New, centrally acting dopaminergic agents with an improved oral bioavailability
Rodenhuis, Nieske

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Chapter 2

Further characterisation of structural requirements for ligands at the dopamine D₂ and D₃ receptor: studies with thienylethylamine as a possible pharmacophore

Abstract

This chapter describes the synthesis and in vitro pharmacology of a novel series of dopamine receptor ligands, in which the classical phenylethylamine pharmacophore is replaced by a thienylethylamine moiety. In general, the novel compounds showed moderate affinity for the dopamine D₂ and D₃ receptors. The results showed that a thienylethylamine moiety can act as a dopamine pharmacophore on these receptors. When the thienylethylamine moiety is fixed in a rigid system the affinity for the dopamine receptor is increased, however, in the tricyclic hexahydrothianaphthoxazine structure, the affinity for the dopamine receptors is diminished.

2.1 Introduction

Dopamine receptor agonists have attracted considerable attention due to their therapeutic potential against several neurological, endocrinological and cardiovascular diseases and drug abuse.116,202-204 Dopamine receptor agonist activities can be found in several classes of compounds including 2-phenylethylamines, aporphines, aminotetralins, naphthoxazines, and ergoline derivatives.

With the application of molecular biological techniques to express receptors in cloned cells at least six different subtypes of dopamine receptors have been identified.21,25 Their characterisation revealed two categories according to sequence homology, gene construction and second messenger system: D₁ and D₅, which are called “D₁-like receptors” and D₂ (D₂L and D₂S), D₃, D₄, which are called “D₂-like receptors”. The mechanism by which dopamine-binding at the receptor induces G-protein activity is unknown but most likely involves a cascade of intermolecular reactions. In particular, charged and conserved amino acid residues found in transmembrane domains should participate in the dopamine recognition. Molecular models and site directed mutagenesis confirm that an Asp in transmembrane domain 3 and two Ser in transmembrane domain 5 are important for the interaction with the amine and the hydroxyl groups of dopamine, respectively.21

Dopamine, and most of the known dopamine receptor agonists, binds with higher affinity to the dopamine D₃ than to the dopamine D₂ receptor. Due to the close homology between the dopamine D₂ and D₃ receptors, especially in the transmembrane domains (~80%), it is difficult to predict dopamine D₂ versus dopamine D₃ receptor selectivity based on receptor models. Malmberg et al.115 suggested that the observed dopamine D₃ receptor selectivity may not be due to a single specific interaction but rather to a small difference in conformation between the dopamine D₃ and D₂ receptors.

McDermed et al.99 elegantly rationalised the heterochirality of the potent dopamine receptor agonists by suggesting a model in which different faces of the compound interact with a putative three-point pharmacophore. An attractive feature of this model is that it allows superposition of the nitrogen atoms, the nitrogen lone pairs, the oxygen atoms, and the aromatic rings, the pharmacophoric elements of several dopamine receptor agonists. In addition, the presence of two lipophilic sites which bind the N-alkyl groups have been postulated.98 Wikström et al.101 have shown with a series of octahydrobenzo[f]quinolines, using a pharmacological in vivo model measuring dopamine D₂ activity, that one of the N-alkyl binding sites can only tolerate N-substituents equal to an n-propyl. Seiler and Markstein205 conclude that this space-limited accessory binding site, which they call “small N-alkyl binding site”, exists in both main groups of dopamine receptors.
Thienylethylamines as possible dopamine pharmacophore

Chart 2.1 Chemical structures of trans-9-hydroxy-N-n-propyl-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (PHNO, 27a), trans-9-hydroxy-4-(2-phenylethyl)-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (27b), trans-9-hydroxy-4-(2-thienylethyl)-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (27c), trans-9-hydroxy-2-N-n-propyl-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (28).

For a more extensive exploitation of this theory, we synthesised some derivatives of the potent dopamine D_2/D_3 agonist PHNO 27a. According to the Wikström/Seiler modification of McDermid’s model N-substituents larger than n-propyl should give compounds inactive at the dopamine D_2/D_3 receptors, while the steric requirements for an R group on the 2-position should be less critical.

The in vitro pharmacology data of the naphthoxazines (Table 2.1) confirmed that an N-substituent should not be larger than an n-propyl, and that there is more structural freedom for a 2-substituent. However, a thienylethyl substituent on the nitrogen (1c) gave a compound with a significantly higher affinity for the dopamine D_3 receptor than the phenylethyl analogue 27b. We hypothesised that the dopamine receptor pharmacophore of compound 27c is the thienylethylamine moiety (pharmacophore 2, chart 2.1) and not the 3-OH-phenylethylamine moiety (pharmacophore 1, chart 2.1). To test this hypothesis the thienylethylamines 29 and 30 were synthesised and tested in vitro.
The *in vitro* pharmacology of compounds 29 and 30 (Table 2.1) showed that the thienylethylamine moiety could behave as a pharmacophore at the dopamine receptor.

It has been known for a long time that the 2-aminotetralin (2-amino-1,2,3,4-tetrahydronaphthalene) structure is pharmacologically important. Initially, 2-aminotetralins were characterised by their sympathomimetic action, causing mydriasis, contraction of the uterus, changes in blood pressure and respiration, and increased intestinal motility in test animals.\textsuperscript{202-204} The 2-aminotetralin system has proved to be a valuable structural base for dopamine receptor, serotonin receptor and adrenoceptor ligands, as well as for compounds that interact with melatonin receptors.\textsuperscript{208,209}

Some of these compounds have been studied by several research groups to elucidate their structure activity relationship for dopamine receptors.\textsuperscript{98,100,101,201,205,210-212} Initially, these studies identified S-(–)-5-hydroxy-2-(N,N-di-\textit{n}-propylamino)tetralin (S-(–)-5-OH-DPAT, S-(–)-9) as the most potent monohydroxy 2-aminotetralin.\textsuperscript{98,100,205} Later S-(–)-5-hydroxy-2-(\textit{n}-propyl-N-2-thienylethylamino)tetralin (S-(–)-N-0437, S-(–)-31) was found to be an even more potent dopamine receptor agonist.\textsuperscript{201} Moreover, R-(+)-7-hydroxy-2-(N,N-di-\textit{n}-propylamino)tetralin (R-(+)-7-OH-DPAT, R-(+)-10) was later shown to have preference for the dopamine D\textsubscript{3} receptor subtype.\textsuperscript{79,213}
Thienylethylamines as possible dopamine pharmacophore

Chart 2.3 Chemical structures of S-(−)-5-hydroxy-2-(N,N-di-n-propylamino)tetralin (S-(−)-5-OH-DPAT, S-(−)-9), R-(+)-7-hydroxy-2-(N,N-di-n-propylamino)tetralin (R-(+)-7-OH-DPAT, R-(+)-10), S-(−)-5-hydroxy-2-(N-n-propyl-N-2-thienylethylamino)tetralin (S-(−)-N-0437, S-(−)-31), 5,6,7,8-tetrahydro-6-(2-propenyl)-4H-thiazolo[4,5-d]azepin-2-amine (BHT920, 32), pramipexole (19) and 6-amino-2-(N,N-di-n-propylamino)-thiazolo[4,5-f]indan (GMC1111, 33).

Their low oral bioavailability and their short duration of action have limited the clinical utility of catechol and phenol-containing drugs. The catechol and phenol rings provide optimal sites for glucuronidation. Thus, for many years, emphasis has been focused on the identification of bioisosteric replacements for catechols and phenols. The idea that neither catecholic nor phenolic hydroxyl groups are an absolute requirement for potent dopamine receptor activity was presented by Andén et al.,183 who showed that the aminothiazolazepine derivative BHT920 (32) is a dopamine autoreceptor agonist, as well as an α2-adrenoceptor agonist. Pramipexole (19), a benzothiazole analogue of the 2-aminotetralins, was found to be a potent dopamine receptor agonist with both dopamine D2 and D3 receptor stimulating properties. It is presently on the market for the treatment of Parkinson’s disease.146,154,155 Another example of an analogue with an aminothiazole moiety is GMC1111 (33), which possesses affinity for the dopamine D2 and D3 receptor, and also inhibits lipid peroxidation.214
Chapter 2

Since it was found that the thienylethylamine moiety might act as a dopamine receptor pharmacophore, we tested whether a thiophene moiety may act as a bioisostere for a phenol in 2-aminotetralins and hexahydronaphthoxazines. Therefore, thiophene analogues of the 2-aminotetralins and hexahydronaphthoxazines, 34-39 were synthesised. All the compounds synthesised were tested in vitro for their affinity at dopamine D$_2$L and D$_3$ receptors. The derivatives with interesting properties were further investigated for their in vivo dopamine receptor activity and bioavailability using the microdialysis technique in freely moving rats.\textsuperscript{215}

2.2 Chemistry

The trans N-arylalkyl substituted hexahydronaphthoxazines 27b and c were synthesised from trans-9-methoxy secondary amine 40 via N-alkylation with the appropriate arylethyl halide or by reductive alkylation. The trans-9-methoxy secondary amine 40 was prepared by using known methods.\textsuperscript{206,216,217} The phenols were achieved through ether cleavage with BBr$_3$ under N$_2$ (Scheme 2.1).
Thienylethylamines as possible dopamine pharmacophore

Scheme 2.1 Reagents: (a) C₆H₅CH₂CH₂Br, K₂CO₃, DMF or 2-thienyl acetic acid, (CH₃)₃N.BH₃, xylene; (b) BBr₃, CH₂Cl₂.

