Design, synthesis and pharmacological evaluation of Enone prodrugs
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Chapter 2

GMC-6650: An orally active dopaminergic prodrug

Abstract

Enone prodrugs of dopaminergic catecholamines represent a new type of prodrug in research on dopamine (DA) agonists. Here, we demonstrate the first benzo[g]quinoline derived enone that induces DA receptor agonist effects just like the aminotetralin derived enones. Significant effects of the (–) enantiomer of [trans-1-propyl-2,3,4,4a,5,7,8,9,10,10a-decahydro-1H-benzo[g]quinolin-6-one] ((–)-2.2a) were observed in microdialysis studies after administration of 1 nmol kg⁻¹ sc and 3 nmol kg⁻¹ po. With a potency comparable to that of the potent DA agonist apomorphine, (–)-2.2a could potentially compete with L-dopa and apomorphine in the treatment of Parkinson’s disease (PD).

2.1 Introduction

To prevent dyskinesias, DA agonist used for treating PD should display a long duration of action. We previously described that \((S)-6-(N,N-di-n-propylamino)-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one\) [\((S)-PD148903, 2.1\)] is bioactivated \textit{in vivo} to its corresponding catecholamine \([\text{\textit{(S)}}]-5,6-di-OH-DPAT, 1.24\) which acts as a potent mixed DA D/D2 full agonist. This DA agonist induced potent dopaminergic behaviour at low doses \((0.03 \text{ mg kg}^{-1} \text{ po})\) in the Ungerstedt rat model of PD. Based on the analysis of brain and plasma samples, it was assumed that the enone was hydroxylated on the \(\alpha\)-ketone position and further converted to the catecholamine \((1.24)\). Furthermore, several analogs of \textbf{2.1} with different \(N\)-alkyl substituents induce similar or more potent dopaminergic effects. Evidently, the aminotetralin derived analogs of \textbf{2.1} contain a ‘template’ for the bioactivation from enones to the corresponding catecholamines, since they do not process any DA agonistic activity by themselves.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{structures.png}
\caption{Molecular structures of \(\text{(S)}\)-PD148903 \textbf{(2.1)}, 5,6-di-OH-DPAT \textbf{(1.24)}, \(-\)-\(N\)-(\(n\)-propyl)-6,7-dihydroxy-benzo[g]quinoline \((\text{TL-334, 1.26d})\), its analogous potential enone prodrug \textit{trans} isomer GMC-6650 \textbf{(2.2a)} and inactive \textit{cis} isomer \textbf{2.2b}.}
\end{figure}

In the structure of the enone prodrug, the \(N,N\)-propyl amine may rotate freely around the C-N bond, rigidification of the general structure \textbf{2.1} to a three-membered ring system should produce an enone that is more restricted to a flat conformation which is more likely to give a more potent DA agonist. It was considered interesting to extend this prodrug concept to benzo[g]quinolines, exemplified by \(N\)-(\(n\)-propyl)-6,7-di-OH-benzo[g]quinoline \((\text{TL-334, 1.26d})\). Like the aminotetralin \textbf{(1.24)}, \text{TL-334 (1.26d)} is a
catecholamine and known as a potent, centrally acting DA receptor agonist.\(^4,5\) Therefore, the tricyclic enone \textit{trans}-1-propyl-2,3,4,4a,5,7,8,9,10,10a-decahydro-1\(H\)-benzo[g]quinoline-6-one (GMC-6650, \textbf{2.2a}) was prepared and pharmacologically evaluated.

### 2.2 Chemistry

#### 2.2.1 Synthetic route \(1^6\)

The target compound GMC-6650 (\textbf{2.2a}) was prepared using the following strategy (Scheme 2.1 and Scheme 2.5).

The starting material 3-(4-methoxyphenyl)-acrylic acid (\textbf{2.3}) was converted to the secondary amine \textbf{2.6} in three steps resulting in high yields (Scheme 2.1). The heterocyclic ring system (A/B) was formed by performing a Birch reduction of the aromatic ring using Li/NH\(_3\) (\(l\)).\(^7,8\) The combination of Na/NH\(_3\) (\(l\)) was also tested, however, less than 50\% of the secondary amine (\textbf{2.6}) was reduced.\(^9\) The greater solubility of lithium in liquid ammonia and a higher reduction potential for lithium enhances the reducing power in this reaction.\(^10\) Upon acidic hydrolysis, cyclization occurred to give compound \textbf{2.7} as a \textit{cis} and \textit{trans} isomers mixture.

![Scheme 2.1 Synthesis of 2.2a in route 1. Reagents and conditions: a) 3 bar \(H_2\), 10\% Pd/C, EtOH, \(RT\); b) i) SOCl\(_2\), DCM, reflux; ii) \(n\)-PrNH\(_2\), DCM, \(RT\); c) LiAlH\(_4\), THF, reflux; d) i) Li/NH\(_3\) (\(l\), THF, \(-60^\circ C\); ii) \(H^+\)/H\(_2\)O.](attachment:image.png)
The process of the construction of the fused heterocyclic ring system is shown in Scheme 2.2. Upon acidic hydrolysis the enol ether (2.9) was converted to the ketone (2.10) and the double bond shifted to give an α,β–unsaturated ketone intermediate (2.11). The amine acted as nucleophile in a conjugate addition linking the nitrogen to the β position. The diastereomers 2.7 were obtained in a ratio of 80:20 as determined by GC analysis. The yield of the reaction was 70%. The major product had a cis configuration as confirmed by the analysis of 1H-NMR spectrum.

Scheme 2.2 The formation of 2.7 via Birch reduction.

Due to a favorable steric requirement for an approach of the nitrogen atom to the conjugated system, the diastereoselectivity can be interpreted by assuming a predominant cis-attack (cis 2.11) in preference to trans-attack (trans 2.11) in the Michael-type addition step\(^\text{11}\) (Figure 2.2).

Figure 2.2 Predominant cis-attack in preference to trans-attack.

It has been described\(^\text{12}\) that cis-2.7 could isomerize to trans form in acidic media through the retro-Michael process (Scheme 2.3), however, in our hands the transformation was not observed.
Interestingly, in the presence of 1% KOH in ethanol at room temperature, the cis-2.7 can been efficiently transformed into the trans-2.7 with a ratio of 11:89 (cis: trans) in 78% yield (Scheme 2.4). The possible pathway for this isomerization maybe involves the cleavage and regeneration of the C8a-C8 linkage via retro-Mannich process.

The third ring (C) was constructed by the introduction of a C4 moiety through a Wittig reaction. The mixture of E and Z isomers (2.8) was used without further purification for the final ring-closure in polyphosphoric acid (PPA) at 100°C. The last cyclization step was highly regioselective at 100°C in 33% (2.2a) and 3% (2.2b) over 2 steps, respectively.

Scheme 2.5 Synthesis of 2.2a in route 1. e) Br(Ph)3P+(CH2)3CO2Et, t-BuOK, DMF, 0°C, RT; f) PPA, 100°C.
It was observed that when the reaction was carried out at 115°C, up to 30% of the benzo[h]quinoline analogue of 2.2 was isolated as side-product (2.12, Figure 2.3).

