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

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Abstract

In the present study, a series of thiophene analogs of 2-aminotetralins and hexahydronaphthoxazines were studied in vivo for their ability to decrease striatal dopamine release, their effects on locomotor activity, and their behavioral characteristics in reserpinized 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, 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 thianaphthoxazines 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.

Keywords: Dopamine receptor agonist; Microdialysis; Bioavailability; Striatum; Bioisosteric replacement; Thiophene analog

1. Introduction

The pharmacological importance of the 2-aminotetralin structure has been known for a long time (Bamberger and Müller, 1888). 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 (Bamberger and Müller, 1888; Kraushaar, 1954; Violland et al., 1971). During the late sixties central dopamine receptor activity of 2-aminotetralins was identified, which led to active synthesis programs around the world (Fig. 1).

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 (Arvidsson et al., 1981; Copinga et al., 1993). 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 (**7**) is a very potent dopamine receptor agonist (Seiler and Markstein, 1982, 1984), which has affinity for both the dopamine D_2 and the dopamine D_3 receptors (Van Vliet et al., 1996). Another potent dopamine D_2/D_3 receptor agonist is 5-hydroxy-2-(*N*-*n*-propyl-*N*-2-(2-thienyl)ethylamino)tetralin (N-0437, **1**), 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 (Calabrese et al., 1998). This accounts for all the hydroxylated 2-aminotetralins, since they undergo considerable inactivation by glucuronidation in the gut and the liver (Swart et al., 1991). Therefore, for many years, the identification of bioisosteric catechol and phenol replacements has been emphasized. 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 (**3**), a benzothiazole

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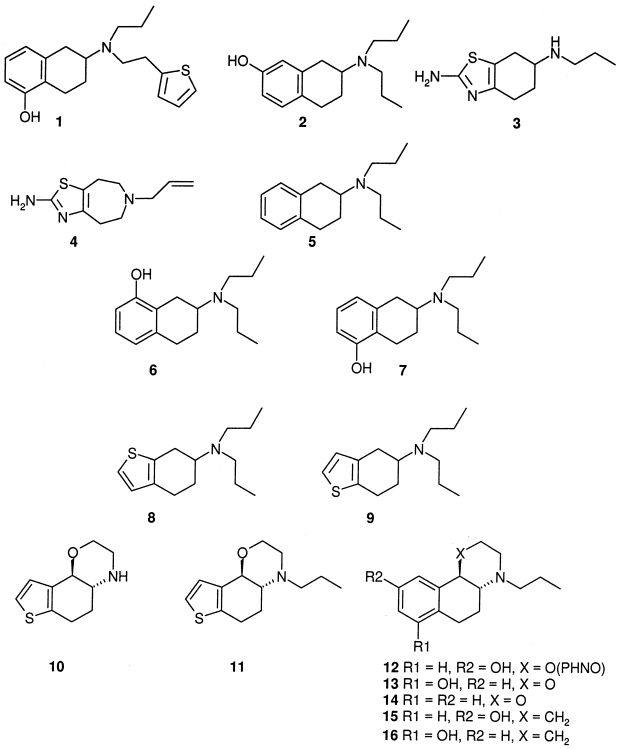

Fig. 1. Chemical structures of 5-hydroxy-2-(*N*-*n*-propyl-*N*-2-thienyl)ethylamino)tetralin (N-0437, **1**), 7-hydroxy-(*N*,*N*-di-*n*-propylamino)tetralin (7-OH-DPAT, **2**), pramipexole (**3**), 5,6,7,8-tetrahydro-6-(2-propenyl)-4*H*-thiazolo[4,5-*d*]azepin-2-amine (BHT920, **4**), 2-(*N*,*N*-di-*n*-propylamino)tetralin (DPAT, **5**), 8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin (8-OH-DPAT, **6**), 5-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin (5-OH-DPAT, **7**), 6-(*N*,*N*-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**8**), 5-(*N*,*N*-di-*n*-propylamino)tetra-hydrobenzo[*b*]thiophene (**9**), trans-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**10**), trans-*N*-*n*-propyl-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**11**), trans-9-hydroxy-4-(*n*-propyl)-2,3,4a,5,6,10b-hexahydro-4*H*-naphth[1,2*b*][1,4]oxazine (**12**), trans-7-hydroxy-4-(*n*-propyl)-2,3,4a,5,6,10b-hexahydro-4*H*-naphth[1,2*b*][1,4]oxazine (**13**), trans-4-(*n*-propyl)-2,3,4a,5,6,10b-hexahydro-4*H*-naphth[1,2*b*][1,4]oxazine (**14**), trans-9-hydroxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (**15**), trans-7-hydroxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (**16**).

analog of the 2-aminotetralins, which is presently on the market as a therapeutic agent for Parkinson's disease (Schneider and Mierau, 1987; Mierau et al., 1995; Guttman, 1997). Also, Andén and coworkers showed that the aminothiazolazepine derivative 5,6,7,8-tetrahydro-6-(2-propenyl)-4*H*-thiazolo[4,5*d*]azepin-2-amine (BHT920, **4**) is a dopamine receptor agonist, with α-adrenoceptor properties (Andén et al., 1983).