The synthesis of 2-phenyl-N-n-propynaphthoxazine 28 is outlined in Scheme 2.2. The racemic trans-aminoalcohol 42 was acylated with 2-chloro-2-phenylacetyl chloride to afford a mixture of diastereomeric trans-amidoalcohols 43. Cyclisation of the chloroacetamide 43 was achieved with NaOH in isopropanol, affording the mixture of trans-lactams 44a, b with the 2-phenyl ring in equatorial or in axial position. The 2-(axial)phenyl trans-lactam and the 2-(equatorial)phenyl-trans-lactam could be separated by column chromatography. Theoretically the morpholine-ring can exist in two conformations namely a pseudo chair or boat conformation. The phenyl-ring can be adjusted in a pseudo-axial and pseudo-equatorial position. The morpholine ring exists probably most of the time in a chair conformation since this is energetically the most favourable conformation, but it is possible that the conformation changes to the boat-conformation. When the morpholine ring exists in a chair conformation with the phenyl-ring in the axial position there is an interaction in space between the protons on C10b and C15/C19. The other epimer with the phenyl-ring in the equatorial position shows an interaction in space between the protons on C10b and C2. The NOESY-experiments showed that the fast eluting compound has an interaction between the protons on C10b and C15/C19, so this is the compound with the phenyl in the axial position. The last eluting compound shows an interaction between the protons on C10b and C2, indicating that the compound has the phenyl-ring in the equatorial position. The 2-equatorial-phenyl isomer was used for the next reaction. After reduction of the amide with BH₃.ME₂S complex, the final step was demethylation, which was achieved by applying BBr₃, giving the final product 28.
The thienylethylamines 29 and 30 were synthesised according to Scheme 2.3. The secondary amine 47 was acylated with 2- or 3-thienylacetyl chloride. As a result of the rotation around a binding with a partial double bond character the aliphatic protons and the aliphatic carbon atoms of 48 and 49 were according to the ¹H- and ¹³C-NMR spectra chemically non-equivalent. The resulting amides were reduced with BH₃·Me₂S complex.

The synthesis of the tetrahydrobenzo[b]thiophenes is outlined in Scheme 2.4. A Grignard-reaction of 50 with ClMgCH₂SiMe₃ followed by quaternisation with methyl iodide gave the 3-(trimethylammoniummethyl)-2-(trimethylsilylmethyl)thiophene 52, which is the precursor of
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2,3-dimethylene-2,3-dihydrothiophene. Treatment of 52 with n-tetrabutylammonium fluoride (TBAF) leads to the formation of 2,3-dimethylene-2,3-dihydrothiophene, which is an unstable intermediate. This intermediate was captured in a Diels-Alder [4 + 2] cycloaddition reaction with methyl acrylate as the dienophile. We did not succeed in the separation of the mixture of regioisomers obtained. Hydrolysis of this mixture of esters gave the carboxylic acids 54a, b in good yield. A Curtius rearrangement gave the mixture of amines 55a, b. Only after conversion into the tertiary amines 34 and 35 was it possible to separate the mixture of regioisomers on a SiO$_2$ column.

**Scheme 2.4** Reagents: (a) ClMgCH$_2$SiMe$_3$, Ni(PPh$_3$)$_2$Cl$_2$, Et$_2$O; (b) CH$_3$I, CH$_3$CN; (c) CH$_2$=CHCO$_2$CH$_3$, TBAF, CH$_3$CN; (d) NaOH; (e) 1) DPPA, Et$_3$N, dioxane; 2) HCl, dioxane, 120 °C; (f) C$_3$H$_7$I, K$_2$CO$_3$, DMF; (g) SiO$_2$ column chromatography, EtOAc:Hexane = 1:9.

The aminomethyltetrahydrobenzo[b]thiophenes (36 and 37) were synthesised from the corresponding carboxylic acids (54a and b) in three steps by standard chemistry (Scheme 2.5). The two isomers could be separated on a SiO$_2$ column after conversion to the tertiary amines.
The hexahydrothianaphthoxazines 38 and 39 were synthesised from the commercially available ketone 58, which was readily converted into the tosyloxime 60 in two steps. Neber rearrangement of 60 with potassium tert-butoxide afforded the desired amino-ketone 61. The amino ketone 61 was readily acylated with chloroacetyl chloride. Reduction of the keto-chloroacetamide 62 with sodium borohydride gave only the trans isomer. The proton on C1 gave a doublet at δ 4.6 ppm with a coupling constant of 7.2 Hz indicating a di-axial coupling. The cis-compound would have a couplings constant of about 3.5 Hz.

The cyclization of the alcohol-chloroacetamide 63 by means of 50 % aqueous NaOH-solution in isopropanol at room temperature gave satisfactory yields of the lactam 64, which was reduced with LiAlH₄ to the oxazine 38. The reduction took place in a low yield. Alkylation of the amine 38 with propyl iodide in DMF afforded the tertiary amine 39 in a good yield.
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Scheme 2.6 Reagents: (a) NH₂OH.HCl; (b) TsCl; (c) t-BuOK/ETOH; (d) HCl/H₂O; (e) ClCH₂COCl, NaOH, CH₂Cl₂; (f) NaBH₄; (g) NaOH; (h) LiAlH₄; (i) C₃H₇I, DMF, K₂CO₃.

2.3 Results and Discussion

The structural requirements for the N-substituents of dopaminergic 7- and 9-hydroxylated octahydrobenzo[f]quinolines (OHB[f]Q) and related compounds have been described previously. On the basis of in vivo biochemical and behavioural data it was demonstrated that the nitrogen should be substituted with maximal an n-propyl group for optimal dopamine receptor activity. Larger N-substituents, (e.g. n-butyl) for the 9-hydroxy-OHB[f]Qs gave a dramatic reduction in potency of these compounds. The Kᵢ values shown in Table 2.1 for the N-substituted 9-hydroxy-hexahydronaphthoxazines (9-OH-HNO) 27b and 28 are in full agreement with the models of McDermed and Wikström. According to these models there is space available for a 2-substituent. Interestingly, however, the dopamine D₃ receptor affinity of 27c (Kᵢ = 83 nM) showed that this compound does not fit these receptor models. This has led us to hypothesise that it is the thienylethylamine moiety of 27c, which confers dopamine D₃ receptor properties to this compound. In the binding model for the dopamine receptors described by Miller et al., the protonated nitrogen of the ligand interacts with the receptor. With the development of molecular biological techniques and the cloning of the dopamine receptors, the amino acid sequence of the different receptors were determined which lead to the assumption that the protonated nitrogen of ligands interacts with Asp 114 (D₂) or Asp 110 (D₃) in transmembrane domain 3 through a reinforced ionic bond (for review see ref. 21). In the hydroxylated 2-aminotetralins and OHB[f]Qs an additional hydrogen bond is formed from the phenolic hydrogen of the ligands to the Ser 193 (D₂) or Ser 192 (D₃) in transmembrane domain 5. If a thiophene ring utilises the same interaction points as the phenol, it may be speculated
that the sulfur atom in the thienylethyl substituent may form a hydrogen bond with the hydroxyl moiety of a Ser residue. Sulfur can only act as a hydrogen bond acceptor and consequently this weaker interaction may provide an explanation for the lower affinity for the dopamine D₃ receptor of compound 27c, as compared to compound 27a. Two alternative explanations for the diminished affinity are I) the non-optimal distance between the hydrogen bond forming moieties on the aromatic site and the nitrogen, II) alternative interaction points in the receptor for these essential atoms in a dopamine receptor.

Table 2.1 Receptor binding data of various dopamine receptor ligands.

<table>
<thead>
<tr>
<th>Compound</th>
<th>D₂L [³H]N-0437</th>
<th>D₃ [³H]Spiperone</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-PHNO ((+)-27a)</td>
<td>6.24</td>
<td>0.21</td>
</tr>
<tr>
<td>(±)-27b</td>
<td>&gt;3676</td>
<td>1566</td>
</tr>
<tr>
<td>(±)-27c</td>
<td>3676</td>
<td>83</td>
</tr>
<tr>
<td>(±)-28</td>
<td>375</td>
<td>12</td>
</tr>
<tr>
<td>29</td>
<td>1080</td>
<td>117</td>
</tr>
<tr>
<td>30</td>
<td>439</td>
<td>108</td>
</tr>
<tr>
<td>34</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>35</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>36</td>
<td>3107ᵇ</td>
<td>60</td>
</tr>
<tr>
<td>37</td>
<td>2037</td>
<td>247</td>
</tr>
<tr>
<td>38</td>
<td>&gt;4780ᵇ</td>
<td>3000</td>
</tr>
<tr>
<td>39</td>
<td>630ᵇ</td>
<td>240</td>
</tr>
<tr>
<td>(−)-5-OH-DPAT (9)²²³</td>
<td>14</td>
<td>0.54</td>
</tr>
<tr>
<td>(+)-7-OH-DPAT (10)²²³</td>
<td>34</td>
<td>0.57</td>
</tr>
<tr>
<td>(±)-N-0437 (31)²²³</td>
<td>0.06</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Footnotes: a Kᵢ values are means of three separate experiments; the results of which did not vary more than 25%. b [³H]NPA was used as radiolabeled ligand.

