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

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Chapter 4

Thiophene analogues of naphthoxazines and 2-aminotetralins: bioisosteres with improved relative oral bioavailability, as compared to 5-OH-DPAT*

Abstract

In the present study, a series of thiophene analogues of 2-aminotetralins and hexahydronaphthoxazines were studied in vivo for their ability to decrease striatal dopamine release, their effects on locomotor activity, and their behavioural characteristics in reserpinised rats, in order to investigate whether a thiophene moiety can act as a bioisostere for the phenol moiety.

In general, the new compounds showed lower in vivo activities than 5-hydroxy-2-(N,N-di-n-propylamino)tetralin (5-OH-DPAT). However, the introduction of the thiophene moiety gave a significant improvement of the relative oral bioavailability, as compared to 5-OH-DPAT.

Our results suggest that the thiophene moiety can act as a bioisostere for a phenol group in hydroxylated 2-aminotetralins. For the hexahydrothianaphthoxazines it was not possible to discriminate between bioisosterism for a phenyl or a phenol moiety. The tetrahydrobenzo[b]thiophenes could be used as lead compounds for the development of novel dopamine receptor ligands with improved relative oral bioavailability.

Chapter 4

4.1 Introduction

The pharmacological importance of the 2-aminotetralin structure has been known for a long time. Initially, aminotetralins were characterised by their sympathomimetic action, i.e. the induction of mydriasis, contraction of the uterus, changes in blood pressure and respiration, and increased intestinal motility in in vivo experiments. During the late sixties central dopamine receptor activity of 2-aminotetralins was identified, which led to active synthesis programs around the world.

The 2-aminotetralin structure has proven to be a valuable structural base, not only for the development of dopamine receptor ligands, but also for the development of serotonin receptor and adrenoceptor ligands, as well as compounds that interact with melatonin receptors. The position of the aromatic hydroxyl group appeared to determine the kind of activity of the 2-aminotetralins, namely, 5- and 7-hydroxy-2-(N,N-di-n-propylamino)tetralin (5- and 7-OH-DPAT) are potent dopamine receptor ligands while 8-hydroxy-2-(N,N-di-n-propylamino)tetralin (8-OH-DPAT) is a very potent and selective serotonin receptor ligand.

