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

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2000

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Chapter 3

Studies with a series of 2-substituted tetrahydrobenzo[b]thiophenes as dopamine receptor agents with selectivity for the dopamine D₃ receptor.

Abstract

This study describes the synthesis and in vitro pharmacology of a series of tetrahydrobenzo[b]thiophenes, which are substituted on the 2-position or which have a methylene bridge between the aliphatic ring and the nitrogen atom. 2-Substitution in 6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene leads to compounds with a 50-100-fold selectivity and a moderate to high affinity for the dopamine D₃ receptor. For the 5-isomer the same introduction gives compounds with no affinity for the dopamine D₂ and D₃ receptor. This difference in affinity between the 5- and 6-isomer is not seen in the parent compounds. An explanation could be that for the 6-isomer the 2-substituted derivatives are structurally comparable with the dopamine D₃ receptor preferring agonist 7-OH-DPAT, while the 2-substituted 5-isomers are structurally more comparable with the low-affinity dopamine receptor ligand 6-OH-DPAT. Lengthening of the distance between the sulfur and the nitrogen atom also gives a dopamine D₃ receptor selective ligand. 2-Substitution of this compound leads to inactive compounds.

In a series of tetrahydrobenzo[b]thiophene analogues, we have identified novel selective dopamine D₃ receptor agents, one of which displays a 100-fold selectivity over dopamine D₂ receptors. These results provide information for the development of pharmacophoric models of the dopamine D₂ and D₃ receptor subtypes that can be used for the future development of selective agonists at these receptor subtypes.
Hydroxylated 2-aminotetralins are potent dopamine receptor agonists. Examples of such are 5-hydroxy-2-(N,N-di-n-propylamino)tetralin (5-OH-DPAT, 9) and 7-hydroxy-2-(N,N-di-n-propylamino)tetralin (7-OH-DPAT, 10). These compounds have no clinical utility because of their low oral bioavailability and their short duration of action, due to glucuronidation in the liver and gut. We have synthesised and tested 6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (34) and 5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (35), which are thiophene analogues of hydroxylated 2-aminotetralins. These tetrahydrobenzo[b]thiophenes turned out to possess a higher relative oral bioavailability than 5-OH-DPAT (chapter 4 and ref. 215). However, the affinity for the dopamine receptors is diminished, as compared to 5-OH-DPAT (chapter 2, ref. 239).

**Chart 3.1** Chemical structures of 6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (34), 2-formyl-6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (65), 2-hydroxymethyl-6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (66), 2-carboxaldehyde-6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (67), 5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (35), 2-formyl-5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (68), 2-hydroxymethyl-5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (69), 6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (36), 2-formyl-6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (70), 2-hydroxymethyl-6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (71), 5-hydroxy-2-(N,N-di-n-propylamino)tetralin (5-OH-DPAT, 9), 7-hydroxy-2-(N,N-di-n-propylamino)tetralin (7-OH-DPAT, 10) and 6-hydroxy-2-(N,N-di-n-propylamino)tetralin (6-OH-DPAT, 72).
Some possible reasons for a diminished affinity for the dopamine receptors can be that: I) a sulfur atom is only a weak hydrogen bond acceptor and is not a hydrogen bond donor as is a hydroxyl moiety, II) there is a non-optimal distance between the hydrogen bond forming moieties on the aromatic ring and the nitrogen, III) there are alternative interaction points for the presumed essential atoms in a dopamine receptor.

In order to investigate the structure-activity relationships of tetrahydrobenzo[b]thiophenes, we synthesized a series of 2-substituted tetrahydrobenzo[b]thiophenes and analogues with a methylene moiety between the aliphatic ring and the nitrogen atom to enlarge the distance between the sulfur and the nitrogen atom.

### 3.2 Chemistry

The synthesis of 5- and 6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (34 and 35) has been described previously (Chapter 2). Using a Vilsmeier-Haack reaction, a formyl moiety was introduced on the 2-position of the thiophene ring. An excess of N-methylformanilide was used which could be easily removed by column chromatography. Reduction of the formaldehydes 65 and 68 with NaBH₄ yielded the hydroxymethyl compounds 66 and 69. From compound 65, the aldoxime 67 was synthesized, using hydroxylamine.HCl in ethanol and 5N NaOH-solution.

