity relative to 2.3 (LogD, calculated (Pallas®): 2.3 = 1.21, 3.11 = 2.11) should warrant a good oral absorption. Thus, either 3.11 fails in the bioactivation process or it is relatively more prone to other metabolism.

Figure 3.1 shows the effect of 3.13, 3.14 and 2.3 at 0.1 mg kg\textsuperscript{-1} \textit{po} in time. Statistical analysis of the graphs using a two-way ANOVA followed by a Student-Newman-Keuls test, shows the effect of 3.14 to be significantly different from 2.3 (p < 0.05). Graphs of 3.13 and 2.3
have a similar profile and are not significantly different at any time. All compounds have a similar rapid onset of action.

![Graph showing pharmacological effect of compounds over time](image)

**Figure 3.1** Pharmacological effect of 0.1 mg kg\(^{-1}\) of 2.3 and two of its analogs po in the Ungerstedt model for Parkinson’s disease expressed as cumulative full contralateral rotations per 15 min over 12h. Each point is the mean ± SEM. for n determinations. \(n = \text{number of animals tested; 3.13 (n = 4); 3.14 (n = 8); 2.3 (n = 10).}

The pharmacological effects are long lasting. Significant activity for 3.13 was measured from 30 to 600 min, for 3.14 from 30 to 720 min, and for 2.3 from 15 to 525 min (all \(p < 0.05\), Wilcoxon signed rank test). A saline curve was not included as it coincides with the x-axis.

The results for 3.13 (Me/Pr) and 3.14 (Et/Pr) relative to 2.3 (Pr/Pr) at both 0.1 mg kg\(^{-1}\) po and 0.3 mg kg\(^{-1}\) po are surprising. Numerous factors like differences in absorption, distribution, bioactivation, potency of the active metabolite or even indirect effects may contribute to these findings. Yet for aminotetralins it has been described that 5,6-di-OH-DPAT had a 133 fold increased potency for in inducing D\(_1/D_2\) related stereotyped behavior in the rat relative to 5,6-di-OH-MethylPAT (respectively the active metabolite of 2.3 and the anticipated active metabolite of 3.13).\(^{23}\) Furthermore an 11 fold increased potency for 5,6-di-OH-DPAT was found over 5,6-di-OH-MethylPAT, in its anti-tremor activity in the reserpinized rat.\(^{23}\) Therefore it is unlikely that the potency of the active metabolites (the aminotetralins) determines the pharmacological
effect induced by the prodrugs. Furthermore, considering absorption and disposition, MAO susceptibility and DA D₂ receptor binding affinity of aminotetralins, N,N-di-n-propyl substitution has been considered optimal for inducing a pharmacological effect.²¹

Therefore, considering the fact that in the Ungerstedt rat model 3.13 and 2.3 are equipotent and 3.14 is more potent than 2.3, it could be that, to some extent, differences in pharmacology are related to differences in bioactivation of 3.13, 3.14 and 2.3. It is thus possible that like the orientation at the chiral center (Chapter 2), also the nature of the N-substituents influences the bioactivation mechanism.

3.4.2 Brain extraction experiments

Whole rat brain tissues were homogenized, extracted and analyzed by GC-MS for metabolites after administration of 30 mg kg⁻¹ sc 3.14 or 10 mg kg⁻¹ sc 2.3a. Vehicle only was administered to provide a blank experiment. Standard solutions of 3.14 and 2.3a were used to determine retention time and fragmentation pattern.

Figure 3.2 shows the mass spectra after analyses: (A) and (B) show the M⁺-ion and fragments of 3.14 and 2.3a respectively, (C) and (D) show the mass spectra of two newly observed analytes. Both M⁺-ions of (C) and (D) correspond with an increase in molecular mass of 16 with respect to their administered precursor. Since fragmentation is similar and none of the compounds above has been detected after saline experiments, it is evident that (C) and (D) are metabolites of the tested compounds. The increase in molecular mass by 16 is most easily explained with the introduction of an oxygen atom into the molecule.