In a number of different in vitro and in vivo models it has been shown that 5-OH-DPAT (9) is a very potent dopamine receptor agonist, which has affinity for both the dopamine D₂ and the dopamine D₃ receptors. Another potent dopamine D₂/D₃ receptor agonist is 5-hydroxy-2-(N-n-propyl-N-2-(2-thienyl)ethylamino)tetralin (N-0437, 31), which has reached the clinical stage as an anti-Parkinson agent. However, its use is limited to subcutaneous and intravenous administration because of its low oral bioavailability. This accounts for all the hydroxylated 2-aminotetralins, since they undergo considerable inactivation by glucuronidation in the gut and the liver. Therefore, for many years, the identification of bioisosteric catechol and phenol replacements has been emphasised. Neither the catecholic nor the phenolic hydroxyl groups appear to be an absolute requirement for potent dopamine receptor activity, as illustrated by the action of pramipexole (19), a benzothiazole analogue of the 2-aminotetralins, which is presently on the market as a therapeutic agent for Parkinson’s disease. Also, Andén and co-workers showed that the aminothiazolazepine derivative 5,6,7,8-tetrahydro-6-(2-propenyl)-4H-thiazolo[4,5-d]azepin-2-amine (BHT920, 32) is a dopamine receptor agonist, with α-adrenoceptor properties.
In an attempt to circumvent the problem of intensive first-pass metabolism, 6- and 5-(N,N-di-n-propylamino)tetrahydrobenzo[b]thiophenes (34, 35) were synthesised. These two compounds possess moderate to high affinity for both the dopamine D₂ and D₃ receptor (Table 4.1). Tricyclic compounds like trans-9-hydroxy-4-(n-propyl)-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (PHNO, 27a) and hydroxylated octahydrobenzo[f]quinolines (77 and 78) also possess high affinity for the dopamine D₂ and D₃ receptor, but they display the same problem as hydroxylated 2-aminotetralins, they undergo considerable glucuronidation in the liver due to the phenol moiety.¹⁶¹ Since these tricyclic compounds could be of interest, trans-2,3,4a,5,6,10b-hexahydro-4H-thianaphth[4,5e][1,4]oxazine (38) and trans-N-n-propyl-2,3,4a,5,6,10b-hexahydro-4H-thianaphth[4,5e][1,4]oxazine (39) were synthesised. Compounds 38 and 39 possessed negligible and low affinity, respectively for the dopamine D₂ and D₃ receptor (Table 4.1).
Chemical structures of 8-hydroxy-2-(N,N-di-\(n\)-propylamino)tetralin (8-OH-DPAT, 74), 5-hydroxy-2-(N,N-di-\(n\)-propylamino)tetralin (5-OH-DPAT, 9), 6-(N,N-di-\(n\)-propylamino)tetrahydrobenzo[b]thiophene (34), 5-(N,N-di-\(n\)-propylamino)tetrahydrobenzo[b]thiophene (35), \textit{trans}-2,3,4a,5,6,10b-hexahydro-4\(H\)-thianaphth[4,5\(e\)][1,4]oxazine (38), \textit{trans}-N-\(n\)-propyl-2,3,4a,5,6,10b-hexahydro-4\(H\)-thianaph[4,5\(e\)][1,4]oxazine (39), \textit{trans}-9-hydroxy-4-(\(n\)-propyl)-2,3,4a,5,6,10b-hexahydro-4\(H\)-naphth[1,2\(b\)][1,4]oxazine (27a), \textit{trans}-7-hydroxy-4-\(n\)-propyl-2,3,4a,5,6,10b-hexahydro-4\(H\)-naphth[1,2\(b\)][1,4]oxazine (75), \textit{trans}-4-\(n\)-propyl-2,3,4a,5,6,10b-hexahydro-4\(H\)-naphth[1,2\(b\)][1,4]oxazine (76), \textit{trans}-9-hydroxy-4-\(n\)-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[f]quinoline (77), \textit{trans}-7-hydroxy-4-\(n\)-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[f]quinoline (78).
Table 4.1. Binding affinities of some dopamine receptor compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
<th>K_i (nM)</th>
<th>D_{2L}</th>
<th>D_{3}</th>
<th>5-HT_{1A}</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-0437 (31)</td>
<td>223</td>
<td>0.06^a</td>
<td>4.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7-OH-DPAT (10)</td>
<td>247</td>
<td>34^a</td>
<td>1.4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pramipexole (19)</td>
<td>155</td>
<td>2.07^d</td>
<td>0.49^d</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5-OH-DPAT (9)</td>
<td>248</td>
<td>6^a</td>
<td>0.66</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8-OH-DPAT (74)</td>
<td>249</td>
<td>3200^b</td>
<td>250</td>
<td>0.9</td>
<td>–</td>
</tr>
<tr>
<td>R-DPAT (R-73)</td>
<td>241</td>
<td>32^c</td>
<td>33</td>
<td>12</td>
<td>–</td>
</tr>
<tr>
<td>S-DPAT (S-73)</td>
<td>241</td>
<td>5.5^c</td>
<td>35</td>
<td>38</td>
<td>–</td>
</tr>
<tr>
<td>34</td>
<td>239</td>
<td>27^b</td>
<td>28</td>
<td>80</td>
<td>–</td>
</tr>
<tr>
<td>35</td>
<td>239</td>
<td>40^b</td>
<td>20</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>38</td>
<td>239</td>
<td>&gt;4780^b</td>
<td>3003</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>39</td>
<td>239</td>
<td>631^b</td>
<td>237</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>27a</td>
<td>206</td>
<td>2.8^e</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>75</td>
<td>206</td>
<td>80^e</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>76</td>
<td>206</td>
<td>110^e</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>


To determine whether a thiophene moiety can act as a bioisostere for a phenol moiety compounds 34, 35, 38, 39 were tested for their effects on dopamine release using the microdialysis technique in freely moving rats. The effects of compounds 34 and 35 were compared with the effects of the prototypic dopamine receptor agonist 5-OH-DPAT. On the basis of structural similarities it was thought that compound 34 could be related to 8-OH-DPAT and compound 35 to 5-OH-DPAT. However, compound 34 also showed affinities for the dopamine D$_2$ and D$_3$ receptors and therefore this compound was also studied for its effects on dopamine release. Since all hydroxylated 2-aminotetralins possess a free phenolic hydroxyl group they are prone to conjugation reactions. However, there is a difference to what extent the compound is glucuronidated depending on the position of the hydroxyl moiety. In this study the compounds are compared with 5-OH-DPAT which is the least glucuronidated of the isomeric dopaminergic monophenolic 2-aminotetralins. The relative oral bioavailabilities of compounds 34 and 35 were determined. No such estimation was made for compounds 38 and 39 since these compounds displayed limited affinity for the dopamine D$_2$ and D$_3$ receptors. In addition, compounds 34 and 35 tested for their locomotor activity and dopamine and serotonin receptor behavioural characteristics in reserpinised rats.
4.2 Materials and methods