![Scheme 3.1](image)

Scheme 3.1 Reagents: (a) POCl₃, N-methylformanilide; (b) NaBH₄, EtOH; (c) NH₄OH.HCl, EtOH, 5N NaOH.

The NMR-data showed that reaction c resulted in two products, the syn and the anti-isomers. Chart 3.2 shows that only the syn-isomer possesses the possibility to form an internal hydrogen bond. Due to this intramolecular hydrogen bond are the protons of the thiophene ring
and the carbon atoms of the thiophene ring and the aldoxime moiety chemically non-equivalent for the syn and anti-isomer. The $^{13}$C-NMR showed that the ratio between the two isomers was 1:2. On TLC there was a slight difference between the $R_f$-values, however, it was not possible to separate the isomers by column chromatography. The fast eluting isomer is probably the syn-isomer, since this isomer has less interaction with the column material due to its intramolecular hydrogen bond. The syn-anti mixture was used for pharmacological testing.

![Chart 3.2](image)

**Chart 3.2** The syn and anti-isomers of compound 67.

The synthesis of the 2-substituted 6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophenes is outlined in Scheme 3.2. First the two enantiomers of 6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (36) were separated. Since the affinity resides in the (+)-enantiomer, only the (+)-enantiomer was used for further reactions. The same reaction procedures were used as for the 2-substituted 5- and 6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophenes.

![Scheme 3.2](image)

**Scheme 3.2** Reagents: (a) POCl$_3$, N-methylformanilide; (b) NaBH$_4$, EtOH.

### 3.3 Results and discussion

Tetrahydrobenzo[b]thiophenes 34 and 35 possess moderate affinity for the dopamine receptors. In order to potentially increase their affinity for the dopamine receptors, substituents were introduced on the 2-position of the tetrahydrobenzo[b]thiophenes. The extra interaction point and the fact that these substituents have better hydrogen bond forming capacities than a
Studies with 2-substituted tetrahydrobenzo[b]thiophenes

sulfur atom may lead to compounds with a higher affinity for the dopamine receptors. For 6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (34) this has led to compounds (65, 66, 67) with moderate to high affinity for the dopamine D₃ receptor. However, the affinity for the dopamine D₂ receptor was dramatically decreased. Therefore, these compounds showed a high selectivity for the dopamine D₃ receptor. The introduction of 2-substituents in 5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (35) gave compounds with low to no affinity for the dopamine D₂ and D₃ receptors. Surprisingly, because the parent compounds 34 and 35 show a comparable affinity for the dopamine receptors. An explanation could be that compounds 65, 66 and 67 are structurally comparable with the dopamine D₃-preferring agonist 7-OH-DPAT (10), while compounds 68 and 69 are structurally more comparable with the low affinity dopamine receptor ligand 6-OH-DPAT (72).

Table 3.1 Receptor binding data of various dopamine receptor ligands.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ki (nM)a</th>
<th>D₂L [³H]Spiperone</th>
<th>D₃ [³H]Spiperone</th>
<th>Ratio D₂L/D₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>27b</td>
<td>28</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>65</td>
<td>&gt;10000c,d</td>
<td>40</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>66</td>
<td>968d</td>
<td>9</td>
<td>108</td>
<td>-</td>
</tr>
<tr>
<td>67</td>
<td>&gt;10000c,d</td>
<td>113</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>35</td>
<td>20d</td>
<td>40</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>68</td>
<td>100/11e</td>
<td>50/19e</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>69</td>
<td>100/-3e</td>
<td>50/6e</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(±)-36</td>
<td>3107d</td>
<td>60</td>
<td>52</td>
<td>-</td>
</tr>
<tr>
<td>(+)-36</td>
<td>100/-7</td>
<td>50/43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(–)-36</td>
<td>100/8e</td>
<td>50/17e</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(+)-70</td>
<td>100/-9e</td>
<td>50/6e</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(+)-71</td>
<td>100/18e</td>
<td>50/14e</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7-OH-DPAT b223</td>
<td>34b</td>
<td>0.57</td>
<td>60</td>
<td>38</td>
</tr>
<tr>
<td>PD128907 b223</td>
<td>42b</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-OH-DPAT 98,205</td>
<td>1200c,f</td>
<td></td>
<td></td>
<td></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]N-0437 was used as radiolabeled ligand. c IC50 value instead of Ki. d [³H]NPA. e First number gives the concentration of the tested compound in nM, second number gives the percentage of displacement of radioligand in %. f [³H]dopamine, IC50 is determined in calf caudate nucleus homogenates.
The most potent and selective compound is derivative 66 with a hydroxymethyl moiety on the 2-position. Introduction of an extra interaction point on the 2-position of 6-(N,N-di-n-propyl)aminotetrahydrobenzo[b]thiophene has a negative effect on the affinity for the dopamine D₂ receptor and no or a positive effect on the affinity for the dopamine D₃ receptor.