To understand where the oxygen atom is inserted into the molecule it is necessary to look at the fragmentation patterns of the molecules. It is known for alkylated amines that the fragments M–29 or M–15 (235→206, 235→220, 249→220, 251→222, 265→236) originate from the loss of an ethyl or methyl moiety from the nitrogen substituent.⁴¹ Six-membered cyclohexenone rings are known to give ‘retro Diels-Alder’ reactions in their fragmentation. A retro Diels-Alder reaction on (A) and (B) of the amine substituted ring gives N,N-dialkyl-N-vinylamines of 113 amu and 127 amu respectively that are also observed in (C) and (D) respectively. Because the fragmentation pattern of the metabolites is not altered for (C) and (D) it is unlikely that an oxygen atom is incorporated in the N,N-di-n-propylamine and the N-ethyl-N-n-propylamine moieties of the molecules or that an N-oxide was formed.
Incorporation of an oxygen atom into the fused ring system seems most plausible. Loss of the N,N-dialkylamine moiety in (A) and (B) leaves a fused ring system with a molecular mass of 149 amu. Instead, in the case of metabolites (C) and (D), this has changed to 147 amu. This could be explained oxidation of the prodrugs to hydroxylated metabolites that dehydrate upon fragmentation (149 + 16 − 18 = 147). In fact, literature suggests a metabolic pathway for testosterone, involving a hydroxylation on its $\alpha'$ position.}

**Figure 3.2** El mass spectra ($M^+$) of (A) 3.14, (B) 2.3a, (C) the observed metabolite of 3.14 and (D) the observed metabolite of 2.3a.
3.4.3 Hypothesized bioactivation mechanism

For 2.3a, the active metabolite in the brain was found to be 5,6-di-OH-DPAT and metabolism of the active prodrug 3.14 is expected to be similar. It is, therefore, conceivable that this α,β-unsaturated-α'-hydroxylated ketone ring system, by in vivo oxidation of the hydroxyl group, is converted to the corresponding α,β-unsaturated-1,2-diketones. Two consecutive keto-enol tautomerizations of this di-ketone will produce the corresponding 5,6-dihydroxy-2-aminotetralin (Scheme 3.4). We have not detected catecholic aminotetralins under this protocol.

\[
\begin{align*}
A: M_w &= 235 \\
B: M_w &= 249 \\
C: M_w &= 251 \\
D: M_w &= 265
\end{align*}
\]

Scheme 3.4 Hypothesized in vivo bioactivation of enone prodrug to their corresponding catechols. Conditions: (a) hydroxylation; (b) oxidation; (c) keto-enol tautomerization.

3.5 CONCLUSIONS

The bioactivation mechanism as observed for 2.3 probably also be applied to analogs with different N-substituents. Compounds 3.12, 3.13, and 3.14 were able to induce a potent and long-lasting dopaminergic pharmacological effect upon oral administration. Even at low doses, these newly synthesized compounds displayed similar or even higher potency relative to 2.3 in alleviating Parkinsonian symptoms. The nature of the N-substituents in 3.12, 3.13, and 3.14 did not affect the onset of action relative to that of 2.3 or 2.3a. Remarkably, N-ethyl-N-n-propyl
substituted 3.14 gave a more potent pharmacological effect than N,N-di-n-propyl substituted 2.3.
This could indicate a correlation between N-substituent and bioactivation efficiency.

The very weak activity of 3.11 was surprising considering the potency of N-0437. The presence of the thiophene ring evidently interferes with metabolism yielding little or no active catecholamine. The inactivity of 3,3-dimethyl substituted analog 3.17 in vivo, demonstrates that bioactivation to a potent dopaminergic compound is inhibited and that the enone structure itself does not induce a dopaminergic effect. Synthesis and testing of an analog of 2.3 with a five-
membered enone ring system may provide further information. Such an enone structure would be most similar to compound 2.3 yet the five-membered ring cannot be transformed to an aromatic ring that is considered essential for binding to the DA receptor. The inactivity of 3.19 and 3.20 could be due to failure in bioactivation or the lack of dopaminergic properties of the corresponding catecholamines.