**Figure 2.3** The structure of benzo[h]quinoline 2.12.

### 2.2.2 Synthetic route 2

An alternative to the synthesis described above was to use the starting material 7-hydroxyquinoline (2.13). The hydrogenation of 7-hydroxy-quinoline (2.13) under high pressure (70 bar H₂) was performed with 5% rhodium on alumina as catalyst in ethanol at 85°C for 6 h (Scheme 2.6). After crystallization from ethyl acetate, 50% total yield of the trans-2.14 was achieved. In contrast, a hydrogenation with rhodium catalyst of quinolines and tetrahydroquinolines under similar condition resulted in decahydroquinolines with preferential cis-isomers on fused ring.

![Scheme 2.6](image)

**Scheme 2.6** Synthesis of 2.17. Reagents and conditions: a) Rh/Al₂O₃, EtOH, 70 bar H₂, 85°C, re-crystallized from ethyl acetate; b) Jones’ reagent, acetone, RT; c) (Ph)₃P⁺(CH₂)₃CO₂Et, t-BuOK, DMF, 0°C; d) PPA, 100°C.

After Jones’ oxidation, trans-octahydro-7(1H)-quinolone (2.15) was afforded in good yield. Similar with the description of Wittig reaction (2.16) and PPA cyclization in Scheme 2.5, the final tricyclic secondary amine 2.17 was formed. The alkylation of this secondary amine 2.17 gave the final product 2.2a.
2.2.3 Synthetic route 3\(^6\)

Another route of the preparation of \(2.2a\) could be accomplished via Diels-Alder reaction (Scheme 2.7).

**Scheme 2.7** Synthesis of \(2.2a\) in route 3. Reagents and conditions: \(\text{a)}\) Ethynyl-magnesium bromide, THF, RT; \(\text{b)}\) 1 N HCl, RT; \(\text{c)}\) refluxing; \(\text{d)}\) Ph\(_2\)O, refluxing, 260°C.

\(2.20\) was prepared in one step from \(2.18\)^{20,21} Amine \(2.22\) was formed from \(2.21\) and \(n\)-propylamine.\(^{22}\) Upon mixing \(2.20\) and \(2.22\) in toluene at 40 bar at 135°C, an instantaneous reaction occurred to give intermediate \(2.23\)^{23,24} which was isolated and characterized by NMR. However, no expected final product \(2.2a\) was obtained under the used conditions. Refluxing the reaction mixture in diphenyl ether (Ph\(_2\)O),\(^{25}\) \(2.2a\) could be prepared in 5% yield. Unfortunately the extent of reaction time and the higher temperature resulted in more ring-opened by-product \(2.24\).

Apparently, it showed that \(2.2a\) was unstable at the used high temperature; the middle ring (A) showed a tendency to aromatize (c.f. apomorphine, \(1.23\)). It should be noted that
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with this route, only 2.2a was formed, no 2.2b was found, although the yield was rather low.

2.2.4 Chiral resolution of racemic GMC-6650

The racemic GMC-6650 (2.2a) was resolved into their enantiomers by semi-preparative HPLC, using a chiral column. Both enantiomers [referred as (−)-2.2a and (+)-2.2a] were isolated in a high optical purity as determined by chiral HPLC (e.e.>99%).

2.2.5 Single crystal X-ray analysis

From the X-ray analysis of enantiopure (−)-2.2a in Figure 2.4, it can be seen that the C7-N bond and C11-C12 bond (torsion angle 176°); C6-C7 and C10-C11 (torsion angle 175°) are in a trans configuration. The absolute configuration was proved as (R, R).

Figure 2.4 The structure of enantiopure (−)-2.2a from X-ray.
Chapter 2

2.3 Pharmacology

2.3.1 In vitro receptor binding

Both enantiomers of GMC-6650 (–)-2.2a and (+)-2.2a were tested for in vitro binding at the DA D₁, D₂, D₃, D₄ receptor. The displacement of the radioligand was measured at a fixed concentration of the test compound (50 or 100 nM). It was estimated whether this concentration of the test compound was above or below the IC₅₀. When at the tested concentration the displacement is below the IC₅₀, the test compound is considered not active at the receptor.

2.3.2 In vivo pharmacology

The potential pharmacological effects of both enantiomers (–)-2.2a and (+)-2.2a were studied by measuring their effects on extracellular DA levels in the corpus striatum, the brain area of interest in PD, using microdialysis in freely moving rats. Microdialysis is a presynaptic model suitable for investigating the pharmacological effects of DA agonists. Stimulation of the autoreceptors leads to a down regulation of DA synthesis and release. The results are expressed in relation to basal DA levels. If the test compound is a DA agonist, it will bind to DA receptors, and the DA release of the striatal dopaminergic neurons will decrease. Measuring the change in release in the striatum as a function of time reflects onset of action, potency and duration of action of the test compound. The control DA release before administration of the test compound is set to 100% and the change in output is expressed in relation to this percentage. In freely moving animals, the potential postsynaptic effects of a test compound can be monitored by simply observing (and possibly video-recording) the behaviour.

2.3.3 Plasma and brain extraction experiments

To obtain further evidence for the bioactivation of these enone compounds, we have given a dose of 10 µmol kg⁻¹ of (–)-2.2a and (+)-2.2a orally to male Wistar rats. After 45 min administration of (–)-2.2a and (+)-2.2a, the blood and brain samples were collected as described. After a usual work-up procedure, using 60% CH₃CN in water for the
precipitation of proteins, the samples were injected to the high-performance liquid chromatography/tandem mass spectrometry system (LC/MS/MS).

2.4 Results and discussion

2.4.1 In vitro receptor binding

*In vitro*, both enantiomers of (−)-2.2a and (+)-2.2a at the tested concentrations were not able to displace 50% of the radioligand used for the receptors tested. Under these test conditions, the IC50 of both the enantiomers for the DA D1 and DA D2 receptors is >100 nM and for the DA D3 and DA D4 receptors it is >50 nM. These results indicate these compounds can be considered inactive *in vitro*. (Table 2.1).

<table>
<thead>
<tr>
<th></th>
<th>DA D1 (SCH23390)</th>
<th>DA D2 (Spiperone)</th>
<th>DA D3 (Spiperone)</th>
<th>DA D4 (Nemonapride)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(−)-2.2a</td>
<td>100/ -1%</td>
<td>100/ 15%</td>
<td>50/ 23%</td>
<td>50/ -4%</td>
</tr>
<tr>
<td>(+)-2.2a</td>
<td>100/ 7%</td>
<td>100/ 3%</td>
<td>50/ 28%</td>
<td>50/ -19%</td>
</tr>
</tbody>
</table>

*a* Tritiated radioligand used.

2.4.2 Microdialysis study

Experiments *in vivo* strongly indicate that (−)-2.2a is converted to a DA agonist.

A significant dose-dependent decrease was observed after administration of 1, 3, 10 nmol kg⁻¹ sc. A maximum DA decrease to 30% of controls was found 120 min after administration of 10 nmol kg⁻¹ sc injection. The DA decrease maintained 50% of controls until the end of the experiment (6 h) (Figure 2.5).
Administration of 3 or 10 nmol kg\(^{-1}\) of (–)-2.2a po induced a significant decrease of 45% and 60% of control values, respectively. After 10 nmol kg\(^{-1}\) po administration of (–)-2.2a, the DA decrease maintained below 50% of basal levels until the end of the experiment (6 h) (Figure 2.6).