To prove the assumption whether the thienylethylamine moiety can act as a pharmacophore, we synthesised compounds 29 and 30, possessing only the thienylethylamine moieties. These compounds possess low to moderate affinity for the dopamine D₃ receptor, but confirmed our assumption that a thienylethylamine moiety can act as a non-optimal pharmacophore at the dopamine D₃ receptor.
It is known from the literature that hydroxylated 2-aminotetralins and hexahydronaphthoxazines are potent dopaminergic agonists, but their oral bioavailability is very low due to glucuronidation in the gut and the liver.\textsuperscript{161}

The thiophene analogues of the 2-aminotetralins (compounds 34 and 35) have considerable affinity for the dopamine D\textsubscript{2} and D\textsubscript{3} receptors, but significantly lower than e.g. 5-OH-DPAT. As stated earlier, one reason could be the less tight H-bonding of the sulfur atom, as compared to a hydroxyl moiety.\textsuperscript{220-222} Calculating the distances between the sulfur and the nitrogen in compounds 34 and 35 in a minimised conformation using the computer program MacroModel shows that these distances are 5.4 Å and 6.0 Å, respectively. The distance between the nitrogen atom and the hydroxyl group in potent dopamine receptor agents should be between 5.5 and 7.4 Å.\textsuperscript{91,108} The distance between the sulfur and the nitrogen atom in the dipropylaminotetrahydrobenzob[thiophenes is comparable with the distance between the oxygen atom of the hydroxyl group and the nitrogen atom in 4-hydroxy-2-aminoindans.\textsuperscript{108} It is known that 2-aminoindans are less potent dopamine receptor ligands than the corresponding 2-aminotetralins.\textsuperscript{97}

The results of compounds 34 and 35 confirmed the hypothesis that a thienylethylamine may act as a pharmacophore, moreover, the semi-rigid system increased the affinity for the dopamine receptor. This is in line with the higher potency of the hydroxylated 2-aminotetralin analogues compared to phenylethylamines.\textsuperscript{97,224} Apparently, the near coplanar arrangement is required for higher dopamine agonist activity. Since a thienylethylamine moiety may act as a pharmacophore, it is stated that a thiophene may be a bioisostere for a phenol moiety. Bioisosteres are groups of molecules which have chemical and physical similarities producing broadly similar biological effects.\textsuperscript{225} The substitution of –CH=CH– by –S– in aromatic rings has been one of the most successful applications of classical isosterism\textsuperscript{182} (an example of such a replacement is found with the atypical antipsychotic olanzapine).

Using microdialysis experiments the relative oral bioavailabilities of the compounds 34, 35 and 5-OH-DPAT could be calculated.\textsuperscript{215} The relative oral bioavailabilities were calculated by comparing the Areas Under the Curve (AUCs) after oral and subcutaneous administration. When there was no significant difference between the AUCs the subcutaneous dose was divided by the oral dose and multiplied by 100 to give the relative oral bioavailability. These data show that, although the affinities of the benzo[b]thiophenes (34 and 35) for the dopamine receptors are lower as compared to 5-OH-DPAT, the relative oral bioavailability is higher. Therefore, the benzo[b]thiophenes are interesting compounds for further research.
Table 2.2  Summary of the microdialysis results of compounds 34, 35 and 5-OH-DPAT. Results are given as Areas Under the Curve (AUCs). Adapted from reference 215.

<table>
<thead>
<tr>
<th>Compound</th>
<th>subcutaneous administration</th>
<th>oral administration</th>
<th>relative oral bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dose (µmol/kg)</td>
<td>AUC</td>
<td>dose (µmol/kg)</td>
</tr>
<tr>
<td>34</td>
<td>0.1</td>
<td>2650 ± 1000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6000 ± 500</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>12446 ± 335</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>1</td>
<td>3150 ± 400</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6700 ± 800</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6700 ± 800</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>9400 ± 960</td>
<td>30</td>
</tr>
<tr>
<td>5-OH-DPAT (9)</td>
<td>0.1</td>
<td>9700 ± 500</td>
<td>10</td>
</tr>
</tbody>
</table>

Footnotes: <sup>a</sup> Experiment lasted 150 min. All other experiments lasted 165 min. All the AUCs of s.c. and p.o. doses of each compound were compared, but only the doses that were not significantly different were put in line in the table.

Compounds 36 and 37 were synthesised to enlarge the distance between the nitrogen and sulfur atom. Compound 36 turned out to have no affinity for the dopamine D<sub>2</sub> receptor and a moderate affinity for the dopamine D<sub>3</sub> receptor. The distance between the sulfur atom and the nitrogen atom in an extended minimised conformation using the computer program MacroModel of compounds 12 and 13 is between 5.7 and 6.7 Å depending on the conformation of the methylene amino group.

Although the distances between the sulfur atom and the nitrogen atom in the hexahydrothianaphthoxazines (38 and 39) are comparable with those in 5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (35), the introduction of a morpholine ring gave a dramatic decrease in the dopamine D<sub>2</sub> and D<sub>3</sub> receptor affinity.

In conclusion, bioisosteric replacement of a phenol by a thiophene moiety gave dopamine receptor agonists with a lower affinity than the corresponding 2-aminotetralins. This loss in affinity is, however, partly compensated by a relative higher oral bioavailability of the tetrahydrobenzo[b]thiophenes 34 and 35.
2.4 Experimental Section

2.4.1 Chemistry

Melting points were determined in open glass capillaries on an Electrothermal digital melting-point apparatus and are uncorrected. $^1$H and $^{13}$C 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), and dd (double doublet). Chemical shifts are given in $\delta$ 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. 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.

*trans*-9-Methoxy-4-(2-phenylethyl)-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (41a). A solution of *trans*-9-methoxy-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (40) (0.42 g, 1.91 mmol), K$_2$CO$_3$ (1.5 g, 10.9 mmol), 2-bromo-phenylethyl (0.39 g, 2.10 mol) in 15 mL of DMF was stirred for 15 h at 60 °C under an atmosphere of nitrogen. The reaction mixture was allowed to cool to RT and poured into water and extracted 3 times with 30 mL of diethyl ether. The combined organic phases were extracted several times with brine, dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. The residue was further purified by flash chromatography using a mixture of CH$_2$Cl$_2$ and MeOH (25/1) as the eluent. After evaporation of the solvent the yield was 0.39 g (63 %) 41a, which was converted to the HCl-salt: mp 239-241 °C; $^1$H NMR (CD$_3$OD) $\delta$ 1.9-2.1 (m, 1H), 2.5-2.7 (m, 1H), 2.9-3.0 (m, 2H), 3.1-3.3 (m, 2H), 3.4-3.6 (m, 3H), 3.7-3.9 (m, 2H), 3.8 (s, 3H), 4.2-4.4 (m, 2H), 4.8 (d, 1H, J = 9.3 Hz), 6.9-7.0 (m, 1H), 7.0-7.2 (m, 2H), 7.3-7.5 (m, 5H); $^{13}$C NMR (CD$_3$OD) $\delta$ 20.3, 24.7, 27.7, 49.7, 51.9, 53.3, 62.2, 62.7, 74.8, 108.4, 113.0, 124.7, 125.7, 127.4, 127.8, 133.4, 135.2, 156.9; Anal (C$_{21}$H$_{25}$NO$_2$.HCl) C, H, N.

*trans*-9-Hydroxy-4-(2-phenylethyl)-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (27b). A 1 M solution of BBr$_3$ in CH$_2$Cl$_2$ was added to a cooled solution (-30 °C) of 41a.HCl (0.22 g, 0.61 mmol) in 30 mL of dichloromethane, under an atmosphere of nitrogen. The reaction was initially stirred for 1 h at this temperature, which was then allowed to rise to RT after which the reaction was stirred a further 3 h. The reaction mixture was then poured into water, made alkaline by the addition of a solution of Na$_2$HCO$_3$. The separated organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. Conversion to the HCl-salt and recrystallization from acetonitril yielded 0.12 g (51 %) 27b: mp of the free base 173-176 °C; $^1$H NMR (CDCl$_3$) $\delta$ 1.6-
1.8 (m, 1H), 2.2-2.4 (m, 2H), 2.6-2.9 (m, 6H), 3.0-3.2 (m, 2H), 3.9-4.1 (m, 2H), 4.4 (d, 1H, J = 9.7 Hz), 6.6-6.8 (m, 1H), 6.9-7.0 (m, 2H), 7.2-7.4 (m, 5H); 13C NMR (CDCl₃) δ 22.5, 25.6, 30.3, 50.7, 53.4, 60.9, 65.4, 77.1, 110.4, 113.2, 124.7, 125.2, 127.0, 127.2, 127.8, 152.8; Anal (C₂₀H₂₃NO₂·2HCl) C, H, N.

trans-9-Methoxy-4-(2-thienylethyl)-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (41b). To a solution of trans-9-methoxy-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (40) (0.5 g, 2.3 mmol) and trimethylamine borane complex (0.34 g, 4.6 mmol) in 30 mL of xylene was added 2-thienyl acetic acid (0.65 g, 4.5 mmol). The mixture was heated under N₂ and refluxed for 15 h. The mixture was poured into water. The organic layer was separated and the aqueous layer was extracted with diethyl ether (2 x 25 mL). The combined organic layers were washed with NaHCO₃-solution and brine, dried over MgSO₄. Evaporation of the solvents yielded an oil which was converted to the HCl-salt and recrystallized from ethanol; yield 0.46 g (62.3%): mp 195.5-197 °C; ¹H NMR (CD₃OD) δ 2.5-2.8 (m, 1H), 3.1-3.3 (m, 1H), 3.5-3.7 (m, 2H), 4.0-4.4 (m, 2H), 4.4-4.6 (m, 2H), 4.4 (s, 3H), 4.8-5.1 (m, 1H), 5.5 (d, 1H, J = 9.5 Hz), 7.5-7.6 (m, 1H), 7.7-7.9 (m, 1H), 8.1 (d, 1H, J = 3.7 Hz); ¹³C NMR (CD₃OD) δ 20.9, 22.7, 25.4, 52.4, 53.8, 63.1, 63.3, 75.4, 109.1, 113.6, 124.0, 125.3, 125.6, 126.4, 128.4, 133.9, 137.3, 157.6; Anal (C₁₉H₂₃NO₂S·HCl) C, H, N.