Metabolism of 3.14 is expected to be similar to that of 2.3a, in which case the active metabolite in the brain was found to be 5,6-di-OH-DPAT. Therefore, it could be possible that hydroxylated metabolite is converted to the catechol. In fact, in vivo α'-oxidation of the hydroxyl group, could give and α,β-unsaturated-1,2-diketones that after two consecutive keto-

3.6 EXPERIMENTAL SECTION

3.6.1 Chemistry

General remarks. Melting points were determined in open glass capillaries on an Electrothermal digital melting-point apparatus and are uncorrected. 1H- and 13C-NMR spectra were recorded at 200 MHz and 50.3 MHz, respectively, on a Varian Gemini 200 spectrometer. The splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Chemical shifts are given in δ units (ppm) and are relative to the solvent. Coupling constants are given in Hertz (Hz). The spectra recorded were consistent with the proposed structures. IR spectra were obtained on an ATI-Mattson spectrometer. Electronic ionization (EI) mass spectra were obtained on a Unicam 610-Automass 150 GC-MS system. Chemical ionization (CI) mass spectra were recorded by the Mass Spectrometry Unit of the University of Groningen. Elemental analyses were performed by the Analytical Chemistry Section at Parke Davis (Ann Arbor, MI) or by the Microanalytical Department of the University of Groningen.
and were within ± 0.4 % of the theoretical values, except where noted. All chemicals used were commercially available (Aldrich or Acros) and were used without further purification.

6-[N-n-Propyl-N-(2-thiophen-2-yl-ethyl)]-amino-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one (3.11). To a stirred solution of 3,4,7,8-tetrahydro-2H,5H-naphthalene-1,6-dione (0.50 g, 3.0 mmol) in dry THF (15 mL) was added 2-thiophen-2-yl-ethylamine (0.35 mL, 3.0 mmol). After stirring for 30 min, NaBH₃CN (0.29 g, 4.5 mmol) was added followed 30 min later by dropwise addition Acetic acid (0.18 mL, 3.0 mmol). After stirring at RT for 3 h the solvent was evaporated. The residue was partitioned between diethyl ether (50 mL) and 50% aqueous Na₂CO₃ (25 mL). The layers were separated and the aqueous layer was extracted with diethyl ether (2 x 50 mL). The combined ethereal layers were dried (Na₂SO₄), filtered and evaporated to give 0.83 g of a colorless oil which was used without further purification. ¹H-NMR (CDCl₃) δ 7.14 (d, 1H), 6.93 (t, 1H), 6.82-6.92 (m, 1H) 2.91-3.10 (m, 4H), 2.75-2.87 (m, 1H), 2.34-2.55 (m, 4H), 1.87-2.28 (m, 8H) ppm; ¹³C-NMR (CDCl₃) δ 198.7, 165.9, 154.5, 142.0, 131.7, 126.7, 125.0, 123.6, 52.2, 47.9, 38.3, 37.5, 31.0, 27.9, 22.1, 20.9 ppm. The oil was dissolved in methanol (60 mL) and propionaldehyde (3.0 mL, 45 mmol) was added. After stirring at RT for 30 min, NaBH₃CN (0.30 g, 4.8 mmol) was added slowly and the mixture was stirred overnight. After stirring at RT for 3 h the solvent was evaporated. The residue was partitioned between diethyl ether (100 mL) and 10% aqueous NaHCO₃ (25 mL). The layers were separated and the aqueous layer was extracted with diethyl ether (4 x 40 mL). The combined ethereal layers were dried (MgSO₄), filtered and evaporated. The resulting colorless oil was purified by column chromatography (silica, dichloromethane/ethanol, 20:1) was subsequently converted to the hydrochloric salt. Yield 0.61 g, 1.7 mmol (58%), mp 189-192°C. IR (KBr) 2943, 2612, 1655, 1389, 850, 706 cm⁻¹; ¹H-NMR (CDCl₃) δ 7.10 (d, 1H), 7.07 (t, 1H), 6.77-6.91 (m, 1H), 3.55-3.71 (m, 3H), 2.73-2.92 (m, 4H), 2.31-2.71 (m, 4H), 2.20 (br d, 2H), 1.87-2.22 (m, 3H), 1.28-1.56 (m, 5H), 0.86 (t, 3H) ppm; ¹³C-NMR (CDCl₃) δ 198.7, 156.3, 141.9, 131.7, 126.4, 124.4, 123.1, 56.0, 52.4, 37.5, 34.4, 31.3, 31.2, 29.8, 24.4, 22.7, 22.1, 22.0, 11.6 ppm; MS (EI) m/z 317 (M⁺); Anal. (C₁₉H₂₇NOS·HCl) C, H and N.