It was found that (+)-2.2a was inactive at a dose of 1 µmol kg\(^{-1}\) sc as shown in Figure 2.7.
Figure 2.7 Effect of (+)-2.2a (1 µmol kg⁻¹ sc) on striatal DA release in freely moving rats. The results are the mean (± SEM) of data obtained from 4 rats.

As anticipated, cis isomer (±)-2.2b did not show any activity in the microdialysis model (Figure 2.8).

Figure 2.8 Effect of cis isomer (±)-2.2b (1 µmol kg⁻¹ sc) on striatal DA release in freely moving rats. The results are the mean (± SEM) of data obtained from 4 rats.

Comparison of these results with previously published data from the Wikström research group,²,³ clearly shows that (−)-2.2a induces strong DA agonistic effects after oral administration. For example, to achieve a 45% decrease of DA level after oral administration, (−)-2.2a is >100-fold more potent than N-propyl-norapomorphine (1.36) and PD-148903 ((−)-2.1).²⁶ After subcutaneous administration, compound (−)-2.2a is >10 times more potent than apomorphine (1.23) and (−)-2.1.²,²⁶ Further comparison of the DA agonistic effects of these compounds show that the onset of action of (−)-2.2a is more gradual and the effect is more long lasting. This may be an important characteristic because this could potentially avoid a steep plasma concentration curve which could lead to adverse effects, like peak-dose dyskinesias. During the microdialysis experiments of (−
2.2a the rats displayed typical DA agonist related behaviour like yawning, sniffing, penile grooming and locomotor activity.\textsuperscript{28,29,30}

2.4.3 Analysis of plasma and brain samples

The identity of (–)-2.2a and (+)-2.2a was confirmed by use of high-performance liquid chromatography/tandem mass spectrometry (LC/MS/MS). Peak 263 was found in the plasma samples from (–)-2.2a administrated rats, but peak 261 was not detected in these plasma samples. Both the peaks 261 and 263 were found in the brain samples. After the administration of (+)-2.2a, only peak 263 was found in plasma and brain samples, however, no peak 261 was found in those samples. Comparing with MS/MS fragmentation and retention time of the standard sample of TL-334 (1.26d, as trans isomer) synthesized in our lab, it is very reasonable to assume that the bioactivation of (–)-2.2a to its corresponding catecholamine was introduced via an intermediate metabolite with a MS 263, and the catecholamine 1.26d is most likely the active form of (–)-2.2a \textit{in vivo} (Scheme 2.7). Apparently, the first step in the bioactivation process is hydroxylation of the $\alpha$-ketone position which is also a known from steroid metabolism.\textsuperscript{31}

\begin{center}
\begin{tabular}{c}
\textbf{Scheme 2.7 Suggested metabolism of 2.2a. Only the (–) enantiomer of 2.2a gave detectable level of a compound believed to be TL-334 (1.26d).}
\end{tabular}
\end{center}

Oral administration of 10 $\mu$mol kg$^{-1}$ of (+)-2.2a did not induce any biochemical or behavioral effects. Peak 263 was found in brain and plasma samples; however, 1.26d was not detected in these samples.
2.5 Conclusions

The bioactivation mechanism of (−) enantiomer of trans-1-propyl-2,3,4,4a,5,7,8,9, 10,10a-decahydro-1H-benzo[g]quinolin-6-one [(−)-2.2a] is expected to be similar to (S)-6-(N,N-di-n-propylamino)-3,4,5,6,7,8-hexahydro-2H-naphthalen-1-one [(−)-2.1]. It is assumed that (−)-2.2a is bioactivated to 1-propyl-1,2,3,4,4a,5,10,10a-octahydrobenzo [g]quinoline-6,7-diol (1.26d) in vivo.

The lack of affinity of (−)-2.2a observed in vitro and the highly potent dopaminergic effects in vivo indicates that the ‘template for bioactivation’ of enones thus may very well extend from bicyclic systems to a tricyclic system.

In microdialysis experiments, (−)-2.2a is active at extremely low doses sc as well as po. The effects observed in this model are stronger than those induced by N-propyl-nonapomorphine and (−)-2.1. Similar to the structure of 2.1, the (R,R) absolute configuration of (−)-2.2a is the essential factor for the activity since the (+)-2.1 did not show activity either. In most cases, the n-propyl group of N-substitution is optimal for high dopaminergic activity.32, 33

The metabolites analysis showed that (−)-2.2a is converted to the corresponding catecholamine in vivo. However, this catecholamine was not detected after the administration of (+)-2.2a.

These properties together make (−)-2.2a an interesting candidate for development into a drug for treating PD.

2.6 Experimental section

2.6.1 Chemistry

**General.** Melting points were determined in open glass capillaries on an Electrothermal digital melting point apparatus and are uncorrected. 1H-NMR spectra were recorded at 300 MHz on a Varian-VXR 300 spectrometer and 13C-NMR spectra were recorded at 50.3 MHz on a Varian Gemini 200 spectrometer. The chemical shifts are given in units (ppm) relative to TMS; the splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), qu (quintuplet), se (sextuplet), m (multiplet). Coupling constant are given in hertz (Hz). The spectra recorded were consistent with the proposed
structures of intermediates and final compounds. IR spectra were obtained on an ATI-Mattson spectro-photometer, and only the important absorptions are given. 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 microanalytical department of the University of Groningen and were within 0.4% of the theoretical values, except where noted. Compounds that were obtained as oils, or as solid in a very small amount, were analyzed by high-resolution mass spectrometry (HRMS), performed on a JEOL MS route JMS-600H by the Department of Chemistry, University of Groningen.

Materials. The starting compounds 3-(4-methoxyphenyl)-acrylic acid, 7-hydroxyquinoline and 3-ethoxy-2-cyclohexen-1-one were purchased from Acros and Aldrich, respectively. (3-Ethoxycarbonylpropyl)-triphenyl-phosphonium bromide (Wittig regent) was prepared according to the published procedure. All other reagents and solvents were also commercially available and were used without further purification with the exception of THF, which was distilled from sodium/bezophenone, and DMF which was distilled from phosphorus pentoxide and dried with 4Å molecular sieves.