4.2.1 Animals

Male Wistar rats (from CDL, Groningen, The Netherlands) weighing 280-320 g were used for microdialysis experiments and rats weighing 180-220 g for the locomotor activity and behavioural characteristics experiments. The rats were housed in Plexiglas cages, eight animals in each cage, with free access to water and food. The cages were placed in a room with controlled environmental conditions (21 °C; humidity 60-65%; lights on at 8 a.m. and off at 8 p.m.). The animals were housed at least one week after arrival prior to surgery and use in the experiments. Animal procedures were conducted in accordance with guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and all protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

4.2.2 Drug treatment

The drugs were dissolved in saline and stored in a concentration of 100 µmol/ml for subcutaneous (s.c.) and 50 µmol/ml for per oral (p.o.) administration and diluted, if necessary, with saline before administration. A volume of 1 ml/kg was administered for s.c. administration and 2 ml/kg for p.o. administration. The drugs that were used were 6-(N,N-di-n-propylamino)tetrahydrobenzo[b]thiophene (34), 5-(N,N-di-n-propylamino)tetrahydrobenzo[b]thiophene (35), 5-hydroxy-2-(N,N-di-n-propylamino)tetralin (5-OH-DPAT, 9), trans-2,3,4a,5,6,10b-hexahydro-4H-thianaphth[4,5e][1,4]oxazine (38) and trans-N-n-propyl-2,3,4a,5,6,10b-hexahydro-4H-thianaphth[4,5e][1,4]oxazine (39). All five drugs were synthesised at the Department of Medicinal Chemistry in Groningen.

4.2.3 Surgery and brain microdialysis

On-line brain microdialysis in freely moving animals has previously been described. In brief, the rats were anaesthetised with midazolam (5 mg/kg s.c.), atropine nitrate (0.1 mg/kg s.c.), ketamine (50 mg/kg i.p.) and xylazine (8 mg/kg i.p.); 10% lidocaine was locally applied. The rats were then mounted into a stereotoxic frame (Kopf). The incisor bar was placed in position so that the skull was held horizontal. 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 tube (ID: 0.22 mm; OD: 0.31 mm) was prepared from polyacrylonitrile/sodium methallyl sulphonate copolymer (AN 69, Hospal, Bologna, Italy). The microdialysis membrane was implanted in the striatum. The dura was removed with a sharp needle. Two anchor screws were positioned in different bone plates nearby. The following co-ordinates 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 successively with ultra pure water, methanol, ultra pure water and Ringer solution (1.2 mM Ca\(^{2+}\)). 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 buprenorphine (0.1 mg/kg i.m.), an analgesic agent. The rats 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 CaCl\(_2\), 1.1 mmol/l MgCl\(_2\)) at 2 µl/min (CMA/102 microdialysis pump, Sweden).

Dopamine was quantitated by high-performance liquid chromatography (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 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).

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.

4.2.4 Locomotor activity as monitored in automated cages and behavioural characteristics

Reserpine (10 mg/kg s.c.) was administered 18 h prior to the start of the experiments. On the day of the experiments the animals were placed alone in Plexiglas boxes during a period of 15 min for habituation. Subsequently, the test compounds were administered subcutaneously. The locomotor activity was registered during a period of 120 min using AUTOMEX II activity monitors (Columbus Instruments, Columbus, OH, USA).

During a period of 60 min the behaviour of the rats was scored manually every 5 minutes. The behaviour scored was repeated sniffing, repeated licking and rearing as dopamine receptor stereotyped behaviour and flat body posture and lower lip retraction as indications of the 5-hydroxytryptamine (5-HT, serotonin) behavioural syndrome. The behaviour was scored when it lasted for more than half the observation period. The effects of the compounds were compared to a saline-treated control group.