6-(N-(3-Fluoro-n-propyl)-N-n-propyl)-amino-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one (3.12). To a stirred solution of 3,4,7,8-tetrahydro-2H,5H-naphthalene-1,6-dione (0.5g, 3.0 mmol) in dry tetrahydrofuran (15 mL) was added n-propylamine (0.28 mL, 3.0 mmol) and
NaBH₃CN (0.29 g, 4.5 mmol). After stirring for 30 min., Acetic acid (0.18 mL, 3.0 mmol) was added dropwise. After stirring at RT for 3 h the solvent was evaporated. The residue was partitioned between diethyl ether (50 mL) and 50% aqueous Na₂CO₃ (25 mL). The layers were separated and the aqueous layer was extracted with diethyl ether (2 x 50 mL). The combined ethereal layers were dried (Na₂SO₄), filtered and evaporated. The compound proved to decompose readily and was therefore used without further purification. Crude yield: 0.7 g, purity: ~90% (GC), MS (EI) m/z 207 (M⁺).

A mixture of the crude product (0.7 g, ~3.0 mmol), Cs₂CO₃ (2.7 g), 1-bromo-3-fluoro-propane (1.22 g, 10.8 mmol) in acetonitrile (25 mL) was heated to 80°C for 36h. After cooling another portion of 1-bromo-3-fluoro-propane (1.22 g, 10.8 mmol) was added and heating was continued for 8h. The reaction mixture was then cooled to RT and diluted with ether (25mL). Filtration and evaporation of the solvents gave 0.5 g of a dark oil, which was purified by column chromatography (silica, dichloromethane/ethanol, 100:1). The purified product was converted to the hydrochloric salt. Yield: 0.14 g, 0.5 mmol (15%), mp 135-139°C. IR (KBr) 2946, 2452, 1660, 1387 cm⁻¹; ¹H-NMR (CDCl₃) δ 4.59 (dt, 1H, J = 5.7 Hz), 4.35 (dt, 1H, J = 5.7 Hz), 2.71-2.77 (m, 1H), 2.12-2.58 (m, 10H), 1.61-2.05 (m, 8H), 1.18-1.48 (m, 3H), 0.82 (t, 3H) ppm; ¹³C-NMR (CDCl₃) δ 198.7, 156.5, 131.7, 82.1 (d, 83.7, 80.4, CH₂F, J = 163.7 Hz), 55.4, 45.5, 45.4, 37.5, 34.3, 31.1, 29.7 (d, 29.8, 29.5, CH₂CH₂F, J = 19.4 Hz), 24.0, 22.7, 22.2, 21.7, 11.5 ppm; MS (EI) m/z 267 (M⁺); Anal. (C₁₆H₂₆FNO·HCl), C, H and N.