3-(4-Methoxyphenyl)-propionic acid N-propyl amide (2.5). 3-(4-methoxyphenyl)-acrylic acid 2.3 (10 g, 55.7 mmol) was dissolved in ethanol (200 mL) and a catalytic amount of 10% Pd/C (120 mg) was added. After shaking for 3 h under 3 bar H2 at RT, the mixture was filtered over Celite® and evaporated and a 10.2 g residue (white solid) was obtained. The residue was refluxed in CH2Cl2 (220 mL) with thionylchloride (10 mL, 137 mmol) for 1.5 h. The volatiles were evaporated and the resulting oil was dissolved in CH2Cl2 (100 mL). This solution was added to a vigorously stirred mixture of 5% aqueous NaOH (230 mL), CH2Cl2 (120 mL) and n-propylamine (7 mL, 85 mmol). After stirring for 1 h, the layers were separated and the aqueous layer was extracted with CH2Cl2 (3 x 80 mL). The combined organic layers were washed with water (3 x 80 mL), brine (3 x 80 mL), dried over MgSO4. Evaporation of the solvent gave the amide 2.5 as a white solid (10.7 g, 87% yield), mp 90-91°C. IR (neat) cm⁻¹ 3303, 2960, 1641, 1542; ¹H-NMR (CDCl3) δ 7.11 (d, 2H, J = 4.4 Hz), 6.81 (d, 2H, J = 4.4 Hz, J = 2.2 Hz), 5.52 (br s, 1H), 3.77 (s, 3H), 3.14 (m, 2H), 2.91 (m, 2H), 2.43 (m, 2H), 1.45 (m, 2H), 0.85 (t, 3H, J = 7.3 Hz) ppm; ¹³C-NMR (CDCl3), δ 170.7, 156.5, 131.4, 127.8, 112.4, 53.7, 39.7, 37.3, 29.4, 21.3, 9.8 ppm; MS (EI) m/z 221 (M⁺).
**N-(3-(4-Methoxyphenyl)-propyl)-N-propyl amine (2.6).** To a stirred mixture of LiAlH₄ (3.6 g, 94.6 mmol) in THF (80 mL) was added dropwise a solution of amide 2.5 (10.4 g, 47.1 mmol) in THF (80 mL). After refluxing for 3.5 h the mixture was cooled to 50°C and excess hydride was destroyed by careful addition of water (3.6 mL), 10% aqueous NaOH (3.6 mL) and water (15 mL). After stirring for 15 min, the slurry was filtered and the white precipitate was washed with ethanol (3 x 10 mL). The combined volatiles (THF and ethanol) were evaporated and the residue was extracted with ethyl acetate (4 x 60 mL), the organic layers were combined, washed with brine (3 x 50 mL), dried over MgSO₄. The solvent was evaporated to dryness to give amine 2.6 as an oil (8.9 g, 91% yield). IR (neat) cm⁻¹ 2931, 2832, 1612, 1513; ¹H-NMR (CDCl₃) δ 7.11 (d, 2H, J = 8.8 Hz), 6.81(d, 2H, J = 8.6 Hz), 3.77 ( br, s, 3H), 2.51-2.66 (m, 6H), 1.98 (br, s, 1H), 1.42-1.83 (m, 4H), 0.90 (t, 3H, J = 7.6 Hz) ppm; ¹³C-NMR (CDCl₃) δ 156.2, 132.6, 127.7, 112.2, 53.7, 50.3, 47.9, 31.2, 30.3, 21.5, 10.3 ppm; MS (EI) m/z 207 (M⁺).

**N-n-Propyl-7-keto-1,2,3,4,4a,5,8,8a-octahydro-[6H]-quinoline (2.7).** Amine 2.6 (12.3 g, 59.4 mmol) was dissolved in dry THF (120 mL), t-BuOH (11.9 mL, 130 mmol). The mixture was cooled to -60°C and liquid NH₃ (120 mL) was introduced. Li metal (1.6 g, 228 mg-atoms) was gradually added in small portions and the blue mixture was stirred at -60°C for 4 h. The color was discharged by addition of a MeOH/aqueous NH₄Cl (sat.) solution (1:1, 40 mL) and the cooling bath was moved. After NH₃ was evaporated, the pH of the slurry was adjusted to 1 by the addition of concentratred hydrochloric acid, and stirred overnight at RT. The mixture was basified to pH >9 (4N NaOH, T<15°C). The aqueous solution was extracted with CH₂Cl₂ (5 x 100 mL) and the combined organic layers were washed with brine (3 x 100 mL) and dried over MgSO₄. Filtration and evaporation of the solvent yielded a dark red oil that was purified by column chromatography (silica, treated with NH₃, CH₂Cl₂/MeOH, gradient), obtaining compound 2.7 as a mixture of trans and cis isomers (1: 4), a light yellow oil, 8.1 g (70% yield), Rᵣ value: trans: 0.72; cis: 0.78 (DCM : MeOH = 5 : 1); trans-2.7: ¹H-NMR (CDCl₃), δ 2.95 (dt, 1H, J = 6.4 Hz, J = 13.9 Hz), 1.11-2.60 (m, 17H), 0.72 (t, 3H, J = 7.3 Hz) ppm; ¹³C-NMR (CDCl₃) δ 207.9, 61.6, 58.9, 52.5, 50.1, 43.5, 38.5, 38.0, 28.7, 22.8, 14.8, 9.3 ppm; MS (EI) m/z 195 (M⁺). Cis-2.7: ¹H-NMR (CDCl₃) δ 3.54 (t, 1H, J = 4.4 Hz), 1.14-2.90 (m, 17H), 0.76 (t, 3H, J = 7.3 Hz) ppm; ¹³C-NMR (CDCl₃) δ 209.0, 62.6, 60.0, 53.5, 51.1, 44.5, 39.5, 39.0, 29.7, 23.8,
trans-N-n-Propyl-7-keto-1,2,3,4,4a,5,8,8a-octahydro-[6H]-quinoline (2.7). The cis-2.7 (4.4 g, 22.6 mmol) was dissolved in 1% KOH ethanol solution (440 mL) and stirred at RT under nitrogen atmosphere for 3 days. The solvent was evaporated and the residue was extracted with CH$_2$Cl$_2$ (3 x 100 mL), washed with brine (3 x 50 mL), dried over Na$_2$SO$_4$. After filtration and evaporation of the solvent, the residue was purified by column chromatography (silica, treated with NH$_3$, CH$_2$Cl$_2$ : MeOH, gradient), yielding 3.4 g mainly trans-2.7 (78% yield, according to GC, about 10% cis left, the mixture was used for further reaction).

N-n-Propyl-2,3,4,4a,5,6,7,8,9,10,10a-decahydrobenzo[g] quinoline-6-one (2.2). To a suspension of t-BuOK (3.8 g, 34 mmol) in dry DMF (6 mL) under N$_2$ at 0°C was added dropwise a solution of (3-ethoxycarbonylpropyl)-triphenylphosphonium bromide (14.1 g, 37.4 mmol) in dry DMF (50 mL). When the addition was completed, the mixture was stirred at 0°C for 30min. A solution of 2.7 (3.3 g, 17.0 mmol) in dry DMF (6 mL) was added dropwise at 0°C under N$_2$. After stirred at 0°C for 4 h, the temperature was allowed to rise to RT and the stirring was continued overnight. Water (150 mL) was added with cooling, and the water layer was extracted with ether (6 x 60 mL), the combined organic layers were washed with brine (2 x 50 mL), dried over MgSO$_4$. The solvent was filtered and evaporated to give a brown oil 2.8. According to GC analysis, 2.8 was a mixture of E and Z isomers, which were used for in the next step with further separation.