trans-9-Hydroxy-4-(2-thienylethyl)-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (27c). Compound 27c was prepared from the methoxy compound 41b by essential the same procedure as described for the preparation of 27b. The yield was 65 %. An analytical sample was recrystallized from ethanol-diethyl ether to provide white crystals: mp free base 173-175 °C, HCl-salt: 231-234 °C; ¹H NMR (CD₃OD) δ 1.8-2.0 (m, 1H), 2.5-2.6 (m, 1H), 2.8-3.0 (m, 2H), 3.3-3.5 (m, 5H), 3.7-3.8 (m, 2H), 4.1-4.4 (m, 2H), 4.7 (d, 1H, J = 9.8 Hz), 6.6-6.7 (m, 1H), 6.9-7.1 (m, 4H), 7.3 (d, 1H, J = 4.9 Hz); ¹³C NMR (CDCl₃) δ 20.3, 22.7, 25.4, 52.4, 53.8, 63.1, 63.3, 75.4, 109.1, 113.6, 124.0, 125.3, 125.6, 126.4, 133.9, 137.3, 157.6; Anal (C₁₈H₂₁NO₂S·HCl) C, H, N.

trans-9-Methoxy-2-phenyl-4-N-n-propyl-2,3,4a,5,6-tetrahydro-4H-naphth[1,2b][1,4]oxazin-3-one (44a) and (44b). To a solution of compound 42 (1.14 g, 4.8 mmol) in 70 mL of dichloromethane was added NaOH (1.0 g) dissolved in 10 mL of water. 2-Chloro-2-phenyl acetyl chloride (1.0 g, 5.3 mmol) dissolved in 10 mL of dichloromethane was slowly added. The reaction mixture was stirred at RT for 2 h. The mixture was then poured into 60 mL of water. The two layers were separated and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with water and thereafter dried over Na₂SO₄. After filtration the solvent was removed under reduced pressure to yield 1.7 g (91 %) oil as a mixture of diastereomers of chloroacetamide 43 and partly cyclized product: ¹H NMR (CDCl₃) δ 0.9 (t, 3H, J = 7.3 Hz), 1.4-1.6 (m, 1H), 1.6-1.9 (m, 2H), 2.35-2.5 (m, 1H), 2.9-3.0 (m, 2H), 3.1-3.3 (m, 1H), 3.7-3.9 (m, 2H), 3.8(s, 3H), 4.8 (d, 1H, J = 9.0 Hz), 5.4 (s, 1H), 6.7-6.8 (m, 1H), 7.0-7.15 (m, 2H), 7.3-7.4 (m, 3H), 7.5-7.6 (m, 2H); ¹³C NMR (CDCl₃) δ 9.7,
Thienylethylamines as possible dopamine pharmacophore

19.7, 23.7, 25.5, 41.6, 53.9, 55.2, 75.2, 79.8, 108.3, 113.2, 124.6, 126.5, 126.7, 127.7, 134.0, 136.8, 156.9, 167.3. The compound was used without further purification.

To a solution of the chloroacetamide 43 (1.7 g, 4.8 mmol) in 200 mL of isopropanol a solution of 1.2 g NaOH in 2.4 mL H₂O was added dropwise at RT. After stirring for 5 h at RT the mixture was neutralised with 1 N HCl. The solvents were evaporated as much as possible and the resulting residue was slurred in 200 mL of water and extracted with 4 x 25 mL of dichloromethane. The organic layer was washed with water, dried over Na₂SO₄ and then reduced to dryness. The residual solid was purified by column chromatography on silica gel 60 using a mixture of ethyl acetate and hexane (1/4) as the eluent resulting in the separation of the two stereoisomers (44a and 44b). Recrystallization from iso-propylacetate gave the lactams as white crystals. Fast eluting compound (44a, axial): yield: 590 mg (38 %): mp 151.5-152 °C; ¹H NMR (CDCl₃) δ 1.0 (t, 3H, J = 7.3 Hz), 1.5-1.9 (m, 3H), 2.3-2.5 (m,1H), 2.8-3.0 (m, 2H), 3.4-3.5 (m, 1H), 3.6-3.8 (m, 2H), 3.8 (s, 3H), 4.6 (d, 1H, J = 9.5 Hz), 5.6 (s, 1H), 6.8 (d, 1H), 7.0 (d, 1H, J = 8.3 Hz), 7.1 (br s, 1H), 7.3-7.4 (m, 3H), 7.6 (d, 2H, J = 7.4 Hz); ¹³C NMR (CDCl₃) δ 11.3, 21.3, 25.2, 26.7, 43.5, 55.2, 57.2, 71.3, 78.2, 109.5, 113.8, 126.0, 127.3, 127.9, 128.4, 129.1, 135.8, 137.2, 157.1, 157.5; IR (NaCl) 1651 cm⁻¹ (CO); MS (EIPI) m/e 351 (M⁺).

Last eluting compound (44b, equatorial): yield: 840 mg (55 %): mp 112.5-113.5 °C; ¹H NMR (CDCl₃) δ 0.9 (t, 3H, J = 7.4 Hz), 1.4-1.6 (m, 1H), 1.6-1.9 (m, 2H), 2.4-2.5 (m,1H), 2.9-3.0 (m, 2H), 3.1-3.3 (m, 1H), 3.8 (s, 3H), 3.8-4.0 (m, 2H), 4.80 (d, 1H, J = 9.3 Hz ,), 5.4 (s, 1H), 6.9 (m, 1H), 7.0-7.2 (m, 2H), 7.5-7.6 (m,3H), 7.7-7.8 (m, 2H); ¹³C NMR (CDCl₃) δ 11.3, 21.3, 25, 27, 43, 55, 57, 77, 81, 110, 115, 126, 127.9, 128, 130, 135.5, 138, 158, 169; IR (NaCl) 1640 cm⁻¹ (CO); MS (EIPI) m/e 351 (M⁺). Anal (C₂₂H₂₅NO₃) C, H, N. The equatorial product is used for the next step.

trans-9-Methoxy-2-phenyl-4-N-n-propyl-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (45).

To a solution of amide 44b (350 mg, 1.0 mmol) in anhydrous THF (25 mL) was added LiAlH₄ (200 mg). The mixture was refluxed for 3 h and then was added successively water (0.2 mL), 4 N NaOH (0.2 mL) and water (0.6 mL). This mixture was refluxed for another 15 min. The solid was filtered off and the filtrate dried over Na₂SO₄ and concentrated to yield 316 mg (94 %) oil. The amine was converted to the HCl-salt. mp 209-210 °C; ¹H NMR (CDCl₃) δ 0.9 (t, 3H, J = 7.3 Hz), 1.5-1.7 (m, 3H), 2.3-2.4 (m, 4H), 2.8-2.9 (m, 3H), 3.1 (dd, 1H, J = 11.7 Hz), 3.8 (s, 3H), 4.6 (d, 1H, J = 9.03 Hz), 4.9 (dd, 1H, J = 10.5 Hz), 6.7-6.8 (m, 1H), 7.0 (d, 1H, J = 8.3 Hz), 7.2 (m, 1H), 7.3-7.5 (m, 5H); ¹³C NMR (CDCl₃) δ 10.5, 17.0, 22.8, 25.8, 53.6, 53.9, 58.1, 60.3, 76.7, 77.5, 108.6, 112.2, 124.6, 125.6, 126.1, 126.8, 127.5, 136.0, 139.2, 156.36; Anal (C₂₂H₂₇NO₃.HCl) C, H, N.

trans-9-Hydroxy-2-phenyl-4-N-n-propyl-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (28). The phenol 28 was prepared from the methoxy compound 45 by essential the same procedure as described for the preparation of 27b from 41a. The yield was 60 %. An analytical sample was recrystallized from acetonitril to provide white crystals: mp 202-
204 °C; $^1$H NMR (CD$_3$OD) $\delta$ 0.9 (t, 3H, $J = 7.1$ Hz), 1.6-1.9 (m, 3H), 2.3-2.4 (m, 1H), 2.7-2.9 (m, 2H), 3.0-3.2 (m, 1H), 3.3-3.5 (m, 3H), 4.2-4.3 (d, 1H, $J = 12.9$ Hz), 4.7 (d, 1H, $J = 9.5$ Hz), 5.5 (br s, 1H), 6.9 (d, 1H, $J = 8.5$ Hz), 6.6-6.7 (m, 1H), 7.1 (s, 1H), 7.3-7.6 (m, 5H); $^{13}$C NMR (CD$_3$OD) $\delta$ 9.8, 15.1, 20.4, 25.0, 49.5, 52.6, 61.9, 68.9, 69.6, 110.6, 114.4, 123.7, 124.9, 127.0, 128.0, 128.2, 133.3, 135.8, 154.7; Anal (C$_{21}$H$_{25}$NO$_2$.HCl.½H$_2$O) C, H, N.

N-n-Propyl-3-thiophen-2-yl-acetamide. To a solution of n-propylamine (5.9 g, 100 mmol) in dichloromethane (50 mL) and 2N NaOH (10 mL) was added dropwise 3-thienylacetyl chloride (2.8 g, 17.4 mmol) dissolved in dichloromethane (10 mL). The reaction mixture was stirred for 2h at RT. The two layers were separated and the aqueous layer was extracted with dichloromethane (10 mL). The combined organic layers were washed with brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure to yield 2.3 g (72 %) oil which solidified upon standing which was recrystallized from ethyl acetate-hexane: $^1$H NMR (CDCl$_3$) $\delta$ 0.8 (t, 3H, $J = 7.3$ Hz), 1.4-1.5 (m, 2H), 3.16-3.22 (m, 2H), 3.6 (s, 2H), 5.6 (br s, 1H), 7.0-7.1 (m, 1H), 7.1-7.2 (m, 1H), 7.3-7.4 (m, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 9.7, 21.2, 36.6, 39.8, 122.2, 125.2, 127.0, 133.5, 169; IR (NaCl) cm$^{-1}$ 1641 (C=O, amide). Anal (C$_9$H$_{13}$NOS) C, H, N.