6-(N-Methyl-N-n-propyl)-amino-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one (3.13). To a stirred solution of 3,4,7,8-tetrahydro-2H,5H-naphthalene-1,6-dione (0.25g, 1.52 mmol) and powdered 4Å molecular sieves (1 g) in toluene (10 mL) was added N-methyl-n-propylamine (0.12 g, 1.54 mmol). The mixture was heated in a sealed flask to 60°C for 18h and after cooling another portion of N-methyl-n-propylamine (0.12 g, 1.54 mmol) was added. Heating is then continued for 8h. Work-up by cooling, filtration, rinsing the residue with ether (5 x 10 mL) and evaporation of the solvents. The resulting yellow/brown solid was dissolved in methanol (15 mL), cooled to 0°C and the pH was adjusted to 4 by adding acetic acid. Then NaBH₃CN (0.11g, 1.65mmol) was added slowly. After stirring at 0°C for 30 min the cooling bath was removed and the reaction mixture was stirred at RT for 2h. The solvent was evaporated and the residue partitioned between 50% aqueous NaHCO₃ (20 mL) and ether (50 mL). After separation of the layers the aqueous layer was extracted with ether (3 x 25 mL). The combined ether layers were
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washed with brine, dried (Na₂SO₄) and evaporated. The resulting oil was purified by column chromatography (silica, dichloromethane/methanol, 20:1) and subsequently converted to the hydrochloric salt. Yield: 0.12 g, 0.45 mmol (30%), mp 140-143°C. IR (KBr) 2937, 2451, 1652, 1390 cm⁻¹; ¹H-NMR (CDCl₃) δ 2.52-2.60 (m, 2H), 2.30-2.42 (m, 4H), 2.20-2.24 (m, 6H), 1.90-1.97 (m, 4H), 1.24-1.50 (m, 4H), 0.85 (t, 3H) ppm; ¹³C-NMR (CDCl₃) δ 198.9, 156.1, 131.7, 58.1, 55.4, 37.5, 37.4, 33.7, 31.1, 24.2, 22.5, 22.1, 20.4, 11.6 ppm; MS (EI) m/z 221 (M⁺); Anal. C₁₄H₂₃NO·HCl) C, H and N.

6-(N-Ethyl-N-n-propyl)-amino-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one (3.14).

To a stirred solution of 3,4,7,8-tetrahydro-2H,5H-naphthalene-1,6-dione (0.75g, 4.30 mmol) and powdered 4Å molecular sieves (2 g) in toluene (15 mL) was added N-ethyl-n-propylamine (0.41 g, 4.70 mmol). The mixture was heated in a sealed flask to 80°C for 18h and after cooling another portion of N-methyl-n-propylamine (0.12 g, 1.54 mmol) was added. Heating is then continued for 8h. Work-up by cooling, filtration, rinsing the residue with ether (5 x 10 mL) and evaporation of the solvents. The resulting yellow/brown solid was dissolved in methanol (25 mL), cooled to 0°C and the pH was adjusted to 4 by adding acetic acid. Then NaBH₃CN (0.37g, 5.85 mmol) was added slowly. After stirring at 0°C for 30 min the cooling bath was removed and the reaction mixture was stirred at RT for 2h. The solvent was evaporated and the residue partitioned between 50% aqueous NaHCO₃ (20 mL) and ether (50 mL). After separation of the layers the aqueous layer was extracted with chloroform (3 x 25 mL). The combined ether layers were washed with brine, dried (Na₂SO₄) and evaporated. The resulting oil was purified by column chromatography (silica, dichloromethane/methanol, 20:1) and subsequently converted to the hydrochloric salt. Yield: 0.51 g, 1.87 mmol (48%), mp 157-158°C. IR (KBr) 2944, 2446, 1652, 1385 cm⁻¹; ¹H-NMR (CDCl₃) δ 2.71-2.78 (m, 1H), 2.51 (q, 2H), 2.27-2.41 (m, 9H), 1.83-1.94 (m, 4H), 1.17-1.44 (m, 3H), 0.97 (t, 3H), 0.85 (t, 3H) ppm; ¹³C-NMR (CDCl₃) δ 198.6, 156.1, 131.7, 55.5, 51.5, 43.9, 37.5, 34.4, 31.2, 24.4, 22.6, 22.1, 21.6, 13.4, 11.5 ppm; MS (EI) m/z 235 (M⁺); Anal. C₁₅H₂₅NO-HCl) C, H and N.