The crude 2.8 was dissolved in CH$_2$Cl$_2$ (25 mL) and was added to PPA (45 g) at 100°C while stirring. After 4 h stirring at 100°C, the reaction mixture was allowed to cool to 80°C when crush ice (100 g) was slowly introduced. The reaction was cooled to RT and extracted with CH$_2$Cl$_2$ to remove the by-product from last step. Ammonia (25% water solution) was added until pH>8 and the solution was extracted with CH$_2$Cl$_2$ (5 x 100 mL) and washed with brine (3 x 30 mL). The combined organic layers were dried, filtered and evaporated. The residue was purified by column chromatography (silica, treated with NH$_3$, CH$_2$Cl$_2$/MeOH, gradient) and resulted in trans isomer 2.2a (1.4 g, 32.6% yield over 2 steps) as a light yellow oil, $^1$H-NMR (CDCl$_3$) $\delta$ 3.51(d, 1H, $J = 11.0$ Hz), 2.65 (m, 2H), 2.21-2.47 (m, 8H), 1.87-2.18 (m, 4H), 1.60-1.79 ppm; MS (EI) m/z 195 (M$^+$); Anal. Calcd for C$_{12}$H$_{21}$NO.HCl: C, 62.19; H, 9.57; N, 6.04. Found: C, 61.93; H, 9.51; N, 6.10.
(m, 3H), 1.37-1.59 (m, 2H), 1.01-1.31 (m, 2H), 0.86 (t, 3H, \(J = 7.3\) Hz) ppm; \(^{13}\)C-NMR (CDCl\(_3\)) δ 197.2, 153.2, 129.9, 59.3, 54, 51.3, 36.2, 36.0, 35.5, 30.0, 29.5, 28.2, 23.8, 20.8, 16.3, 16.6 ppm; MS (EI) m/z 247 (M\(^+\)); Anal. Calcd for C\(_{16}\)H\(_{25}\)NO.HCl: C, 67.71; H, 9.23; N, 4.93. Found: C, 67.24; H, 9.16; N, 4.91. Cis isomer 2.2b 0.13 g (3% over 2 steps) as a light yellow oil. \(^1\)H-NMR (CDCl\(_3\)) δ 3.03 (t, 1H, \(J = 6.1\) Hz), 2.52-2.61 (m, 1H), 2.34-2.49 (m, 6H), 2.21-2.30 (m, 4H), 2.02 (m, 3H), 1.18-1.68 (m, 7H), 0.87 (t, 3H, \(J = 7.3\) Hz) ppm; \(^{13}\)C-NMR (CDCl\(_3\)) δ 197.6, 152.5, 128.8, 55.2, 53.3, 45.2, 36.4, 31.9, 29.9, 26.4, 24.0, 23.5, 21.0, 18.8, 10.5 ppm; MS (EI) m/z 247 (M\(^+\)). The products were subsequently converted to the hydrochloric salt and re-crystallized from ethanol/diethyl ether. Melting points: Trans isomer of 2.2a·HCl mp: 237°C; Cis isomer of 2.2b·HCl mp: 186°C.

7-Hydroxy-trans-decahydroquinoline (2.14). 7-hydroxyquinoline 2.13 (1.5 g, 10.3 mmol) was hydrogenated over 5% rhodium on alumina (1.5 g) in abs. EtOH (35 mL), at 80°C and 70 H\(_2\) bar for 6 h. The catalyst was filtered off though Celite\(^8\), and the filtrate was evaporated in vacuo to give a grey solid (1.5 g). Re-crystallization from ethyl acetate gave 2.14 (0.8 g, 50% yield) as light green needles, mp 169-171°C (lit.169-171°C\(^{16}\); \(^1\)H-NMR (CDCl\(_3\)) δ 3.64, (m, 1H), 3.04 (dqu, 1H, \(J = 1.9\) Hz, \(J = 11.9\) Hz), 2.59 (td, 1H, \(J = 2.2\) Hz, \(J = 11.9\) Hz), 1.87-2.15 (m, 5H), 1.42-1.72 (m, 4H), 0.93-1.33 (m, 4H) ppm; \(^{13}\)C-NMR (CDCl\(_3\)) δ 67.9, 58.0, 45.4, 41.4, 40.6, 34.0, 30.0, 28.2, 25.4 ppm; MS (EI) m/z 155 (M\(^+\)).

trans-Octahydro-7(1H)-quinolone (2.15). Jones’ reagent (4 mL) was added rapidly to a solution of 2.14 (800 mg, 5.16 mmol) in warm acetone (100 mL) while stirring. The resulting mixture was stirred at RT for 1 h. Iso-propanol (1.5 mL) was dropped to the reaction until the excess Jones’ reagent was reduced. Aqueous 1N NaOH solution was added until pH>9, and the mixture was concentrated in vacuo to remove acetone. Water (5 mL) was added to the remaining mixture, which was extracted with CHCl\(_3\) (4 x 40 mL). The combined organic layers were washed with brine, dried over Na\(_2\)SO\(_4\). After filtration and evaporation of the solvent, a solid 2.15 (660 mg, 84% yield) was obtained. \(^1\)H-NMR (CDCl\(_3\)) δ 3.04 (dqu, 1H, \(J = 1.9\) Hz, \(J = 11.9\) Hz), 2.59 (td, 1H, \(J = 3.2\) Hz, \(J = 11.9\) Hz), 2.14-2.49 (m, 5H), 1.76-1.98 (m, 2H), 0.93-1.74 (m, 5H) ppm; \(^{13}\)C-NMR (CDCl\(_3\)) δ
208.5, 58.9, 46.8, 44.7, 39.7, 39.5, 29.5, 28.7, 24.5 ppm; MS (EI) m/z 153 (M⁺); HRMS 153.1161 (obsd). calcd. for C₉H₁₅NO 153.1154.

Ethyl 4-[trans-octahydro-7(1H)-quinolinyldene]butanoate (2.16). To a cooled (0°C) suspension of t-BuOK (966 mg, 8.63 mmol) in dry DMF (1.5 mL), a solution of (3-ethoxycarbonylpropyl)-triphenyl-phosphonium bromide (3.55 g, 9.42 mmol) in dry DMF (7 mL) was added dropwise under N₂. When the addition was complete, the mixture was stirred at 0°C for 30 min. A solution of 2.15 (660 mg) in dry DMF (2 mL) was added dropwise at 0°C under N₂. After stirred at 0°C for 4 h, the temperature was allowed to rise to RT and the stirring was continued overnight. Water (20 mL) was added with cooling, and the suspension was extracted with n-hexane (6 x 50 mL), the combined organic layers were washed with brine (3 x 30 mL), dried over MgSO₄. After filtration an evaporation, a semi-solid crude 2.16 (1.2 g) was obtained.

N-2, 3, 4, 4a, 5, 6, 7, 8, 9, 10, 10a-Decahydrobenzo[g]quinolin-6-one (2.17). The crude 2.16 (1.2 g) was dissolved in CH₂Cl₂ (5 mL) and was added dropwise to PPA (12 g) at 100°C while stirring. After stirring at 100°C for 4 h, the syrup was allowed to cool to 80°C when crush ice 20 g was slowly introduced. The reaction was cooled to RT, ammonia (25% aqua solution) was added until pH > 8, and the solution was extracted with CH₂Cl₂ (5 x 30 mL). The combined organic layers were washed with brine (3 x 30 mL), dried over MgSO₄. After filtration and evaporation of the solvent, the obtained residue was purified by column chromatography (silica, NH₃-treated, CH₂Cl₂/MeOH, gradient) resulting in 2.17 as a light yellow oil (410 mg, 39% yield over two steps). ¹H-NMR (CDCl₃) δ 3.01 (d, 1H, J = 11.7Hz), 2.60 (m, 1H), 2.12-2.48 (m, 8H), 1.85-1.90 (m, 4H), 1.40-1.56 (m, 3H), 0.98-1.21 (m, 2H) ppm; ¹³C-NMR (CDCl₃) δ 197.3, 153.3, 130.1, 55.1, 45.2, 37.9, 36.33, 36.2, 30.1, 29.4, 27.7, 25.2, 20.8 ppm; MS (Cl) m/z 206 (M+1); HRMS 205.1479 (obsd.), calcd. for C₁₃H₁₉NO 205.1467.