Also the introduction of a methylene group between the six-membered ring and the nitrogen in 6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (34) gives a compound with a moderate affinity for the dopamine D₃ receptor and no affinity for the dopamine D₂ receptor. This affinity resides in the (+)-enantiomer of the compound. Introduction of substituents on the 2-position of 6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (36) gives compounds (70, 71) without affinity for the dopamine D₂ and D₃ receptors.

Literature data have shown that a phenol or catechol is not essential for binding at the dopamine receptors since a number of non-phenolic compounds possess affinity for the dopamine receptors. Examples of such compounds are pramipexole, non-hydroxylated 2-aminotetralins, conjugated enynes, heterocyclic bioisosteres of 3-OH-N-phenylpiperazine, and indolylicyclohexanes. Recurrent phenomena in all these compounds are a conjugated system and a nitrogen which can be protonated to bind to the receptor.

The selectivity for the dopamine D₃ receptor is difficult to explain, since there is a high homology in the amino acid sequence in the transmembrane domains between the dopamine D₂ and D₃ receptors. Most receptor models developed deal with dopamine D₂-like receptors and make no difference between D₂ and D₃ receptors. Malmberg et al. found that the putative interacting amino acids in the dopamine D₂ and D₃ receptors are not found on identical positions in the receptors.

Also the hypothesis of Hübner et al. does not seem to be the most likely explanation, since there is only a marginal difference in amino acids between the dopamine D₂ and D₃ receptors. They hypothesised that the selectivity of their conjugated enynes could be explained from the fact that at, especially, the dopamine D₃ receptor the ability of the more lipophilic enyne system facilitates hydrophobic interactions.

Despite several reports on dopamine receptor ligands, until now no distinguishing models for the dopamine D₂ and D₃ receptors have been found. Therefore, more knowledge of pharmacophores, interacting points and receptor conformations are necessary to be able to develop distinguishing dopamine D₂ or D₃ receptor models, which can help to develop other selective agents.

In conclusion, in a series of tetrahydrobenzo[b]thiophene analogues, we have identified novel selective dopamine D₃ receptor agents, one of which displays a 100-fold selectivity over dopamine D₂ receptors.
3.4 Experimental Section

3.4.1 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). Chemical shifts are given in δ units (ppm) and are relative to the solvent. Coupling constants are given in Hertz (Hz). The spectra recorded were consistent with the proposed structures. IR spectra were obtained on a ATI-Mattson spectrometer. Electronic ionisation (EI) mass spectra were obtained on a Unicam 610-Automass 150 GC-MS system. 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.