4.2.5 Statistics

Data of the microdialysis experiments were converted into percentage of the basal levels. The basal levels were determined from four consecutive samples (less than 20% variation), and set at 100%. During a period of 165 min after administration of the test compound the dopamine
release was measured. Microdialysis data were analysed using one-way Analysis of Variance (ANOVA) for repeated measurements, followed by Dunnett’s Method post-hoc test. The relative oral bioavailabilities were determined by comparing the Area Under the Curves (AUCs) after p.o. and s.c. administration. When the AUCs were not significantly different, the relative oral bioavailability, as expressed in percent, was determined by dividing the s.c. dose by the p.o. dose and multiplying by 100. Statistical analysis of the AUCs was performed by a t-test. The data of the locomotor activity experiments were analysed using Two Way Repeated Measures ANOVA on One Factor Balanced Design, followed by Student-Newman-Keuls Method post-hoc test. In all cases a significance level of 0.05 was applied.

4.3 Results

4.3.1 In vivo microdialysis

The basal dialysate concentrations in the striatum for the experiments were $11.1 \pm 0.96$ fmol/min ($n = 62$).

The results of the microdialysis experiments of the compounds 34, 35, 5-OH-DPAT, 38 and 39 are shown in Figure 4.1-4.4. S.c. administration of all compounds, except compound 38, induced a dose-dependent and significant decrease in the release of dopamine in the striatum. Furthermore, compounds 34, 35 and 5-OH-DPAT also induced a significant decrease in the release of dopamine in the striatum after p.o. administration. Effects of compounds 38 and 39 were not studied upon p.o. administration.

The significant decrease in dopamine release induced by s.c. administration of a dose of 0.1 µmol/kg of compound 34 lasted from $t = 30$ min to $t = 45$ min with a maximum decrease of 20 % of control values. For a dose of 1 µmol/kg this was from $t = 30$ min to $t = 105$ min with a maximum decrease of 60 % of control values and for a dose of 10 µmol/kg the significant decrease lasted from $t = 30$ min to $t = 165$ min with a maximum of 75 % of control values (Figure 4.1A). Figure 4.1B shows that the significant decrease after p.o. administration of compound 34 in a dose of 1 µmol/kg lasted from $t = 15$ min to $t = 60$ min with a maximum decrease of 25 % and in a dose of 10 µmol/kg from $t = 30$ min to $t = 135$ min with a maximum decrease of 40 % of control values.
A

**Figure 4.1** Effect of s.c. (A) and p.o. (B) administration of 6-(N,N-di-n-propylamino)tetrahydrobenzo[b]thiophene (34) on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. (n = 4). * P<0.05 (Dunnett’s test).

Compound 35, upon s.c. administration, induced a significant decrease in dopamine release of maximally 35 %, 55 %, and 65 % after doses of 1, 10 and 30 µmol/kg, respectively (Figure 4.2A). The decrease induced by a dose of 1 µmol/kg lasted only 30 min, while the doses of 10 and 30 µmol/kg both induced decreases in dopamine release that lasted until 165 min after administration. The significant effect of administration of a dose of 10 µmol/kg p.o. and a dose
of 30 µmol/kg p.o. of compound 35 lasted from t = 30 min to t = 90 min for both doses with a maximum decrease of 50 % and 60 % of control values, respectively (Figure 4.2B).

Figure 4.2  Effect of s.c. (A) and p.o. (B) administration of 5-(N,N-di-n-propylamino)tetrahydrobenzof[b]-thiophene (35) on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. (n = 4). * P<0.05 (Dunnett’s test).

For comparison, administration of a s.c. dose of 0.1 µmol/kg and a p.o. dose of 10 µmol/kg of 5-OH-DPAT (9) induced very similar effects. Both treatments induced a significant decrease in dopamine release from 15 to 165 min with a maximum decrease in dopamine release of 70 %
and 75% of control values for a s.c. dose of 0.1 µmol/kg and a p.o. dose of 10 µmol/kg, respectively (Figure 4.3).

Figure 4.3  Effect of s.c. and p.o. administration of 5-OH-DPAT on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. (n = 4). * P<0.05 (Dunnett’s test).

Figure 4.4 shows that \textit{trans}-2,3,4a,5,6,10b-hexahydro-4\textit{H}-thianaphth[4,5\textit{e}][1,4]oxazine (38) had no significant effect on the release of dopamine in the striatum in a dose of 100 µmol/kg s.c.