N-n-Propyl-(3-thiophen-2-yl-ethyl)-amine (47). N-n-Propyl-3-thiophen-2-yl-acetamide (1.0 g, 5.5 mmol) was dissolved in anhydrous THF (25 mL) and 2 M BH$_3$.Me$_2$S (5.5 mL, 10.9 mmol) in anhydrous THF (20 mL) was slowly added at RT. The mixture was stirred at RT for 30 min and subsequently refluxed for 1 h. The mixture was allowed to cool to RT and successively MeOH (3.5 mL), H$_2$O (3.5 mL) and 4 N HCl (3.5 mL) was added and the mixture was stirred for another 30 min at RT. The solvent was evaporated and the residue dissolved in H$_2$O, washed with diethyl ether and the aqueous layer was made alkaline with NaHCO$_3$ and extracted with diethyl ether. The combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered and the solvent was evaporated to yield 0.75 g (67 %) yellow oil: $^1$H NMR (CDCl$_3$) $\delta$ 0.9 (t, 3H, $J = 7.3$ Hz), 1.5 (q, 2H, $J = 7.3$ Hz), 1.5-1.6 (m, 2H), 2.5 (t, 2H, $J = 7.2$ Hz), 2.8 (s, 4H), 6.9-7.0 (m, 2H), 7.2-7.3 (m, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 10.2, 21.6, 29.2, 48.7, 50.2, 119.4, 124.0, 126.6, 138.8; Anal Calcd (Obsd) for C$_9$H$_{15}$NS.¾H$_2$O: C: 59.14 (59.60), H: 9.10 (8.68), N: 7.66 (7.30).

The amine was converted to the HCl-salt and recrystallized from diethyl ether-isopropanol: mp 207-210 °C.

N-n-Propyl-(3-thiophen-2-yl-ethyl)-thiophen-2-yl-acetamide (48) To a solution of amine 47.HCl (500 mg, 2.4 mmol) dissolved in dichloromethane (50 mL) and 10 % NaOH (10 mL) was added 2-thienylacetylchloride (2 mL). The mixture was stirred for 3 h at RT and poured into water. The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The oil was purified over a SiO$_2$-column with dichloromethane as eluent. Evaporation of the dichloromethane yielded 660 mg (93 %) oil: $^1$H NMR (CDCl$_3$) $\delta$ 0.9 (t, 3H, $J = 7.3$ Hz), 1.4-1.7 (m, 2H), 2.8-2.9 (m, 2H), 3.1-3.2 (m, 1H), 3.3-3.4 (m, 1H), 3.5-3.7 (m, 4H), 6.8-7.0 (m, 4H), 7.2-7.3 (m, 2H); $^{13}$C NMR (CDCl$_3$) $\delta$ 9.7 (C10), 9.9 (C10), 19.3 (C9 or C12), 20.8 (C9 or C12), 26.7 (C9 or C12), 28.1 (C9 or C12), 33.3 (C6),
33.4 (C6), 46.0 (C8 or C11), 46.2 (C8 or C11), 47.6 (C8 or C11), 49.1 (C8 or C11), 119.8, 120.3, 123.2, 124.1, 124.5, 124.8, 125.2, 126.5, 126.7, 135.2, 136.5, 137.8, 168.2 (C7); Anal (C13H19NOS2) C, H, N.

**N-n-Propyl-(2-thiophen-2-yl-ethyl)-thiophen-3-ylethyl-amine (29).** N-n-Propyl-(3-thiophen-2-yl-ethyl)-thiophen-2-yl-acetamide 48 (0.5 g, 1.70 mmol) was dissolved in anhydrous THF (40 mL) and 2 M BH₃.Me₂S (3 mL) in anhydrous THF (10 mL) was slowly added at RT. The mixture was stirred at RT for 30 min and subsequently refluxed for 3 h. The mixture was allowed to cool to RT and successively MeOH (3 mL), H₂O (3 mL) and 12 N HCl (3 mL) was added and the mixture was stirred for another 30 min at RT. The solvent was evaporated and the residue dissolved in H₂O, washed with diethyl ether and the aqueous layer was made alkaline with NaHCO₃ and extracted with diethyl ether. In both diethyl ether layers compound was present and therefore all the organic layers were combined. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and the solvent was evaporated to yield 0.33 g (69.3 %) light yellow solid: mp 141.5-142.5 °C; ¹H NMR (CDCl₃) δ 0.9 (t, 3H, J = 7.3 Hz), 1.5 (q, 2H, J = 7.3 Hz), 2.5-2.6 (m, 2H), 2.8-2.9 (m, 6H), 3.0-3.1 (m, 2H), 6.8 (m, 1H), 6.9-7.0 (m, 3H), 7.1-7.2 (m, 1H), 7.2-7.3 (m, 1H); ¹³C NMR (CDCl₃) δ 10.5, 19.0, 26.4, 26.6, 53.5, 54.4, 54.5, 119.1, 121.8, 123.1, 123.7, 125.1, 126.9, 139.4, 141.7. Anal (C₁₅H₂₁NS₂.ditoluoyl tartaric acid) C, H, N.

**N-n-Propyl-(3-thiophen-2-yl-ethyl)-thiophen-3-yl-acetamide (49).** N-n-Propyl-(3-thiophen-2-yl-ethyl)-amine 47 (1.0 g, 6.0 mmol) was dissolved in dichloromethane (50 mL) and 10 % NaOH-solution (10 mL) and 3-thienyl acetyl chloride (1.0 g, 6.2 mmol) in dichloromethane (20 mL) were added. The reaction mixture was stirred for 2 h at RT. The two layers were separated and the organic layer was washed with 3 N HCl-solution and water, dried over Na₂SO₄ and the solvent was evaporated to yield 1.2 g (85.7 %) yellow oil: ¹H NMR (CDCl₃) δ 0.9 (dt, 3H, J = 7.3 Hz), 1.4-1.7 (m, 2H), 2.7-2.9 (m, 2H), 3.0-3.2 (m, 1H), 3.3-3.4 (m, 1H), 3.5-3.7 (m, 4H), 6.8-7.0 (m, 4H), 7.2-7.3 (m, 2H); ¹³C NMR (CDCl₃) δ 9.7 (C10), 9.9 (C10), 19.3 (C9 or C12), 20.7 (C9 or C12), 26.7 (C9 or C12), 28.0 (C9 or C12), 34.3 (C6), 45.6 (C8 or C11), 46.0 (C8 or C11), 47.6 (C8 or C11), 48.9 (C8 or C11), 119.7, 120.2, 120.4, 120.5, 124.0, 124.3, 124.7, 126.5, 126.8, 127.2, 133.5 (C4 or C13), 133.6 (C4 or C13), 136.8 (C4 or C13), 137.8 (C4 or C13), 169.1 (C7); IR (NaCl) cm⁻¹ 1642 (C=O, amide). Anal C₁₅H₁₉NS₂.½H₂O C, H, N.

**N-n-Propyl-(3-thiophen-2-yl-ethyl)-thiophen-3-yl-ethyl-amine (30).** This compound was synthesised in 67 % yield according to the method used for compound 29: mp 117-119 °C; ¹H NMR (CDCl₃) δ 0.9 (t, 3H, J = 7.3 Hz), 1.5-1.8 (m, 2H), 2.8-3.0 (m, 4H), 3.0-3.2 (m, 4H) 6.9-7.1 (m, 4H), 7.2-7.3 (m, 2H); ¹³C NMR (CDCl₃) δ 10.1, 14.8, 23.0, 53.0, 58.5, 119.9, 124.6, 126.5, 136.7. Anal (C₁₅H₁₉N₂S₂.HCl.½H₂O) C, H, N.

**3-(Dimethylaminomethyl)-2-(trimethylsilylmethyl)thiophene (51).** A solution of trimethylsilylmethylmagnesiumchloride, prepared from magnesium (8.9 g, 0.37 mol), a crystal iodine and chloromethyltrimethylsilane (40.9 g, 0.34 mol), in anhydrous diethyl ether (20 mL)
was added dropwise to a cooled solution of 2-bromo-3-(dimethylaminomethyl)-thiophene (50) (47 g, 0.21 mol), bis(triphenylphosphine)nickel(II)chloride (1.4 g, 2.6 mol-%) in anhydrous diethyl ether (500 mL). After refluxing for 20 h the mixture was cooled and slowly water (160 mL) and aqueous saturated NH₄Cl-solution (160 mL) were added. The two layers were separated and the aqueous layer was extracted with diethyl ether. The combined diethyl ether layers were washed with brine, dried over Na₂SO₄, filtered and the solvent was evaporated to yield 37 g (76 %) of an oil: ¹H NMR (CDCl₃) δ 0.02 (s, 9H), 2.16 (s, 6H), 2.2 (s, 2H), 3.2 (s,2H), 6.9 (s, 2H); ¹³C NMR (CDCl₃) δ -1.7, 18.1, 45.0, 56.5, 119.3, 129.1, 132, 138 (compare ref. 218).