2-Formyl-6-(N,N-di-$n$-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (65). A mixture of phosphorous oxychloride (1.29 g, 8.4 mmol) and N-methylformanilide (1.14 g, 8.4 mmol) was stirred for 0.5 h at RT. Then 6-(N,N-di-$n$-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (34) (1.0 g, 4.2 mmol) was added and the mixture was stirred for 4 h at RT after which it was poured into water. The aqueous layer was extracted with dichloromethane and the combined organic layers were extracted with 4 N HCl. The acidic layer was basified and extracted with dichloromethane. The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The product was purified over a SiO$_2$ column using CH$_2$Cl$_2$: MeOH = 20:1 as the eluent yielding 1.1 g (99 %) of a yellow oil: $^1$H NMR (CDCl$_3$) δ 0.9 (t, 6H, J = 7.3 Hz), 1.4 (q, 4H), 1.6-1.8 (m, 1H), 1.9-2.1 (m, 1H), 2.4-2.5 (m, 4H), 2.6-2.8 (m, 3H), 2.9-3.1 (m, 2H), 7.4 (s, 1H), 9.8 (s, 1H); $^{13}$C NMR (CDCl$_3$) δ 10.3, 20.7, 23.9, 24.1, 26.7, 51.1, 55.5, 135.3, 135.5, 139.4, 146.2, 181.2; IR (NaCl) cm$^{-1}$ 1667 (C=O). A sample was converted to the HCl-salt for analysis, Anal Calcd (Obsd) for C$_{15}$H$_{23}$NOS.HCl.1H$_2$O: C: 56.32 (56.33), H: 8.19 (8.01), N: 4.38 (4.72).

2-Hydroxymethyl-6-(N,N-di-$n$-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (66). To a cooled solution of 2-formyl-6-(N,N-di-$n$-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (65) (200 mg, 0.75 mmol) in ethanol (5 mL) was added dropwise NaBH$_4$ (95 mg, 2.5 mmol) in ethanol (5 mL). After stirring for 30 minutes at RT the solvent was evaporated. The residue was dissolved in water and extracted with dichloromethane. The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The resulting oil was dissolved in anhydrous ether and 1 N HCl in ether was added to yield 144 mg (63%): $^1$H NMR (CDCl$_3$) δ 0.9 (t, 6H, J = 7.3 Hz), 1.5 (q, 4H, J = 7.3 Hz), 1.6-1.8 (m, 1H), 1.9-
Chapter 3

2.1 (m, 1H), 2.4-2.5 (m, 4H), 2.6-2.9 (m, 4H), 3.0-3.1 (m, 1H), 4.7 (s, 2H), 6.6 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 10.4, 20.4, 24.1, 24.3, 25.6, 51.2, 56.3, 58.6, 124.3, 133.2, 139.7; Anal Calcd (Obsd) for C$_{13}$H$_{25}$NOS.0.3 H$_2$O: C: 66.03 (65.80), H: 9.46 (9.31), N: 5.13 (5.15).

2-Cardboxaldehyde-6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (67). To a solution of 2-formyl-6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene.HCl (65) (100 mg, 0.33 mmol) and hydroxylamine (80 mg, mol) in 5 mL ethanol was added 0.8 mL 5 N NaOH-solution. After stirring for 3 h at RT the solvent was evaporated, water was added and extracted with dichloromethane. The organic layer was dried over Na$_2$SO$_4$ and evaporated to yield 90 mg yellow oil (97 %). The compound consisted of a syn and anti-isomer which could not be separated. An analytical sample was converted to the HCl salt. $^1$H NMR (CDCl$_3$) $\delta$ 0.9 (t, J = 7.2 Hz, 6H), 1.5 (q, J = 7.2 Hz, 4H), 1.6-1.8 (m, 1H), 2.0-2.2 (m, 1H), 2.4-2.6 (m, 4H), 2.6-3.2 (m, 5H), 6.8 (s, $\frac{1}{2}$H), 7.0 (s, $\frac{1}{2}$H), 7.5 (s, $\frac{1}{2}$H), 8.1 (s, $\frac{1}{2}$H); $^{13}$C NMR (CDCl$_3$) $\delta$ 11.6, 21.4, 25.0, 25.4, 26.7, 52.5, 57.3, 128.6, 129.4, 131.3, 134.1, 135.0, 140.9, 144.5; IR (NaCl) cm$^{-1}$ 1615 (C=N–OH); The $^{13}$C-NMR showed that the ratio between the two isomers was 1:2. On TLC, with as eluent CH$_2$Cl$_2$:MeOH = 20:1, there was only a slight difference between the R$_f$-values. Anal Calcd (Obsd) for C$_{15}$H$_{24}$N$_2$OS.HCl: C: 56.85 (56.46), H: 7.95 (8.06), N: 8.84 (8.75).