Compound 39 in a s.c. dose of 1 µmol/kg had no effect on the release of dopamine, while s.c. administration of 10 µmol/kg induced a significant decrease in the release of dopamine in the striatum from t = 30 min to t = 60 min with a maximum decrease of 40%. The significant effect of s.c. administration of 50 µmol/kg lasted from t = 15 min to t = 150 min with a maximum of 65% (Figure 4.4).
Figure 4.4 Effect of s.c. administration of trans-2,3,4a,5,6,10b-hexahydro-4H-thianaphth[4,5e][1,4]-oxazine (38) and trans-N-n-propyl-2,3,4a,5,6,10b-hexahydro-4H-thianaphth[4,5e][1,4]oxazine (39) on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. (n = 4). * P<0.05 (Dunnett’s test).

The relative oral bioavailabilities, as determined by comparing the AUC after s.c. and p.o. administration, of 6-(N,N-di-n-propylamino)tetrahydrobenzo[b]thiophene (34), 5-(N,N-di-n-propylamino)tetrahydrobenzo[b]thiophene (35) and 5-OH-DPAT (9) were calculated from Figures 4.1-4.3, and are shown in Table 4.2. For compounds 34 and 35 the relative oral bioavailabilities were ≥ 10 %, while for the reference compound 5-OH-DPAT it was 1 %. In order to verify the fact that the decrease induced by a dose of 10 µmol/kg p.o. was not already induced by a lower dose, we have found that a dose of 1 µmol/kg p.o. of 5-OH-DPAT induced a decrease in the release of dopamine in the striatum of only 50-55 %. Furthermore, microdialysis experiments in our laboratory with the (−)-enantiomer of 5-OH-DPAT also showed that the relative oral bioavailability was about 1-3 % (Chapter 7).
Table 4.2 AUCs of the microdialysis experiments of 6-(N,N-di-n-propylamino)tetrahydrobenzo[b]thiophene (34), 5-(N,N-di-n-propylamino)tetrahydrobenzo[b]thiophene (35) and 5-OH-DPAT (9) after s.c. and p.o. administration.

<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>10</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>
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<td></td>
<td>10</td>
<td>6700 ± 800</td>
<td>30</td>
</tr>
<tr>
<td>35</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.

4.3.2 Locomotor activity in reserpinised rats

Both compounds 34 and 35 induced a significant increase in locomotor activity in reserpinised rats (Figure 4.5). The basal level of locomotor activity of reserpinised rats is maximally 48.5 ± 18.8 counts per 15 min (n = 4) as measured upon s.c. administration of saline. When comparing their maximum effect on locomotor activity as measured by the number of counts over 15 min, compound 34, in a dose of 10 µmol/kg, induced an increase in locomotor activity to 600 counts/15 min, which returned to basal levels after 60 min. In a dose of 30 µmol/kg the maximum effect was 750 counts/15 min, which returned to basal levels after 90 min. The effects of compound 35 in the same doses were less pronounced than those for compound 34. In a dose of 10 µmol/kg compound 35 induced an increase in locomotor activity to maximally 300 counts/15 min and in a dose of 30 µmol/kg the maximum effect was 650 counts/15 min The latter returned to basal levels after 105 min.
4.3.3 Behaviour in reserpinised rats

Table 4.3 shows that s.c. administration of compound 34 induced both dopamine receptor stereotyped behaviour (sniffing, licking and rearing) and a 5-HT behavioural syndrome (flat body posture and lower lip retraction) in the reserpinised rats. Administration of compound 35 induced dopamine receptor but no serotonin receptor behaviour.

Table 4.3 Behaviour after s.c. administration of the tetrahydrobenzo[b]thiophenes 34 and 35 represented as the number of animals which showed the behaviour of the total number of animals used in the experiment.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose µmol/kg s.c.</th>
<th>Sniffing</th>
<th>Licking</th>
<th>Rearing</th>
<th>Flat body posture</th>
<th>Lower lip retraction</th>
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<tr>
<td>34</td>
<td>10</td>
<td>4/4</td>
<td>0/4</td>
<td>0/4</td>
<td>2/4</td>
<td>2/4</td>
</tr>
<tr>
<td>34</td>
<td>30</td>
<td>4/4</td>
<td>2/4</td>
<td>3/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>35</td>
<td>10</td>
<td>4/4</td>
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<td>30</td>
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</table>
4.4 Discussion