3-(Trimethylammonium)-2-(trimethylsilylmethyl)thiophene iodide (52). To a stirred solution of 3-(dimethylaminomethyl)-2-(trimethylsilylmethyl)thiophene (51) (3 g, 0.013 mol) in acetonitril (10 mL) was added iodomethane (1.3 mL, 0.021 mol). After refluxing for 1 h the mixture was cooled to RT and diethyl ether was added. The mixture was filtered to yield 3.45 g (71%) of a yellow solid compound: ¹H NMR (DMSO) δ 0.0 (s, 9H), 2.5 (s, 2H), 3.0 (s, 9H), 4.4 (s, 2H), 7.1 (d, J = 5.4 Hz, 1H), 7.3 (d, J = 5.4 Hz, 1H); ¹³C NMR (DMSO) δ -1.4, 18.5, 51.7, 60.3, 122.3, 123.1, 130.8, 147.7 (compare ref. 218).

5- And 6-methylcarboxylate-4,5,6,7-tetrahydrobenzo[b]thiophene (53a, b). To a stirred solution of 2-(trimethylsilylmethyl)-3-(trimethylammonium)thiophene iodide (52) (10 g, 0.03 mol) and methylacrylate (136 mL) in acetonitril (270 mL) was added dropwise a solution of tetrabutylammoniumfluoride trihydrate (17.1 g, 0.05 mol) in acetonitril (540 mL) in 2 h. After the addition was complete the solution was concentrated and diethyl ether was added until no more precipitate was formed. The mixture was filtered and the solvent was evaporated. Bulb-to-bulb distillation at 100 °C (0.05 mm Hg) yielded 4.2 g (81 %) oil: ¹H NMR (CDCl₃) δ 1.8-2.0 (m, 1H), 2.15-2.3 (m, 1H), 2.6-3.1 (m, 5H), 3.7 (s, 3H), 6.7 (d, J = 5.1 Hz, 1H), 7.1 (d, J = 5.1 Hz, 1H); 5-isomer ¹³C NMR (CDCl₃) δ 23.9, 26.1, 27.8, 39.6, 51.6, 122.3, 127.2, 133.3, 134.3, 175.3; 6-isomer ¹³C NMR (CDCl₃) δ 24.4, 25.6, 27.1, 40.3, 51.6, 122.3, 127.1, 133.3, 134.3, 175.3; the ratio 6-isomer/5-isomer = 2:1, which was determined by ¹³C NMR; IR (NaCl) cm⁻¹ 1739 (C=O) (compare ref. 218).

4,5,6,7-Tetrahydrobenzo[b]thiophene-5 and 6-carboxylic acid (54a, b). A solution of 5- and 6-methylcarboxylate-4,5,6,7-tetrahydrobenzo[b]thiophene (53a, b) (4.29 g, 22 mmol) in an aqueous 16% NaOH-solution (35 mL) was refluxed for 45 min. After cooling to RT an aqueous 2 N HCl-solution was added until the pH was 1. A white solid was formed which dissolved in dichloromethane. The aqueous layer was saturated with NaCl and extracted with dichloromethane. The combined organic layers were dried over Na₂SO₄, filtered and the solvent was evaporated to yield 3.87 g (97 %) pale yellow solid: mp 82.5-84.5 °C; ¹H NMR (CDCl₃) δ 1.8-2.1 (m, 1H), 2.2-2.3 (m, 1H), 2.7-3.1 (m, 5H), 6.8 (d, J = 5.1 Hz, 1H), 7.1 (d, J = 5.1 Hz, 1H); 5-isomer ¹³C NMR (CDCl₃) δ 23.7, 25.8, 27.5, 39.5, 122.3, 127.2, 133.0, 134.3, 181.5; 6-isomer ¹³C NMR (CDCl₃) δ 24.3, 25.3, 26.8, 40.1, 122.3, 127.1, 133.0, 134.3, 181.2. ¹³C NMR (compare ref. 218) determined a 2:1 ratio of the 6-isomer:5-isomer.
5-And 6-ammonium-4,5,6,7-tetrahydrobenzo[b]thiophene hydrochloride (55a, b). To a solution of mixture 54a, b (4.26 g, 23 mmol) and triethylamine (3.62 g, 24 mmol) in dioxane (120 mL) was added diphenylphosphoryl azide (5.3 mL, 24 mmol) at 5°C. The reaction was stirred overnight at RT. Diethyl ether (300 mL) and water (300 mL) were added to the reaction mixture. After separation of the two layers the organic layer was washed with an 1% NaOH solution, dried over Na₂SO₄ and concentrated under reduced pressure yielding a red residue which was used without purification for the next step. To the residue was added aqueous 1N HCl (110 mL) and dioxane (110 mL) and heated for 2 h at 120 °C. The mixture was allowed to cool to RT and the mixture was basified (pH ~10) with 4N NaOH and extracted with diethyl ether and dried over Na₂SO₄. Filtration and evaporation of the organic solvents gave a brown oil, which dissolved in diethyl ether. Slow addition of an ethereal HCl solution gave a light brown solid. The yield of the obtained mixture of regioisomers (55a, b) was 2.6 g (58%): mp 192.8-194.1 °C. ¹H NMR (CDCl₃) δ 1.5-1.7 (m, 1H), 1.9-2.0 (m, 1H), 2.4-3.0 (m, 4H), 3.1-3.3 (m, 1H), 6.7 (d, 1H, J = 5.1 Hz), 7.0 (d, 1H, J = 5.1 Hz). 5-isomer ¹³C NMR (CDCl₃) δ 21.9, 31.8, 34.0, 45.9, 121.0, 126.0, 132.1, 132.4; 6-isomer ¹³C NMR (CDCl₃) δ 22.4, 31.1, 33.3, 46.4, 120.9, 125.7, 132.1, 132.9; IR (KBr) 3300 cm⁻¹ and 1618 cm⁻¹ (NH₂). ¹³C NMR (compare ref. 218) determined a 2:1 ratio of the 6-isomer:5-isomer.

5-And 6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (34 and 35). To a stirred solution of 5- and 6-ammonium-4,5,6,7-tetrahydrobenzo[b]thiophene chloride (55a, b) (2.5 g, 0.013 mol) and K₂CO₃ (7.05 g, 0.05 mol) in DMF (370 mL) was added 1-iodopropane (9 mL, 0.092 mmol). After stirring for 24 h at 50 °C the solution was poured into water and extracted 5 times with diethyl ether. The combined diethyl ether layers were washed 6 times with brine, dried over Na₂SO₄, filtered and evaporated to yield 3 g (84%). Now it is possible to separate both isomers by column chromatography with as eluent ethyl acetate : Hexane (1: 9). After evaporation of the solvent the isomers were dissolved in anhydrous diethyl ether and 1 N HCl in diethyl ether was added to yield 1.3 g (36%) of the 6-isomer and 650 mg (18%) of the 5-isomer. 6-Isomer: mp 134-135 °C; ¹H NMR (CDCl₃) δ 1.0 (t, J = 7.3 Hz, 6H), 1.8 (q, J = 7.6 Hz, 4H), 2.0-2.2 (m, 1H), 2.3-2.4 (m, 1H), 2.7-3.0 (m, 2H), 3.0-3.4 (m, 6H), 3.7-3.9 (m, 1H), 6.8 (d, J = 5.1 Hz, 1H), 7.2 (d, J = 5.1 Hz, 1H); ¹³C NMR (CDCl₃) δ 9.6, 18.0, 18.2, 23.5, 23.6, 24.7, 52.2, 52.7, 60.5, 123.4, 126.3; Anal (C₁₄H₂₃NS.HCl) C, H, N. 5-Isomer: mp 134-136 °C; ¹H NMR (CDCl₃) δ 1.0 (t, J = 7.2 Hz, 6H), 1.8 (q, J = 7.3 Hz, 4H), 2.0-2.2 (m, 1H), 2.3-2.4 (m, 1H), 2.8-3.0 (m, 2H), 3.0-3.3 (m, 6H), 3.7-3.9 (m, 1H), 6.8 (d, J = 5.1 Hz, 1H), 7.2 (d, J = 5.1 Hz, 1H); ¹³C NMR (CDCl₃) δ 9.6, 18.0, 18.3, 23.1, 23.4, 25.4, 52.2, 52.7, 60.2, 123.4, 126.7, 131, 133; Anal (C₁₄H₂₃NS.HCl.¼H₂O) C, H, N.

5- And 6- (N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydro-benz[b]thiophene (36, 37). To a cooled and stirred solution of 4,5,6,7-tetrahydrobenzo[b]thiophene-5 and 6-carboxylic acid (54a, b) (2.7 g, 0.015 mol) in anhydrous dichloromethane (40 mL) was added under nitrogen dropwise oxalyl chloride (5.9 mL, 8.9 g, 0.068 mol). The mixture was stirred overnight at RT and evaporated to yield 2.9 g (96%) oil of compound 56a, b: ¹H NMR (CDCl₃) δ 1.9-2.2
(m, 1H), 2.3-2.5 (m, 1H), 2.7-3.3 (m, 5H), 6.8 (d, J = 5.0 Hz, 1H), 7.1 (d, J = 5.0 Hz, 1H); 5-isomer $^{13}$C NMR (CDCl$_3$) δ 23.5, 26.3, 28.0, 51.6, 123.0, 127.0, 132.0, 134.3, 176.1; 6-isomer $^{13}$C NMR (CDCl$_3$) δ 24.0, 25.8, 27.4, 52.0, 123.0, 127.0, 131.8, 134.1, 176.1. $^{13}$C NMR determined a 2:1 ratio of the 6-isomer:5-isomer. The reaction product was used for the next step without further purification and analysis.