3-Ethynyl-2-cyclohexen-1-one (2.20). To a stirred solution of 0.5 M ethynyl-magnesium bromide in THF (50 mL) was added 3-ethoxy-2-cyclohexen-1-one 2.18 (1.88 g, 13.4 mmol) in THF (7 mL) under N₂. The mixture was stirred at RT for 20 h and was
acidified with 1N HCl (100 mL). After stirring for 30 min, the water layer was extracted with CH$_2$Cl$_2$ (4 x 40 mL), the combined organic layers were washed with water (2 x 10 mL), brine (3 x 10 mL), and dried over MgSO$_4$. Filtration and evaporation of the solvent gave a yellow oil that was purified by column chromatography (silica, ethyl acetate/hexane, gradient), yielding 2.20 as an oil (1.4 g, 87% yield). $^1$H-NMR (CDCl$_3$) $\delta$ 6.21 (s, 1H), 3.48 (s, 1H), 2.34-2.42 (m, 4H), 1.94-2.00 (m, 2H); MS (EI) m/z 120 (M$^+$).

1-(N-$n$-Propylamine)-4-pentene (2.22). $^2$ 5-bromo-1-pentene 2.21 (750 mg, 5 mmol) was added to $n$-propylamine (1.62 g, 15 mmol), and this mixture was refluxed for 3 days. The excess $n$-propylamine was evaporated in vacuo and the residue was dissolved in CH$_2$Cl$_2$ (30 mL). 5% NaOH solution was added until the solution was adjusted to pH = 9 while cooling with ice-bath. The organic layer was separated and the aqua layer was extracted with CH$_2$Cl$_2$ (3 x 30 mL). The combined organic layers were washed with brine (3 x 20 mL) and dried over Na$_2$SO$_4$. The solvent was filtered and evaporated to obtain 2.22 (550 mg, 86% yield). $^1$H-NMR (CDCl$_3$) $\delta$ 5.69-5.89 (dd, 1H, $J$ = 10.3 Hz), 4.89-5.02 (m, 2H), 2.50-2.62 (m, 4H), 2.00-2.11 (m, 2H), 1.43-1.63 (m, 5H), 0.85-0.92 (t, 3H, $J$ = 7.3 Hz) ppm; $^{13}$C-NMR (CDCl$_3$), $\delta$ 136.9, 113.1, 50.3, 47.8, 30.0, 27.6, 21.6, 10.2 ppm.

7-[3-(Propylamino)propyl]-3,4-dihydro-1(2$H$)-naphthalenone (2.23). A solution of 2.20 (118 mg, 1 mmol) in toluene was added to a solution of 2.22 (125 mg, 1 mmol) in toluene. The mixture was stirred under 40 bar pressure at 135°C for 3 h. After cooling, the solvent was evaporated in vacuo, and the residue was extracted with CH$_2$Cl$_2$ (3 x 10 mL), washed with brine (3 x 10 mL), dried over MgSO$_4$. After filtration and evaporation of the solvent, the obtained residue was purified by column chromatography, yielding 2.23 as a yellow oil (120 mg, 49% yield). $^1$H-NMR (CDCl$_3$), $\delta$ 6.81 (d, 1H, $J$ = 13.7Hz), 5.69-5.85 (m, 2H), 4.98-5.09 (m, 3H), 3.05-3.17 (m, 4H), 2.30-2.43 (m, 4H), 1.90-2.10 (m, 4H), 1.48-1.72 (m, 4H), 0.86-0.90 (t, 3H, $J$ = 7.3 Hz) ppm; $^{13}$C-NMR (CDCl$_3$), $\delta$ 197.0, 159.9, 143.1, 135.8, 115.8, 114.1, 95.9, 35.8, 29.3, 25.3, 24.3, 21.2, 19.7, 9.8 ppm; MS (EI) m/z 247 (M$^+$).
A solution of 2.20 (362 mg, 3.02 mmol) in diphenylether (3 mL) was added to a solution of 2.22 (383 mg, 3.02 mmol) in diphenylether (3 mL) under N₂ at RT and stirred overnight. The solution was heated to reflux for 1h and cooled to RT, followed by the addition of 1N HCl (50 mL). The aqua layer was washed with CH₂Cl₂ (3 x 15 mL). The water layer was adjusted with 4N NaOH until pH = 9, and extracted with CH₂Cl₂ (5 x 20 mL). The combined organic layers were washed with brine (3 x 20 mL), dried over MgSO₄. After filtration and evaporation of the solvent, the obtained residue was purified with column chromatography (silica treated with NH₃, CH₂Cl₂/MeOH, gradient), giving 2.2a (40 mg, 5% yield), with by-product 2.24, 140 mg. ¹H-NMR (CDCl₃), δ 7.83 (s, 1H), 7.15-7.32 (m, 2H), 2.88-2.94 (m, 4H), 2.54-2.70 (m, 7H), 2.04-2.16 (m, 2H), 1.78-1.93 (qu, 2H, J = 7.8 Hz), 1.44-1.62 (se, 2H, J = 7.4 Hz), 0.86-0.93 (t, 3H, J = 7.3 Hz) ppm; ¹³C-NMR (CDCl₃) δ 197.1, 140.6, 138.8, 132.1, 131.0, 127.4, 125.1, 50.0, 47.5, 37.7, 31.6, 29.5, 27.8, 21.8, 21.2, 10.2 ppm; MS (Cl) m/z 246.1 (M+1).

Preparative chromatographic resolution of 2.2a. A 20 mg/mL solution of diastereomer 2.2a in 2-propanol was injected into a HPLC system using a Waters 510 HPLC pump fitted with a Chiralpack AD preparative column (250 x 10 mm), 40 µL per injection. Mobile phase was a mixture produced by an ISCO model 2360 gradient programmer and consisted of 99% n-hexane and 1% 2-propanol [containing 0.1% triethylamine (w/w)]. Flow rate of the mobile phase was 3.5 mL min⁻¹. The two enantiomers were detected by a Waters 486 millipore tunable absorbance detector (λ = 254 nm) and were recorded on paper using a Kipp & Zonen flatbed recorder (chart speed 5 mm min⁻¹). After evaporation of the mobile phase, the optical rotation of the two fractions was determined using a Perkin Elmer 241 polarimeter. First fraction: [α]d²⁰ = +209° (c=0.034, methanol). Second fraction: [α]d²⁰ = -206° (c=0.032, methanol). The purity of both enantiomers were analyzed by an analytical column (250 x 4.6 mm) (e.e > 99.5% for both enantiomers). Both enantiomers were converted to their corresponding maleate salts and were re-crystallized from ethanol/ diethyl ether. Melting points: (−)-2.2a·maleate mp: 192°C; (+)-2.2a·maleate mp: 186°C.
2.6.2 X-ray crystallographic data