In the present study we investigated the effects of the bioisosteric replacement of a phenol moiety by a thiophene moiety. The effects of the compounds on dopamine release were determined using the microdialysis technique in freely moving rats. Systemic administration of compounds 34, 35 and 39 but not 38 induced a decrease in the release of dopamine in the striatum, which results from the dopamine receptor agonistic properties of the compounds, as the release of dopamine is under the control of dopamine autoreceptors.\textsuperscript{189} Compound 39 was less effective compared to compounds 34 and 35 in decreasing dopamine release in the striatum and compound 38 was without effect, which is in line with the differences of the four compounds in binding affinities found for the dopamine D\textsubscript{2} and D\textsubscript{3} receptor. All compounds were less potent than 5-OH-DPAT, again in agreement with the higher affinity at the dopamine D\textsubscript{2} and D\textsubscript{3} receptors of the latter compound. The role of the dopamine D\textsubscript{3} receptor as an autoreceptor is still under debate,\textsuperscript{61,64} while it is generally accepted that the dopamine D\textsubscript{2} receptor functions as an autoreceptor.\textsuperscript{63}

The affinity of compounds 34 and 35 for the dopamine D\textsubscript{2} and D\textsubscript{3} receptors is lower than that of 5-OH-DPAT, probably due to the fact that the sulfur atom in the thiophene ring is only a weak hydrogen bond acceptor unlike the hydroxyl moiety of a phenol, which is a strong hydrogen bond acceptor and donor. The fact that compound 39 was more effective compared to compound 38 is most likely due to a better fit into the receptor of the n-propyl substituent of compound 39 than the hydrogen of compound 38.

For a compound to display dopamine receptor activity the distance between the nitrogen atom and the H-bond forming group is of importance. Previous studies indicated that the distance between the nitrogen and the hydroxyl moiety in dopamine receptor agents should be between 5.5 and 7.4 Å.\textsuperscript{91,108} For 5-OH-DPAT and 8-OH-DPAT the distance between the nitrogen and the hydroxyl moiety in a minimised conformation using the computer program MacroModel is 6.6 and 5.2 Å, respectively (Chapter 2). For 5-OH-DPAT this has been formerly published by Malmberg et al.\textsuperscript{115} This difference in distances might explain the difference in dopamine receptor activity of the two compounds, i.e. 5-OH-DPAT fits into the dopamine receptor, while the distance in 8-OH-DPAT seems to be too small. The distances between the sulfur and the nitrogen atom of compounds 34 and 35 in a minimised conformation are 5.4 and 6.0 Å, respectively (Chapter 2). This might explain why compound 34 displays dopamine receptor activity beside its serotonin receptor activity different than 8-OH-DPAT.

The relative oral bioavailability of compounds 34, 35 and 5-OH-DPAT was determined by comparing the effects on the dopamine output after s.c. and p.o. administration, i.e. applying a pharmacodynamic method. Compounds 34 and 35 showed relative oral bioavailabilities of about 10 % and >10 %, respectively. The reference compound 5-OH-DPAT had a relative oral bioavailability of about 1 % (Table 4.2). Thus, the structural changes did influence the oral bioavailability in a positive manner. For hydroxylated 2-aminotetralins glucuronidation is the
main route of metabolism. The thiophene ring is not a target for glucuronidation, which most likely explains the higher relative oral bioavailability of compounds 34 and 35, as compared to 5-OH-DPAT.

The effects of compounds 34 and 35 on postsynaptic dopamine receptors were determined using a locomotor activity measure and looking at the behavioural characteristics after administration of the drugs. Compounds 34 and 35 induced a significant increase in locomotor activity in reserpinised rats, which again confirms that these compounds are dopamine receptor agonists. The behavioural scoring (Table 4.3) showed that compound 34 induced dopamine receptor stereotyped behaviour (sniffing, licking and rearing), as well as the 5-HT behavioural syndrome (flat body posture and lower lip retraction). Compound 35, on the other hand, only induced dopamine receptor activity. Thus, the behavioural models confirm that both compounds are active at postsynaptic dopamine receptors. In the microdialysis experiments and in the locomotor activity experiments compound 34 was in low doses more potent than compound 35. The behavioural scoring, however, does not show this difference in potency. It is speculated that this might have been caused by the fact that the serotonergic activity of compound 34 attenuated the dopamine receptor activity of this compound which was not the case for compound 35.