6-(N-Methyl-N-propargyl)-amino-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one (3.15). To a stirred solution of 3,4,7,8-tetrahydro-2H,5H-naphthalene-1,6-dione (0.25g, 1.40 mmol) and powdered 4Å molecular sieves (1 g) in toluene (10 mL) was added N-methyl-propargylamine (0.13 mL, 1.54 mmol). The mixture was heated in a sealed flask to 80°C for 18h
and after cooling another portion of N-methyl-n-propylamine (0.12 g, 1.54 mmol) was added. Heating is then continued for 8h. Work-up by cooling, filtration, rinsing the residue with ether (5 x 10 mL) and evaporation of the solvents. The resulting yellow/brown solid was dissolved in methanol (25 mL), cooled to 0°C and the pH was adjusted to 4 by adding acetic acid. Then NaBH₃CN (0.14 g, 2.10 mmol) was added slowly. After stirring at 0°C for 30 min the cooling bath was removed and the reaction mixture was stirred at RT for 2h. The solvent was evaporated and the residue partitioned between 50% aqueous NaHCO₃ (20 mL) and ether (50 mL). After separation of the layers the aqueous layer was extracted with chloroform (3 x 25 mL). The combined ether layers were washed with brine, dried (Na₂SO₄) and evaporated. The resulting oil was purified by column chromatography (silica, dichloromethane/methanol, 30:1) and subsequently converted to the hydrochloric salt. Yield: 0.18 g, 0.71 mmol (51%), mp 120-123°C. IR (KBr) 3188, 2931, 2467, 1666, 1389 cm⁻¹; ¹H-NMR (CDCl₃) δ 3.40 (d, 2H), 2.53-2.63 (m, 1H), 2.17-2.45 (m, 10H), 1.86-2.11 (m, 4H), 1.11-1.27 (m, 2H) ppm; ¹³C-NMR (CDCl₃) δ 198.6, 154.8, 131.6, 73.1, 56.3, 42.9, 38.4, 37.4, 35.4, 31.0, 25.2, 22.1, 21.7 ppm; MS (El) m/z 217 (M⁺); Anal. (C₁₄H₁₉NO·HCl) C, H and N.

6-(N,N-di-n-Propylamino)-3,3-dimethyl-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one (3.17). Dimedone (3.16) (5.0 g, 35.7 mmol), paraformaldehyde (1.1 g, 35.7 mmol), dipropylamine (3.8 g, 37.5 mmol) and powdered 4Å molecular sieves (18 g) were mixed in toluene (55 mL). Under vigorous stirring the mixture was heated to 85°C for 1h. Acetone (2.71 mL, 37.1 mmol) was introduced and heating continued for 50h. After cooling the mixture was filtered and the residue was rinsed thoroughly (ethyl acetate). The organic layer was concentrated to about 50 mL and washed with 4N HCl (5 x 50 mL). The combined acidic layers were basified to pH = 9 using 4N NaOH and then were extracted with ethyl acetate (5 x 50 mL). The combined organic layers were washed with brine, dried (MgSO₄) and evaporated to give a red solid (3.85 g, crude). The solid was dissolved in methanol (50 mL) and acetic acid (2.0 mL) was added. After cooling the mixture to 0°C, NaBH₃CN (0.90 g, 14.3 mmol) was added slowly. The reaction mixture was allowed to warm up to RT overnight. Work-up by evaporation of the solvent and partitioning of the residue between 50% aqueous NaHCO₃ (20 mL) and ether (50 mL). After separation of the layers the aqueous layer was extracted with chloroform (3 x 25 mL). The combined ether layers were washed with brine, dried (Na₂SO₄) and evaporated. The resulting oil was purified by column chromatography (silica,
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dichloromethane/methanol, 20:1) and subsequently converted to the hydrochloric salt. Yield: 2.26 g, 7.2 mmol (21%), mp 98-102°C. IR (KBr) 2965, 2425, 1655, 1393 cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) 2.57-2.82 (m, 2H), 2.43 (t, 3H), 1.91-2.21 (m, 8H), 1.38-1.50 (m, 6H), 1.01 (s, 3H), 0.96 (s, 3H), 0.85 (t, 6H) ppm; \(^{13}\)C-NMR (CDCl\(_3\)) \(\delta\) 198.9, 153.7, 130.5, 55.9, 52.3, 51.1, 45.3, 34.5, 32.9, 29.1, 27.0, 24.2, 22.4, 21.6, 11.6 ppm; MS (El) \(m/\zeta\) 277 (M\(^+\)); Anal. (C\(_{18}\)H\(_{28}\)N\(_2\)O\(_2\)·HCl·1/2H\(_2\)O) C, H and N.