The (−)-2.2a (30 mg, as its HCl salt) was dissolved in abs. EtOH (1 mL) while heating. Ether was gently added on top of ethanol until a diffuse cloud formed in the border formed by the two phases. The two layers were kept still as such overnight, platelet, colorless crystals were formed. The crystallographic data for the structure (−)-2.2a in this paper has been deposited to the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-273828. Copies of the data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or form the Cambridge Crystallographic Data Centre (CCDC). 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (int) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Selected data on the geometry

Standard deviations in the last decimal place are given in parentheses.

\[
[C_{16}H_{26}NO]^+ \cdot \text{Cl}^-, M_r = 283.84, \text{orthorhombic, } P2_12_12, a = 11.0021(8), b = 19.541(2), c = 7.1000(5) \text{ Å, } V = 1526.4(2) \text{ Å}^3, Z = 4, D_x = 1.235 \text{ gcm}^{-3}, F(000) = 616, \mu = 2.44 \text{ cm}^{-1}, \lambda(\text{MoK}_\alpha) = 0.71073 \text{ Å, } T = 100(1) \text{ K, 11933 reflections measured, } \text{GooF} = 1.036, \text{wR}(F^2) = 0.0745 \text{ for 3005 unique reflections and 276 parameters and } R(F) = 0.0344 \text{ for 2649 reflections obeying } F_o \geq 4.0 \sigma(F_o) \text{ criterion of observability.}
\]

\[
\begin{align*}
\text{Interatomic Distances (Å)} \\
\text{O} & - \text{C1} & 1.217(3) & \text{C5} & - \text{C13} & 1.339(3) \\
\text{N} & - \text{C7} & 1.520(2) & \text{C6} & - \text{C7} & 1.526(3) \\
\text{N} & - \text{C8} & 1.506(3) & \text{C7} & - \text{C11} & 1.525(3) \\
\text{N} & - \text{C14} & 1.499(3) & \text{C8} & - \text{C9} & 1.507(3) \\
\text{C1} & - \text{C2} & 1.501(3) & \text{C9} & - \text{C10} & 1.508(3) \\
\text{C1} & - \text{C13} & 1.486(3) & \text{C10} & - \text{C11} & 1.513(3) \\
\text{C2} & - \text{C3} & 1.519(3) & \text{C11} & - \text{C12} & 1.531(3) \\
\text{C3} & - \text{C4} & 1.519(3) & \text{C12} & - \text{C13} & 1.500(3) \\
\text{C4} & - \text{C5} & 1.500(3) & \text{C14} & - \text{C15} & 1.513(3) \\
\text{C5} & - \text{C6} & 1.510(3) & \text{C15} & - \text{C16} & 1.505(3)
\end{align*}
\]

\[
\text{Bond Angles (deg.)}
\]
Chapter 2

C7  -N -C8  111.48(14)  N  -C7  -C11  111.72(15)
C7  -N -C14  111.59(14)  C6  -C7  -C11  109.47(16)
C8  -N -C14  110.19(15)  N  -C8  -C9  111.58(17)
O  -C1  -C2  122.31(19)  C8  -C9  -C10  110.75(17)
O  -C1  -C13  120.59(19)  C9  -C10  -C11  110.60(17)
C2  -C1  -C13  117.05(18)  C7  -C11  -C10  113.59(17)
C1  -C2  -C3  112.27(18)  C7  -C11  -C12  107.53(16)
C2  -C3  -C4  109.94(17)  C10  -C11  -C12  110.55(17)
C3  -C4  -C5  112.67(18)  C11  -C12  -C13  113.36(17)
C4  -C5  -C6  115.1(18)  C1  -C13  -C5  120.84(18)
C4  -C5  -C13  122.80(18)  C1  -C13  -C12  116.70(18)
C6  -C5  -C13  122.08(18)  C5  -C13  -C12  122.39(18)
C5  -C6  -C7  112.37(17)  N  -C14  -C15  114.23(16)
N  -C7  -C6  109.75(16)  C14  -C15  -C16  109.67(17)

Torsion Angles (deg.)

C8  -N  -C7  -C6  172.44(15)
C8  -N  -C7  -C11  50.8(2)
C14  -N  -C7  -C6  -63.88(19)
C14  -N  -C7  -C11  174.51(16)
C7  -N  -C8  -C9  -56.1(2)
C14  -N  -C8  -C9  179.48(15)
C7  -N  -C14  -C15  168.30(17)
C8  -N  -C14  -C15  -56.1(2)
O  -C1  -C2  -C3  151.18(19)
C13  -C1  -C2  -C3  151.18(19)
O  -C1  -C13  -C5  176.49(19)
O  -C1  -C13  -C12  -67.3(2)
C2  -C1  -C13  -C5  -1.0(3)
C2  -C1  -C13  -C12  -178.20(17)
C1  -C2  -C3  -C4  55.7(2)
C2  -C3  -C4  -C5  -48.8(2)
C3  -C4  -C5  -C6  -163.21(17)
C3  -C4  -C5  -C13  17.8(3)
C4  -C5  -C6  -C7  -167.07(16)
C13  -C5  -C6  -C7  11.9(3)
C4  -C5  -C13  -C1  8.1(3)
C4  -C5  -C13  -C12  -174.87(18)
C6  -C5  -C13  -C1  -170.78(17)
GMC-6650: An orally active dopaminergic prodrug

C6 -C5 -C13 -C12  6.3(3)  
C5 -C6 -C7 -N  -170.84(15)  
C5 -C6 -C7 -C11 -47.9(2)  
N -C7 -C11 -C10 -49.9(2)  
N -C7 -C11 -C12 -172.59(16)  
C6 -C7 -C11 -C10 -171.71(16)  
C6 -C7 -C11 -C12  65.6(2)  
N -C8 -C9 -C10  59.0(2)  
C8 -C9 -C10 -C11 -56.4(2)  
C9 -C10 -C11 -C7  52.6(2)  
C9 -C10 -C11 -C12  173.60(17)  
C7 -C11 -C12 -C13 -47.7(2)  
C10 -C11 -C12 -C13 -172.18(17)  
C11 -C12 -C13 -C1 -170.14(17)  
C11 -C12 -C13 -C5  12.7(3)  
N -C14 -C15 -C16 -172.44(17)  

Table 2.2 Geometry of intra- and intermolecular hydrogen bonds (Å,°) with s.u.'s in parentheses

<table>
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<td>N --H1 Cl</td>
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<td>3.0395(16)</td>
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</tbody>
</table>

Translation of ARU-code to Equivalent Position Code

[3556. ] = 1/2+x,1/2-y,1-z

2.6.3 Pharmacology

Animals. Animals used for the biochemical and behavior activity experiments were male rats of a Wistar derived strain (Harlan, the Netherlands) weighing 300-350 g. The rats were placed in a room at controlled environmental conditions (21°C, humidity 60-65%; lights on at 8 a.m. and off at 8 p.m.). At least 1 week after arrival the rats were used in the experiments. Animal procedures were conducted in accordance with guidelines published
Drugs. All enones were tested as their hydrochloride salts unless noted otherwise. The drugs were dissolved in physiological (0.9%) saline immediately before use. All in vivo experiments were performed at the Animal Laboratory Unit of the University of Groningen, The Netherlands.