In an attempt to circumvent the problem of intensive first-pass metabolism, 6- and 5-(*N*,*N*-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophenes (**8, 9**) were synthesised. These two compounds possess moderate to high affinities for both the dopamine D_2 and D_3 receptor (Table 1). Tricyclic compounds like *trans*-9-hydroxy-4-(*n*-propyl)-2,3,4a,5,6,10b-hexahydro-4*H*-naphth[1,2*b*][1,4]oxazine (PHNO, **12**) and hydroxylated octahydrobenzo[*f*]quinolines (**15, 16**) also possess high affinity for the dopamine D_2 and D_3 receptor, but they display the same problem as hydroxylated 2-aminotetralins, they undergo considerable glucuronidation in the liver due to the phenol moiety (Swart et al., 1991). Since these tricyclic compounds could be of interest, *trans*-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth-[4,5*e*][1,4]oxazine (**10**) and *trans*-*N*-*n*-propyl-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**11**) were synthesised. Compound **10** and **11** possessed neglectable and low affinity respectively for the dopamine D_2 and D_3 receptors (Table 1).

To determine whether a thiophene moiety can act as a bioisostere for a phenol moiety compounds **8–11** were tested for their effects on dopamine release using microdialysis. The effects of compounds **8** and **9** 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 **8** could be related to 8-OH-DPAT and compound **9** to 5-OH-DPAT. However, compound **8** 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 (Swart et al., 1991). 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 monophenolic 2-amino-tetralins. The relative oral bioavailabilities of compounds **8** and **9** were determined (see Section 2). No such estimation was made for compounds **10** and **11**, since these compounds displayed limited affinities for the dopamine D_2 and D_3 receptors. In addition, compounds **8** and **9** were tested for their locomotor activity and dopamine and serotonin receptor behavioral characteristics in reserpinized rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (from Harlan, Zeist, the Netherlands) weighing 280–320 g were used for microdialysis experiments and rats weighing 180–220 g for the locomotor activity and behavioral 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 AM and off at 8 PM). The animals were housed at least 1 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.

Table 1
Binding affinities of some dopamine receptor compounds

Compound	Reference	K_i (nM)		
		D_{2L} agonist	D_3 [^{3}H]spiperone	5-HT_{1A} [^{3}H]8-OH-DPAT
N-0437 (**1**)	Van Vliet et al. (1996)	0.06[a]	4.0	–
7-OH-DPAT (**2**)	Pugsley et al., 1995)	34[a]	1.4	–
pramipexole (**3**)	Mierau et al. (1995)	2.07[b]	0.49[b]	–
5-OH-DPAT (**7**)	Glase (1995)	6[a]	0.66	–
8-OH-DPAT (**6**)	Zhuang (1993)	3200[c]	250	0.9
R-DPAT (R-**5**)	Yu et al. (1996)	32[d]	33	12
S-DPAT (S-**5**)	Yu et al. (1996)	5.5[d]	35	38
8	unpublished data	27[c]	28	80
9	unpublished data	40[c]	20	–
10	unpublished data	> 4780[c]	3003	–
11	unpublished data	631[c]	237	–
12	Dijkstra et al. (1985)	2.8[e]		
13	Dijkstra et al. (1985)	80[e]		
14	Dijkstra et al. (1985)	110[e]		

[a][^{3}H]N-0437.
[b][^{3}H]spiperone, high affinity binding.
[c][^{3}H]N-*n*-propylnorapomorphine.
[d][^{3}H]quinpirole.
[e]IC_{50} (nM) in rat striatal membrane homogenates using [^{3}H]DP-5,6-ADTN.

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 (**8**), 5-(*N*,*N*-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**9**), 5-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin (5-OH-DPAT, **7**), *trans*-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**10**) and *trans*-*N*-*n*-propyl-2,3,4a,5,6,10b-hexahydro-4*H*-thianaphth[4,5*e*][1,4]oxazine (**11**). All five drugs were synthesised at the Department of Medicinal Chemistry in Groningen.