2-Formyl-5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (68). This compound was synthesised in 53 % yield yellow oil according to the method used for compound 65. $^1$H NMR (CDCl$_3$) $\delta$ 0.9 (t, J = 7.3 Hz, 6H), 1.5 (q, 4H, J = 7.1 Hz), 1.6-1.8 (m, 1H), 2.1-2.2 (m, 1H), 2.4-2.6 (m, 4H), 2.6-2.7 (m, 1H), 2.7-3.0 (m, 2H), 3.0-3.2 (m, 2H), 7.3 (s, 1H), 9.8 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 10.3, 20, 21.4, 24.1, 24.0, 24.5, 26.3, 51.1, 55.2, 136.0, 181.2; IR (NaCl) cm$^{-1}$ 1669 (C=O); MS (EIPI) m/e 265 (M+).

2-Hydroxymethyl-5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (69). This compound was synthesised according to the method used for compound 66. Purification over a SiO$_2$ column using CH$_2$Cl$_2$:MeOH=9:1 yielded 146 mg yellow oil (87%). $^1$H NMR (CDCl$_3$) $\delta$ 0.9 (t, 6H, J = 7.3 Hz), 1.5 (q, 4H, J = 7.5 Hz), 1.6-1.8 (m, 1H), 2.0-2.2 (m, 1H), 2.4-2.6 (m, 4H), 2.6-2.8 (m, 3H), 2.9-3.1 (m, 2H), 4.7 (s, 2H), 6.6 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 10.4, 20.3, 23.8, 24.6, 26.3, 51.2, 55.7, 58.6, 124.9; MS (EIPI) m/e 267 (M+).

Resolution of 6-(N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (36). To a solution of 4 g (15.9 mmol) free amine 36 in 60 mL of isopropyl acetate was added dropwise a solution of 6.4 g (15.9 mmol) of (+)-di-p-toluoyl-D-tartaric acid in isopropyl acetate (60 mL). The precipitate was collected and three times recrystallised from ethanol/diethyl ether to give 3.8 g (36.5 %) of the diastereomeric salt (mp 173-174 °C); [$\alpha$]$_{589}^{21}$ = + 114.5°.

This salt was stirred with 1 N NaOH and 50 mL chloroform was added to the suspension. The layers were separated and the aqueous layer extracted with chloroform. The organic layers were washed with water, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting oil was converted to the HCl salt. The product was dried in vacuo to afford 1.4 g (30.6
Studies with 2-substituted tetrahydrobenzo[b]thiophenes

% of (+)-12. HCl as a white solid. An analytical sample was recrystallized from isopropanol-ether; mp 112-113 °C, $[\alpha]^{21}_{D} = +62.4^\circ$ (c 0.1, MeOH).

The filtrate of the salt formation and the filtrate of the first recrystallization were combined. After evaporation under vacuo and treatment of the residue with NaOH solution gave impure (–)-amine. This amine (2.0 g), 3.2 g (–)-di-p-toluoyl-L-tartaric acid and 400 mL of methanol was heated to solution. According to the procedure as described above, 1.3 g (28.4 %) of (–)-13. HCl was obtained with (mp 113-114 °C); $[\alpha]^{21}_{D} = -62.5^\circ$.

(+)-2-Formyl-6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (70). This compound was synthesised according to the method used for compound 65. Purification over a SiO₂ column using CH₂Cl₂:MeOH=9:1 yielded 421 mg crude product. 156 mg was purified twice over a SiO₂ column with as eluent CH₂Cl₂:MeOH=20:1 yielding 32 mg (13 %) pure and 50 mg (21 %) not completely pure yellow oil. ¹H NMR (CDCl₃) $\delta$ 0.9 (t, 6H, J = 7.2 Hz), 1.3-1.6 (m, 5H), 1.9-2.1 (m, 2H), 2.2-2.5 (m, 7H), 2.6-2.8 (m, 1H), 2.8-2.9 (m, 1H), 2.9-3.1 (m, 1H), 7.3 (s, 1H), 9.8 (s, 1H); ¹³C NMR (CDCl₃) $\delta$ 10.4, 18.7, 23.2, 25.7, 29.3, 55.2, 58.3, 135.8, 181.3; IR (NaCl) cm⁻¹ 1682 (C=O); MS (EIPI) m/e 279 (M⁺).