Bioisosteres are groups or molecules, which have chemical and physical similarities producing broadly similar biological effects. The substitution of -CH=CH- by -S- in aromatic rings has been one of the most successful applications of classical isosterism. Since the dopamine D2 and D3 receptor binding affinity of compounds 34 and 35 are comparable to DPAT (73), it could be suggested that a thiophene moiety is just a bioisostere for a benzene moiety rather than for a phenol moiety. If this hypothesis were correct, the in vivo activity of compounds 34 and 35 should have been the same. However, compound 35 did not induce the 5-HT behavioural syndrome in reserpinised rats, whereas compound 34 and DPAT (73) both possess serotonin and dopamine receptor properties. When the thiophene moiety is considered as a bioisostere for a phenol it is clear that there are similarities between compounds 34 and 35 and their alleged corresponding hydroxylated 2-aminotetralins, i.e. 8- and 5-OH-DPAT, but not all pharmacological aspects are identical. Due to differences in distances between the nitrogen atom and H-bond accepting or donating moieties of the different compounds, there is not a hydroxyl position in a hydroxylated 2-aminotetralin that exactly corresponds with the sulfur position in the thiophene analogues. This is, however, a general phenomenon of isosteric replacement; even though it represents a subtle structural change it might result in a modified profile, i.e. some properties of the parent molecule remain unaltered, others will be changed.

Compounds 38 and 39 were synthesised as possible bioisosteric analogues for PHNO (27a) or one of its analogues. After changing the structure of the hexahydronaphthoxazines (27a, 75, 76) to the hexahydrothianaphthoxazines (38, 39) the position of the sulfur atom would suggest that the hexahydrothianaphthoxazines are bioisosteric analogues for trans-7-hydroxy-4-n-propyl-2,3,4a,5,6,10b-hexahydro-4H-naphth[1,2b][1,4]oxazine (75) which is not a potent dopamine
receptor ligand. However, small structural changes in the basic structure may have large influences on the dopamine receptor activity of compounds. For instance, in the series of the hydroxylated 2-aminotetralins the position of the hydroxyl moiety on the benzene ring determines the dopamine receptor activity of the compounds. For these compounds there is an order of dopamine receptor potency: 5-OH-DPAT > 7-OH-DPAT > 6-OH-DPAT > 8-OH-DPAT, the latter displaying negligible dopamine receptor affinity. On the other hand, in the series of the hexahydronaphthoxazines (27a, 75, 76) only the 9-hydroxy analogue possesses potent dopamine receptor activity, while in the series of the benzo[f]quinolines (77 and 78) both the 7- and 9-hydroxy isomers are potent dopamine receptor ligands. Also this study shows that the structural changes of compounds 34 and 35 result in a higher dopamine receptor activity of compound 34 compared to compound 35, which was unexpected based on the ranking of the monohydroxy 2-aminotetralins.

Still, the binding data indicate that indeed compounds 38 and 39 are ligands with low dopamine receptor affinity. Despite this low affinity for the dopamine D₂ and D₃ receptors the compounds were tested since it was not clear whether or not possible active metabolites could be formed in vivo. Sulfur atoms in molecules may be oxidised in vivo to sulfoxides, which may be active compounds. For instance, in the case of pergolide the sulfoxide metabolite retains its dopamine receptor activity. The pharmacological data, however, show that the dopamine receptor activity of compounds 38 and 39 resembles the low dopamine receptor efficacy of compound 75 or its non-hydroxylated analogue 76. Given the small difference in binding affinities between compound 75 and its non-hydroxylated analogue 76 and their comparable, low efficacy, it is not possible to determine whether a thiophene moiety is a bioisostere for a phenol or a phenyl moiety using the hexahydrothianaphthoxazines 38 and 39.

Because of the diminished activity of compounds 34 and 35, compared to 5-OH-DPAT, it is now an interesting challenge to develop new compounds based on the structure of tetrahydrobenzo[b]thiophenes, which possess the same, improved oral bioavailability as do our compounds 34 and 35, but with a higher affinity and activity at the dopamine D₂ and D₃ receptor. These compounds will be of great interest for the development of new drugs in Parkinson’s disease therapy.

In conclusion, we have shown that a thiophene moiety may qualitatively function as a bioisostere for a phenol moiety in hydroxylated 2-aminotetralins. For the hexahydrothianaphthoxazines it was not possible to discriminate between bioisostericism for a phenyl or a phenol moiety. The tetrahydrobenzo[b]thiophenes (34 and 35) possess higher relative oral bioavailabilities than 5-OH-DPAT.