2.3. Surgery and brain microdialysis

On-line brain microdialysis in freely moving animals has previously been described (Westerink, 1992). 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 stereotaxic 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 poly-

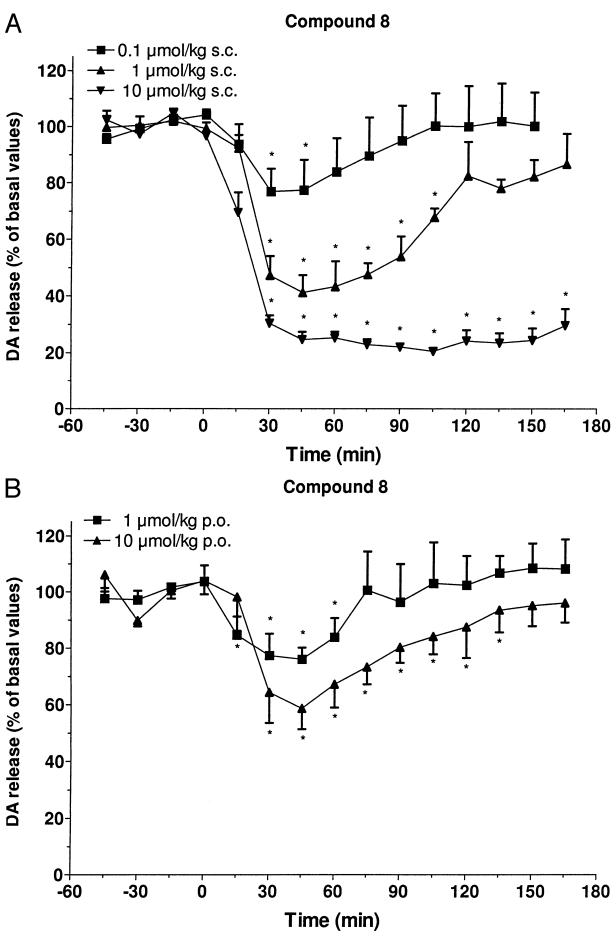

Fig. 2. Effect of s.c. (A) and p.o. (B) administration of **8** on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. ($n = 4$). * $P < 0.05$ (Dunnett's test).

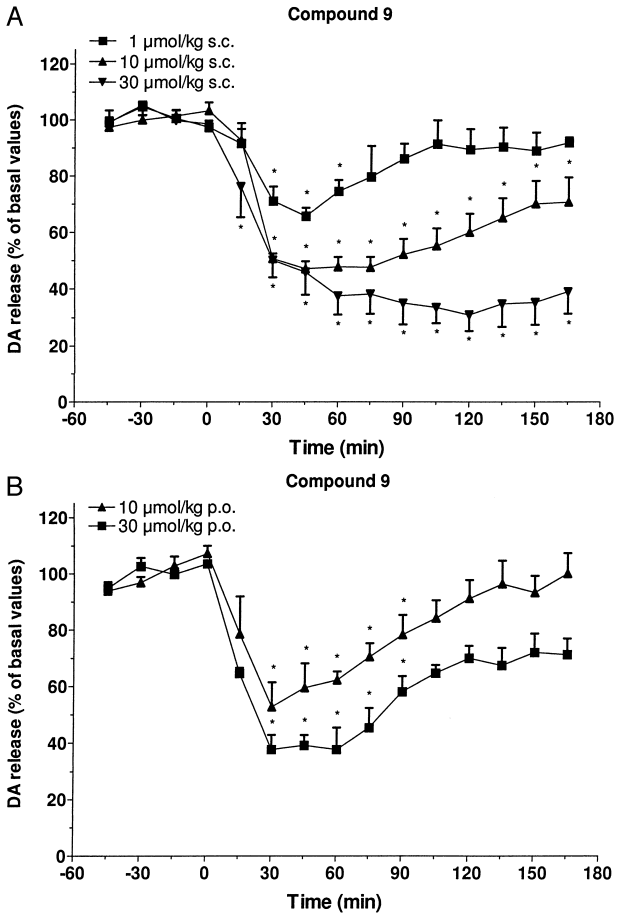

Fig. 3. Effect of s.c. (A) and p.o. (B) administration of **9** on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. ($n = 4$). $^{*}P < 0.05$ (Dunnett's test).

acrylonitrile/sodium methallyl sulfonate 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 coordinates were used according to the atlas of Paxinos and Watson (1982): 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.) as 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.

2.4. Locomotor activity as monitored in automated cages and behavioral 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 behavior of the rats was scored manually every 5 min. The behavior scored was repeated sniffing, repeated licking and rearing as dopamine receptor stereotyped behavior and flat body posture and lower lip retraction as indications of the 5-hydroxytryptamine (5-HT, serotonin) behavioral syndrome. The behavior 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.