To a stirred solution of 4,5,6,7-tetrahydrobenzo[b]thiophene-5 and 6-carbanoyl chloride (56a, b) (1.8 g, 8.8 mmol) in dichloromethane (100 mL) was added dropwise a mixture of di-n-propylamine (1.8 mL, 1.3 g, 0.013 mmol) and triethylamine (1.3 mL, 0.95 g, 0.010 mmol) in dichloromethane. After stirring for 3 h at RT the mixture was evaporated and the residue was dissolved in diethyl ether. The diethyl ether layer was extracted 4 times with 4N HCl, dried over Na$_2$SO$_4$ and evaporated. Purification with column chromatography with as eluent ethyl acetate:Hexane (1: 9) yielded 1.66 g (71%) oil of compound 57a, b: $^1$H NMR (CDCl$_3$) δ 0.9 (t, J = 7.4 Hz, 6H), 1.5-1.6 (m, 4H), 1.9-2.0 (m, 2H), 2.7-3.0 (m, 5H), 6.7 (d, J = 5.1 Hz, 1H), 7.0 (d, J = 5.1 Hz, 1H); 5-isomer $^{13}$C NMR (CDCl$_3$) δ 10.9, 20.2, 24.3, 28.3, 30.5, 32.9, 56.7, 60.2, 121.7, 127.5, 135.2, 135.3; Anal (C$_{15}$H$_{25}$NS.¼H$_2$O) C, H, N. 6-isomer: mp 79-81 °C; $^{13}$C NMR (CDCl$_3$) δ 10.9, 20.2, 24.8, 27.6, 29.8, 33.8, 56.7, 60.2, 121.6, 127.2, 135.2, 135.3. Anal (C$_{15}$H$_{25}$NS.C$_4$H$_4$O$_4$) C, H, N.

4-Oxim-4,5,6,7-tetrahydrothianaphthene (59). 226-228 4-Keto-4,5,6,7-tetrahydrothianaphthene (58) (10.0 g, 66.0 mmol) was dissolved in ethanol (120 mL) and water (12 mL). To this solution was added sodium acetate (11 g, 134 mmol) and hydroxylammoniumchloride (8.67 g, 125 mmol). This mixture was refluxed for 3 h and then cooled to RT. Cold water was added and the precipitate obtained was filtered, washed with water and dried: yield 13.06 g (118 %) not pure. Recrystallization of the white precipitate from ethanol gave white crystals; mp 125-127 °C; IR (KBr) 3289 cm$^{-1}$ (C=N); $^1$H-NMR (CDCl$_3$) δ 2.0 (t, 2H, J = 6.3 Hz), 2.8-2.9 (m, 4H), 7.1 (d, 1H, J = 5.3 Hz), 7.3 (d, 1H, J = 5.4 Hz); $^{13}$C-NMR (CDCl$_3$) δ 22.2, 22.6, 24.8, 122.9, 123.1, 131, 143.7, 153.1; Anal (C$_8$H$_9$NOS) C, H, N.
4-Tosyloxim-4,5,6,7-tetrahydrothianaphthene (60). A solution of 4-oxim-4,5,6,7-tetrahydrothianaphthene (59) (4.0 g, 23.9 mmol) in 25 mL pyridine was cooled to about 10°C in an ice-bath. p-Toluene sulfonyl chloride (10.3 g, 53.9 mmol) was added slowly in small portions. This mixture was stirred for 2 h at about 10°C and then 2 h at RT. Then the mixture was poured into ice water. The precipitate obtained was filtered, washed with water and dried. The yield was 7.84 g (100%) not pure. Recrystallization of the white precipitate from ethyl acetate gave white crystals; mp 130-132°C; IR (KBr) 1596 cm⁻¹ (C=N); ¹H-NMR (CDCl₃) δ 1.9 (t, 2H, J = 6.2 Hz), 2.4 (s, 3H), 2.8-2.9 (m, 4H), 7.0 (d, 1H, J = 5.2 Hz), 7.2 (d, 1H, J = 5.3 Hz), 7.4 (d, 2H, J = 8.3 Hz), 7.9 (d, 2H, J = 8.8 Hz); ¹³C-NMR (CDCl₃) δ 21.8, 22.3, 23.8, 24.6, 123.2, 123.4, 128.8, 128.9, 129.5, 133, 145, 148, 159; Anal (C₁₅H₁₅NO₃S₂·½H₂O) C, H, N.

4-Keto-5-amino-4,5,6,7-tetrahydrothianaphthene (61). A solution of potassium tert-butoxide (5.7 g, 50.8 mmol), ethanol (43 mL) and toluene (107 mL) was cooled to 0-5°C. To this solution was added 4-tosyloxim-4,5,6,7-tetrahydrothianaphthene (60) (10.0 g, 31.6 mmol). This mixture was stirred for 2 h at 0-5°C and then stirred for 2 h at RT. The precipitate (potassium tosylate) obtained was filtered and washed with diethyl ether. To the filtrate was added 5 mL 37% HCl. After stirring some time a precipitate arises of the ketamine.HCl 61. The precipitate was filtered and washed with diethyl ether, the yield was 4.5 g (71%) before recrystallization, after recrystallization of the precipitate from ethanol-diethyl ether yellow crystals were obtained; mp 197-199°C; IR (KBr) 3430 cm⁻¹ (NH), ~ 3000 cm⁻¹ (NH), 1600 cm⁻¹, 1500 cm⁻¹ (NH), 1676 cm⁻¹ (C=O); ¹H-NMR (CD₂OD) δ 2.3-2.5 (m, 1H), 2.6-2.7 (m, 1H), 3.3-3.4 (m, 2H), 4.3-4.4 (dd, 1H, J = 13.7 Hz), 7.4 (s, 2H); ¹³C-NMR (CD₂OD) δ 24.6, 30.2, 55.7, 124.9, 126.4, 135, 158, 188.

4-Keto-5-chloroacetamide-4,5,6,7-tetrahydrothianaphthene (62). To a solution of 4-keto-5-amino-4,5,6,7-tetrahydrothianaphthene (61) (5.3 g, 21.1 mmol) in dichloromethane (290 mL) was added a solution of NaOH (7.1 g, 0.18 mol) in water (61 mL). To this stirred mixture was added chloroacetylchloride (5.9 g, 4.2 mL, 51.9 mmol) and the mixture was stirred another 3 h. After the reaction was complete the organic layer was separated. To the aqueous layer was added water (145 mL) and 4 N HCl until the aqueous layer was neutral. The aqueous layer was extracted with dichloromethane (3 x 25 mL). The combined organic layers were washed with brine, dried over Na₂SO₄ and evaporated. Recrystallization from ethyl acetate-hexane yielded 6.0 g (95%) brown crystals; mp 130-132°C; IR (KBr) 3334 cm⁻¹ (NH), 1681 cm⁻¹ (C=O), 1639 cm⁻¹ (C=O, amide); ¹H-NMR (CDCl₃) δ 1.9-2.1 (m, 1H), 2.8-2.9 (m, 1H), 3.15-3.25(m, 1H), 4.1 (s, 2H), 4.5-4.65 (m, 1H), 7.1 (d, 1H, J = 5.3 Hz), 7.3 (d, 1H, J = 5.4 Hz), 7.6 (m, 1H); ¹³C-NMR (CD₂OD) δ 24.6, 31.3, 42.6, 55.9, 124.5, 124.7, 135.6, 155.7, 166.4, 189 Anal (C₁₀H₁₀NO₂SCl) C, H, N.

4-Hydroxy-5-chloroacetamide-4,5,6,7-tetrahydrothianaphthene (63). A solution of 4-keto-5-chloroacetamide-4,5,6,7-tetrahydrothianaphthene (62) (4.0 g, 16.4 mmol) in methanol (90 mL) under nitrogen was cooled to 5-8°C in an ice-bath. While stirring the solution NaBH₄ (1.5 g, 39.6 mmol) was added in portions. The solution was stirred another h at 5-8°C. To the
mixture was added 1 N HCl to remove excess of NaBH₄ and then the solvent was evaporated. Recrystallization of the crude product from ethyl acetate-hexane yielded 3.55 g (88%) brown crystals: mp 148-150 °C; IR (KBr) ~ 3200 cm⁻¹ (OH), ~ 3000 cm⁻¹ (NH), 1646 cm⁻¹ (C=O, amide); ¹H-NMR (CDCl₃) δ 1.85-2.05 (m, 1.5H), 2.15-2.3 (m, 1.5H), 2.8-3.0 (m, 2H), 4.1 (s, 2H), 4.2 (m, 1H), 4.6 (d, 1H, J = 7.17 Hz), 6.7 (br s, 1H), 7.1 (d, 1H, J = 5.0 Hz), 7.2 (d, 1H, J = 5.1 Hz); ¹³C-NMR 23.3, 27.6, 42.9, 54.1, 69.8, 124.1, 127.4, 127.5, 128, 169; Anal (C₁₀H₁₂NO₂SCl) C, H, N.