6-(N,N-di-n-Propyl)amino-8-phenyl-3,4,5,6,7,8-hexahydro-2\(^H\)-naphthalen-1-one (3.19) 1,3-cyclohexadion (1.0 g, 8.9 mmol), benzaldehyde (0.94 g, 8.9 mmol), di-n-propylamine (0.94 g, 9.3 mmol) and powdered 4Å molecular sieves (5 g) were mixed in toluene (15 mL). Under vigorous stirring the mixture was heated to 85°C for 1h. Acetone (0.65 mL, 8.9 mmol) was introduced and heating continued for 54h. After cooling the mixture was filtered and the residue was rinsed thoroughly (ethyl acetate). The organic layer was evaporated to give a red oil (2.19 g, crude) which was dissolved in methanol (30 mL) and acetic acid (2.0 mL) was added. After cooling the mixture to 0°C, NaBH\(_3\)CN (0.56 g, 8.9 mmol) was added slowly. The reaction mixture was allowed to warm up to RT overnight. Work-up by evaporation of the solvent and partitioning of the residue between 50% aqueous NaHCO\(_3\) (30 mL) and diethyl ether (20 mL). The combined organic layers were washed with brine, dried (Na\(_2\)SO\(_4\)) and evaporated. The resulting oil was purified by column chromatography (silica, dichloromethane/ methanol, 20:1) and subsequently converted to the hydrochloric salt that was recrystallized from ethanol/diethyl ether. Yield: 0.88 g, 2.4 mmol (27%), mp 165-167 °C. IR (KBr) 2936, 2877, 2451, 1662, 1454 cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) 7.03-7.29 (m, 5H), 3.78 (br s, 1H), 2.85 (dt, 1H), 2.13-2.39 (m, 10H), 1.89-2.04 (m, 3H), 1.21-1.47 (m, 5H), 0.85 (t, 6H) ppm; \(^{13}\)C-NMR (CDCl\(_3\)) 197.3, 158.4, 147.1, 135.0, 128.2, 127.9, 127.4, 126.4, 125.4, 55.2, 52.2, 42.2, 37.7, 36.9, 35.0, 31.9, 22.0, 21.5, 11.6 ppm; MS (El) \(m/\zeta\) 326 (M+1); Anal. (C\(_{22}\)H\(_{31}\)NO·HCl) C, H and N.

6-(N,N-di-n-Propyl)amino-8-(thiophen-2-yl)-3,4,5,6,7,8-hexahydro-2\(^H\)-naphthalen-1-one (3.20) 1,3-cyclohexadion (1.0 g, 8.9 mmol), 2-thiophenecarboxaldehyde (1.0 g, 8.9 mmol), dipropylamine (0.94 g, 9.3 mmol) and powdered 4Å molecular sieves (5 g) were mixed in toluene (15 mL). Under vigorous stirring the mixture was heated to 85°C for 1h. Acetone (0.65 mL, 8.9 mmol) was introduced and heating continued for 20h. After cooling the mixture
was filtered and the residue was rinsed thoroughly (ethyl acetate). The organic layer was evaporated to give a red oil (2.6 g, crude). The oil was dissolved in methanol (30 mL) and acetic acid (2 mL) was added. After cooling the mixture to 0°C, NaBH₃CN (0.56 g, 8.9 mmol) was added slowly. The reaction mixture was allowed to warm up to RT overnight. Work-up by evaporation of the solvent and partitioning of the residue between 50% aqueous NaHCO₃ (30 mL) and diethyl ether (20 mL). After separation of the layers the aqueous layer was extracted with chloroform (3 x 50 mL). The combined ether layers were washed with brine, dried (Na₂SO₄) and evaporated. The resulting oil was purified by column chromatography (silica, dichloromethane/methanol, 20:1) and subsequently converted to the hydrochloric salt that was recrystallized from ethanol/diethyl ether. Yield: 1.0 g, 3.3 mmol (37%), mp 156-157°C. IR (KBr) 2940, 2870, 1670, 1435 cm⁻¹; ¹H-NMR (CDCl₃) δ 6.99-7.05 (m, 1H), 6.79-6.89 (m, 2H), 2.88 (br t, 1H), 2.23-2.49 (m, 12H), 1.89-2.00 (m, 2H), 1.39-1.65 (m, 5H), 0.87 (t, 6H) ppm; ¹³C-NMR (CDCl₃) δ 195.7, 162.3, 140.1, 133.5, 125.1, 121.6, 120.6, 54.0, 51.0, 36.4, 35.6, 35.5, 33.3, 30.5, 28.0, 20.1, 10.3 ppm; MS (EI) m/z 331 (M⁺); Anal. C₂₀H₂₉NOS·HCl C, H, N and Cl.