Surgery and brain microdialysis. On-line brain microdialysis in freely moving animals has previously been described. In brief, the rats were anaesthetized with N₂O (40%), isoflurane (2%) and O₂ (60%); 10% lidocaine was locally applied. The rats were mounted into a stereotaxic frame (Kopf). The incisor bar was placed in position so that the skull was held horizontally. The skull was exposed and burr holes were drilled. A Y-shaped dialysis probe was used for the experiments, with an exposed tip length of 3 mm. The dialysis cannula (ID: 0.22 mm; OD: 0.31 mm) was prepared from polyacrylonitrile/sodium methallyl sulfonate copolymer (AN 69, Hospal, Bologna, Italy). The microdialysis cannula was implanted in the striatum. The dura was removed with a sharp needle. Two anchor screws were positioned in different bone plates nearby in order to fix the cannula. The following coordinates were used according to the atlas of Paxinos and Watson: AP+1.0, LM ±3.0 relative to bregma, and VD−6.0 below dura. Before insertion into the brain the dialysis probe was perfused with a Ringer solution. The dialysis probe was positioned in the burr hole under stereotaxic guidance. The probe was cemented in this position with dental cement. After the surgery, the rats received Finadine (1 mL/kg i.p.), an analgesic agent, and were housed solitary. The experiments were performed in conscious rats 17-48 h after implantation of the cannula. The striatum was perfused with a Ringer solution (147 mmol/L NaCl, 4 mmol/L KCl, 1.2 mmol/L CaCl₂, 1.1 mmol/L MgCl₂) at 2 µl/min (CMA/102 microdialysis pump, Sweden). The dialysate contents of DA and its metabolites were quantitated by HPLC with electrochemical detection with a detection limit of approximately 5 fmol/sample. An HPLC pump (LKB, Pharmacia) was used in conjunction with an electrochemical detector (Antec, Leiden) working at 625 mV versus an Ag/AgCl reference electrode, the detection was done with a Coulochrome detector set to +300 mV for DOPAC and HIAA and -300 mV for DA. The analytical column was a Supelco Supelcosil LC-18 column (3 µm particle size). The mobile phase consisted of a mixture of 4.1 g/L sodium acetate (Merck), 85 mg/L octane sulphonic acid (Aldrich), 50 mg/L EDTA (Merck), 1 mM tetramethylammonium chloride (Acros), 8.5 % methanol (Labscan) and ultra pure water (pH = 4.1 with glacial acetic acid). Drugs were dissolved in saline and injected sc or were given orally. Data were
converted into percentage of basal levels. The basal levels were determined from four consecutive samples (less than 20% variation), and set at 100%. The DA release was measured during 6 h after the rats were administration of the compound. After the experiments the rats were sacrificed and the brains were removed. After removal the brains were kept in 4% paraformaldehyde solution until they were sectioned to control the location of the dialysis probes.

**Receptor binding.** The *in vitro* binding affinity experiments were performed at Lundbeck AC/S, Copenhagen, Denmark.

(–)-2.2a and (+)-2.2a were tested for *in vitro* binding at the DA D1, D2, D3, and D4 receptors. The displacement of the radioligand was measured at a fixed concentration of the test compound (50 nM or 100 nM). It was estimated whether this concentration of the test compound was above or below the IC50 of the radioligand. When at the tested concentration the displacement of the radioligand was below the IC50, the test compound is considered inactive at the receptor.

**D1 binding.** By this method the inhibition of drugs of the binding of [3H]-SCH-23390 (1.16) (0.20 nM, Kd 0.45 nM) to DA D1 receptors in membranes from rat corpus striatum is determined *in vitro*. Method was been described by Hyttel and Larsen.35, 36

**D2 binding.** By this method the inhibition of drugs of the binding of [3H]-spiperone (0.50 nM, Kd 0.20 nM) to DA receptors in membranes from rat corpus striatum is determined *in vitro*. Method and results were described by Hyttel and Larsen.37, 38

**D3 Binding.** By this method the inhibition by drugs of the binding of [3H]-spiperone (0.3 nM, Kd 0.45 nM) to membranes of human cloned DA D3 receptors expressed in CHO-cells in determined *in vitro*. A modified method from R.G. Mackenzie et al was used39. CHO-cells expressing the human cloned D3 DA receptor were harvested and the cell suspension centrifuged at 1000 rpm for 7 min at 4°C. The supernatant was frozen. At the day of experiment the cell pellet was thawed at room temperature and diluted in assay buffer (25 Mm TRIS-HCl pH 7.4 + 6.0 mM MgCl2 +1.0 mM EDTA) to the desired concentration. 50 µL displacer (10 µM Haloperidol, text compound or assay buffer) and 230 µL buffer was added to a 96 well deep plate. Then 50 µL 0.3 nM [3H]-spiperone was added. The reaction was initiated by addition of 670 µL membrane suspension (test concentration 26 µg protein/670 µL). Packard GF/C unifilter (96 well) was pretreated with 0.1% PEI-solution 10-15 min before filtration. After 60 min of incubation at 25°C the reaction was terminated by filtration at Tomtec unifilter. The filters were washed twice with ice cold assay buffer. The filters were dried for 1.5 hours at 50°C, 35 µL
scintillation liquid was added and bound radioactivity was counted in Wallac Tri-Lux scintillation counters.

**D₄ binding.** By this method the inhibition of drugs of the binding of [³H]-Nemonapride (0.50 nM, Kᵦ 0.20 nM) to cloned human DA D₄ receptors is determined in vitro. Method and results were described by Meier *et al.*

**The plasma and brain extraction experiments.** Two male Wistar rats were used for this experiment. One of the rats was given 10 µmol/kg (-) 2.2a p.o. and the other rat was given 10 µmol/kg (+)-2.2a p.o. The rat given (-)-2.2a showed DA agonist stereotyped behaviour in the form of sniffing and chewing 10 min following the oral administration. The rat given (+)-2.2a showed no stereotyped behaviour throughout the experiment. At 65 min, the two test animals were killed with an overdose of chloral hydrate. A heart puncture was performed and a large volume of blood was collected in EDTA vacutainer tubes (Becton Dickinson, France). The sample was then centrifuged for 10 min at 3000 rpm in a Chilspin MSE centrifuge (Chilspin, MSE, England). The supernatants were transferred to a glass tube and was kept in the freezer at −18°C until the day of analysis. In addition, the whole brains were taken out, weighed, dissected, frozen on dry ice and homogenized in 10 mL 60 % acetonitrile / 40 % water containing 0.1 % formic acid and 0.01 % mercaptoethanol. The homogenized brain was centrifuged for 10 min at 3000 rpm in a Chilspin MSE centrifuge (Chilspin, MSE, England). The supernatant was transferred to a glass tube and was kept in the freezer at −18°C until the day of analysis.

### 2.7 References and notes


6 Most synthesis work of this route was done by Batiaan J. Venhuis. Enone prodrug of catecholamines. *PhD thesis*, Rijksuniversiteit Groningen, the Netherlands, 2002, Chapter 5, pp135-159.