**trans-2,3,4a,5,6,9b-Hexahydro-4H-thianaph[4,5e][1,4]oxazine-3-one (64).** To a solution of 4-hydroxy-5-chloroacetamide-4,5,6,7-tetrahydrothianaphthene (63) (5.2 g, 21.16 mmol) in isopropanol (275 mL) was added dropwise 50% NaOH solution (3.6 mL) at RT. The solution was stirred for 15 h. After the reaction was complete the solvent was evaporated until almost dry. The suspension was diluted with water (180 mL), neutralised with 10% HCl and extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na₂SO₄ and evaporated. Recrystallization of the crude product from ethyl acetate-hexane yielded 2.0 g (45%) brown crystals; mp 242-244 °C; IR (KBr) 3313 cm⁻¹ (NH), 1638 cm⁻¹ (C=O); ¹H-NMR (CDCl₃) δ 1.9-2.1 (m, 1H), 2.1-2.2 (m, 1H), 2.9-3.0 (m, 2H), 3.6-3.7 (m, 1H), 4.4 (d, 2H, J = 2.7 Hz), 4.47-4.53 (m, 1H), 7.0 (d, 1H, J = 5.2 Hz), 7.2 (d, 1H, J = 5.2 Hz), 7.9 (br s, 1H); ¹³C-NMR 23.5, 27.6, 53.8, 68.3, 76.1, 124.2, 124.5, 133.9, 136.3, 170.2; Anal (C₁₀H₁₁NO₂S) C, H, N.

**trans-N-n-Propyl-2,3,4a,5,6,9b-Hexahydro-4H-thianaph[4,5e][1,4]oxazine (39).** To a solution of trans-2,3,4a,5,6,9b-hexahydro-4H-thianaph[4,5e][1,4]oxazine-3-one (64) (1.3 g, 6.2 mmol) in anhydrous tetrahydrofuran (195 mL) was cooled to about 5 °C. To this solution was added LiAlH₄ (845 mg, 22.3 mmol). This mixture was refluxed for 2 h and then cooled to RT. Then successively water (0.9 mL), 4 N NaOH-solution (0.9 mL) and water (2.7 mL) were added to remove excess of LiAlH₄. The mixture was filtered, washed with diethyl ether and then the filtrate was dried over Na₂SO₄ and evaporated. Recrystallization from isopropanol-anhydrous diethyl ether yielded 245 mg (17%) white crystals; mp 264-266 °C; IR (KBr) 3213 cm⁻¹ (NH), ¹H-NMR (CDCl₃) δ 1.2-1.3 (m, 1H), 2.1-2.3 (m, 1H), 2.4-2.6 (m, 1H), 2.9-3.1 (m, 2H), 3.2-3.3 (m, 1H), 3.3-3.5 (m, 2H), 3.7-3.8 (m, 1H), 3.9-4.0 (m, 1H), 4.8-4.9 (m, 1H), 7.0 (d, 1H, J = 5.4 Hz), 7.3 (d, 1H, J = 5.4 Hz); ¹³C-NMR (CDCl₃) δ 23.9, 26.1, 45.2, 58.0, 64.8, 76.3, 125.1, 125.5, 134.5, 136.5; Anal (C₁₀H₁₃NOS.HCl.¼H₂O) C, H, N.
The mixture was poured into water (20 mL) and extracted with diethyl ether (5 x 20 mL). The combined extracts were washed with brine (6 x 20 mL), dried over Na₂SO₄ and evaporated. The resulting oil was dissolved in anhydrous diethyl ether and diethyl ether saturated with gaseous HCl was added to prepare the HCl-salt in a yield of 94.35 mg (80%): mp 238-240 °C; ¹H-NMR (CDCl₃) δ 1.1 (t, 3H, J = 7.3 Hz), 1.7-2.1 (m, 3H), 2.6-2.8 (m, 1H), 3.0-3.2 (m, 3H), 3.3-3.7 (m, 4H), 4.1-4.2 (m, 2H), 4.8 (d, 1H, J = 9.1 Hz), 7.0 (d, 1H, J = 5.1 Hz), 7.2 (d, 1H, 5.2 Hz); ¹³C-NMR (CDCl₃) δ 10.9, 17.6, 23.4, 24.1, 52.6, 55.4, 65.0, 65.6, 76.7, 125.3, 125.5, 135, 147; Anal (C₁₃H₁₉NOS.HCl) C, H, N.

2.4.2 Distance calculation

Conformational analyses were performed on a Silicon Graphics O₂ Workstation R5000 chipset, running IRIX 6.3. Conformational analyses were performed in MacroModel version 6.5 using the Monte Carlo Multiple Minimum (MCMM) search protocol. All ligands were considered in their protonated, positively charged forms. N-n-propyl groups were truncated to N-methyl groups during the conformational analyses in order to reduce the number of torsion angles. All minimizations were performed within the MM3* force field while simulating a distance-dependent GB/SA water continuum as implemented in MacroModel. Prior to submitting them to the MCMM protocol all ligands were minimised with default options. The starting conformations for the ligands were independently submitted to the MCMM protocol. To search conformational space 1000-5000 MC steps were performed on each starting conformation, dependent on the number of torsion angles. Starting conformations for each step were systematically generated using the SUMM option. The number of torsion angles to be varied in each MC step was set between 2 and n-1, n being the total number of variable torsion angles. Ring closure bonds were defined in the 6-membered non-aromatic rings in order to allow torsion angles within these rings to be varied as well. Ring closure distances were limited to 0.5-2.0 Å. The randomly generated structures were minimised using the Truncated Newton Conjugate Gradient (TNCG) minimizer, allowing for 250 iterations per structure, until an initial gradient of 0.01 kcal/Å mol⁻¹ was reached. Least squares superimposition of all non-hydrogen atoms was used to eliminate duplicate conformations. For non-chiral ligands, specifying the NANT options prevented rejection of mirror images. The minimum energy conformations thus obtained were submitted to a final minimisation, using the Full Matrix Newton Raphson (FMNR) minimiser, allowing for 1000 iterations per structure, until a final gradient of 0.002 kcal/Å mol⁻¹ was reached. An energy cut-off of 12 kcal/mol was applied to the search results. (For ligands containing a ‘chiral’ protonated nitrogen atom, the search results of the independent analyses performed on the starting conformations with inverted nitrogen atoms were combined and subsequently filtered on energy (ΔE ≤ 3.0 kcal/mol) using the filter mode.) After the minimisation the distances were calculated.
2.4.3 Pharmacology

**Cell lines expressing dopamine receptor isoforms.** A cell line expressing the human dopamine D<sub>2L</sub> was purchased from Dr. O. Civelli, Oregon Health Sciences University. The D<sub>2L</sub> receptor cDNA was subcloned into the expression vector, pRc/CMV. The plasmids were transfected by electroporation into CHO K1 cells. A single stable transfectant, resistant to the antibiotic G418, was isolated and selected for use in the binding studies. The human dopamine D<sub>3</sub> receptor cDNA cloned in the pcDNAIneo plasmid was obtained from Dr. K. O’Malley and stably transfected into CHO K1 cells by a modified calcium phosphate precipitation technique<sup>237</sup> and transfectants were selected in G418, isolated and screened for expression of human D<sub>3</sub> receptors by radioligand binding as previously described.<sup>50</sup>

**Cell culture and preparation of cell membranes.** CHO K1 cells expressing either human dopamine D<sub>2L</sub> and D<sub>3</sub> receptors were grown in 162 cm<sup>2</sup> culture flasks in F12 medium (Gibco Laboratories, Grand Island, N.Y., USA) supplemented with 10 % foetal bovine serum (FBS, Hyclone, Logan, UT) in an atmosphere of 5 % CO<sub>2</sub>/95 % air at 37 ºC. Cells were grown until confluent after which growth medium was removed and replaced with 0.02 % EDTA in a phosphate-buffered saline solution (Sigma Chemical Co. St. Louis, MO, USA) and scraped from the flasks. The cells were centrifuged at about 1000 x g for 10 min at 4 ºC and then resuspended in TEM buffer (25 mM Tris-HCl, pH 7.4 at 37 ºC, 1 mM EDTA, and 6 mM CaCl<sub>2</sub>) for D<sub>2L</sub> and D<sub>3</sub> and homogenised with a Brinkman Polytron homogenizer at setting 5 for 10 sec. The membranes were pelleted by centrifugation at 20000 x g at 4 ºC for 20 min, then the pellets were resuspended in appropriate buffer at 1 ml/flask and stored at -70 ºC until used in the receptor binding assay.

**Receptor binding assays: D<sub>2L</sub> and D<sub>3</sub> dopamine receptors.** A cell membrane preparation (400 µL) was incubated in triplicate with 50 µL [³H]N-0437 (2nM for D<sub>2L</sub>) or [³H]spiperone (0.5 nM for D<sub>3</sub>), 50 µL buffer, or competing drugs where appropriate to give a final volume of 0.5 mL. After 60 min incubation at 25 ºC, the incubations were terminated by rapid filtration through Whatmann GF/B glass fibre filters (soaked for 1 hr in 0.5 % polyethyleneimine) on a Brandel MB-48R cell harvester, with 3 washes of 1 mL ice-cold buffer. Individual filter discs containing the bound ligand were placed in counting vials with 4 mL of scintillation fluid (Ready Gel, Beckman Instrument Inc., Fullerton, CA, USA) and then counted in a Beckman LS-6800 liquid scintillation counter at an efficiency of 45 %. Non-specific binding was defined in presence of 1 µM of haloperidol.

**Data calculation.** Saturation and competition binding data were analysed using the iterative non-linear least square curve-fitting Ligand program. In competition experiments, apparent K<sub>i</sub> values were calculated from IC50 values by method of Cheng and Prusoff.<sup>238</sup> Experimental compounds were made up as stock solutions in dimethyl sulfoxide (DMSO). The final concentration of 0.1 % DMSO used in the incubation mixture had no effect on the specific binding. Each observation was carried out in triplicate. To allow these calculations, K<sub>d</sub> values
were measured for the interaction of various ligands with the receptor. These were: 
$[^3\text{H}]$spiperone binding, human D$_3$, 0.15 ± 0.02 (n=3); $[^3\text{H}]$N-0437 binding, human D$_{2L}$, 2.24 ± 0.05, nM (n=3).