(+)-2-Hydroxymethyl-6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (71). This compound was synthesised according to the method used for compound 66 starting with 0.25 g crude 2-formyl-6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (70). Purification over a SiO₂ column using CH₂Cl₂:MeOH=9:1 yielded 57 mg yellow oil. ¹H NMR (CDCl₃) $\delta$ 0.9 (t, 6H, J = 7.2 Hz), 1.3-1.6 (m, 5H), 1.9-2.1 (m, 3H), 2.3-2.7 (m, 6H), 2.7-3.0 (m, 3H), 4.7 (s, 2H), 6.6 (s, 1H); ¹³C NMR (CDCl₃) $\delta$ 10.2, 23.2, 26.2, 26.8, 28.6, 29.2, 54.8, 58.6, 64.3, 124.6; MS (EIPI) m/e 281 (M⁺).

3.4.2 Pharmacology

For the compounds 6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (34), 2-formyl-6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (65), 2-hydroxymethyl-6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (66), 2-carboxaldoxim-6-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (67), 5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (35) and (±)-6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene ((±)-36) the binding experiments were performed according to the method described in Chapter 2.

For the compounds 2-formyl-5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (68), 2-hydroxymethyl-5-(N,N-di-n-propyl)amino-4,5,6,7-tetrahydrobenzo[b]thiophene (69), (±)-6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydro-benzo[b]thiophene ((±)-36), (–)-6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (–)-36), (+)-2-formyl-6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (70) and
(+)-2-hydroxymethyl-6-(N,N-di-n-propyl)aminomethyl-4,5,6,7-tetrahydrobenzo[b]thiophene (71) the binding experiments were performed by Lundbeck, Denmark.

\[^{3}H\text{Spiperone (D}_2\text{ binding)}\] By this method the inhibition of drugs of the binding of \[^{3}H\text{Spiperone (0.5 nM, K}_d\text{ 0.20 nM)}\] to dopamine D\textsubscript{2} receptors in membranes from rat corpus striatum is determined in vitro. Method and results are described by Hyttel and Larsen.\textsuperscript{245}

\[^{3}H\text{Spiperone (D}_3\text{ binding)}\] By this method the inhibition by drugs of the binding of \[^{3}H\text{Spiperone (0.3 nM, K}_d\text{ 0.45 nM)}\] to membranes of human cloned dopamine D\textsubscript{3} receptors expressed in CHO-cells is determined in vitro. Method modified from R.G. MacKenzie et al.\textsuperscript{50}

CHO-cells expressing the human cloned D\textsubscript{3} dopamine receptor are harvested and the cell suspension centrifuged at 1000 rpm for 7 min at 4°C. The supernatant is frozen. At the day of experiment the cell pellet is thawed at room temperature and diluted in assay buffer (25 Mm TRIS-HCl pH 7.4 + 6.0 mM MgCl\textsubscript{2} + 1.0 mM EDTA) to the desired concentration.

50 µl Displacer (10 µM Haloperidol, test compound or assay buffer) and 230 µl buffer is added to a 96 well deep plate. Then 50 µl 0.3 nM \[^{3}H\text{spiperone is added. The reaction is initiated by addition of 670 µL membrane suspension (test concentration 26 µg protein/670 µl). Packard GF/C unifilter (96 well) is pretreated with 0.1 % PEI-solution 10-15 min before filtration. After 60 min. of incubation at 25°C the reaction is terminated by filtration at Tomtec unifilter. The filters are washed twice with ice cold assay buffer. The filters are dried for 1.5 hours at 50°C, 35 µl scintillation liquid is added and bound radioactivity is counted in Wallac Tri-Lux scintillation counters.