3.6.2 Pharmacology

General remarks. All compounds were tested as hydrochloride salts unless noted otherwise. All in vivo experiments were performed at Parke-Davis Pharmaceutical Research (Ann Arbor, MI, USA). Brain extraction experiments were performed at the laboratory animal unit of the Rijksuniversiteit Groningen, The Netherlands.

The Ungerstedt model for Parkinson’s disease.²⁵ Contralateral turning experiments were essentially according to the original reference by Ungerstedt and Arbuthnott. Briefly, rats were lesioned in right medial forebrain bundle (P4.8mm, L1.1mm, V-8.2mm from bregma) with 8 mg / 4 mL of 6- hydroxydopamine HBr in saline with ascorbic acid 1 mg/ml added. After 3 weeks recovery, completeness of lesion was assessed with apomorphine 50 mg kg⁻¹ sc. Only animals rotating more than 100 turns in an hour were used in subsequent experiments. Rats were removed from home cages in morning, weighed, dosed and placed into harnesses in rotator apparatus. Rats sit in stainless steel, flat bottomed, hemispheric bowls and are connected via the harness and a flexible spring tether to an automated data collection system. Data is presented as full rotations in contralateral directions. Rats are used once weekly.
**Brain extraction.** The experiments were largely adapted from Feenstra et al.\(^4^3\) Male Wistar rats were administered 30 mg kg\(^{-1}\) sc of 3.14 or 10 mg kg\(^{-1}\) sc of 2.3a, or a saline solution. After 30 min all prodrug treated animals showed stereotypical dopaminergic behavior. The rats were then stunned, decapitated and the whole brain was removed quickly and kept in liquid N\(_2\). Tissue homogenates were prepared with a Teflon pestle in 5 mL glass tubes filled with 0.5 mL 0.1M HClO\(_4\) solution. The homogenates were poured into polypropylene tubes. The pestle and glass tubes were rinsed with 0.5 mL 0.1M HClO\(_4\) solution that was added to the homogenate (total of about 3 mL). The homogenates were centrifuged at 3000 g at 4\(^\circ\)C for 15 min. The supernatants were completely decanted into glass tubes. The pH was adjusted to 11 by addition of saturated Na\(_2\)CO\(_3\) (300 \(\mu\)L) solution. To the alkaline solution was added diethyl ether (3 mL) and this was shaken vigorously for 30 min. Tubes were then centrifuged at 3000 g at 4\(^\circ\)C for 5 min and 80\% of the organic layer was removed with a pipette. The extraction procedure was repeated three times and the organic layers were combined and evaporated under a stream of N\(_2\) gas at RT. The residue was dissolved in toluene (150 \(\mu\)L). Analyses was performed by splitless injection of 4 \(\mu\)L of the solution into a Unicam 610-Automass 150 GC/MS system, fitted with an Alltech CpSil, 10 meter column. Injection at 275 \(^\circ\)C, column at 100-320\(^\circ\)C, ramp rate 10 \(^\circ\)C /min, EI mass detection.

### 3.7 REFERENCES AND NOTES


36. When a primary amine reacts with 3.1, the initially formed imine readily rearranges to the enamine in which the electrons of six chemical bonds have become conjugated.