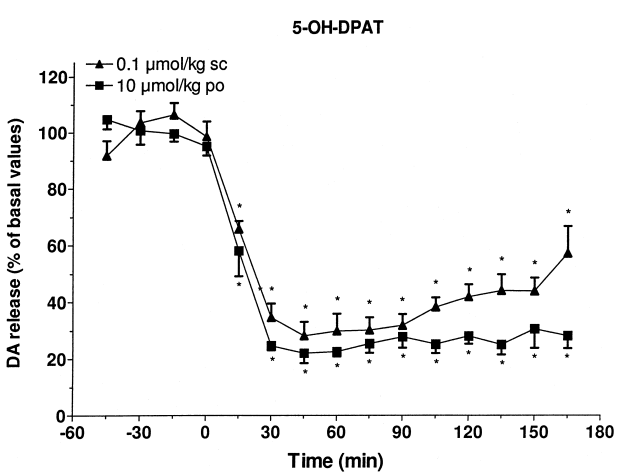

Fig. 4. Effect of s.c. and p.o. administration of 5-OH-DPAT on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. ($n = 4$). $^{*}P < 0.05$ (Dunnett's test).

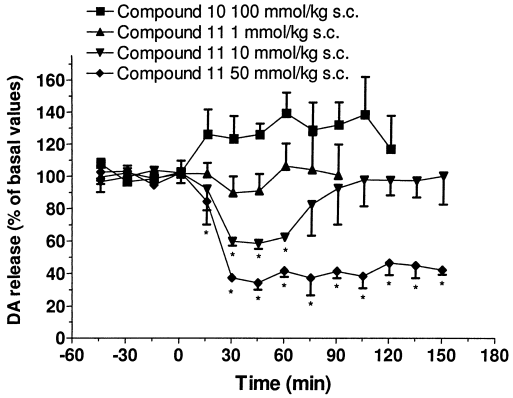

Fig. 5. Effect of s.c. administration of **10** and **11** on striatal dopamine release in freely moving rats. Data are presented as mean ± S.E.M. ($n = 4$). $^{*}P < 0.05$ (Dunnett's test).

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 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 post hoc test. In all cases a significance level of 0.05 was applied.

3. Results

3.1. In vivo microdialysis

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

The results of the microdialysis experiments of the compounds **8,9**, 5-OH-DPAT, **10** and **11** are shown in Figs. 2–5. S.c. administration of all compounds, except compound **10**, induced a dose-dependent and significant decrease in the release of dopamine in the striatum. Furthermore, compounds **8,9** and 5-OH-DPAT also induced a significant decrease in the release of dopamine in the striatum after p.o. administration. Effects of compounds **10** and **11** 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 **8** lasted from $t = 30$ to $t = 45$ min with a maximum decrease of 20% of control values. For a dose of 1 μmol/kg this was from $t = 30$ 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 $t = 30$ to $t = 165$ min with a maximum decrease of 75% of control values (Fig. 2A). Fig. 2B shows that the significant decrease after p.o. administration of compound **8** 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.

Compound **9**, 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 (Fig. 3A). The decrease induced by a dose of

Table 2
AUCs of the microdialysis experiments of **8,9** and **7** after s.c. and p.o. administration

Compound	Subcutaneous administration		Oral administration		Relative oral bioavailability (%)
	Dose (μmol/kg)	AUC	Dose (μmol/kg)	AUC	
8	0.1	2650 ± 1000[a]	1	2730 ± 390[a]	10
	1	6000 ± 500	10	3700 ± 950	10
	10	12446 ± 335			
9	1	3150 ± 400	10	4100 ± 750	10
	10	6700 ± 800	10	4100 ± 750	100
	10	6700 ± 800	30	7000 ± 450	30
	30	9400 ± 960	30	7000 ± 450	100
5-OH-DPAT (**7**)	0.1	9700 ± 500	10	11500 ± 300	1

[a] 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.

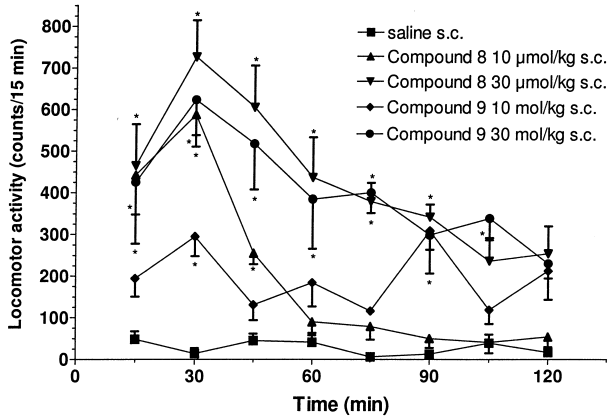

Fig. 6. The effect of **8** and **9** on the locomotor activity of reserpinized rats. Data are presented as mean ± S.E.M. ($n = 4$). $^{*}P < 0.05$ (Student–Newman–Keuls test).

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 **9** lasted from $t = 30$ min to $t = 90$ min for both doses with a maximum decrease of 50% and 60% of control values, respectively (Fig. 3B).

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 (**7**) 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 (Fig. 4).

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

Compound **11** in an 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 $t = 15$ to $t = 150$ min with a maximum decrease of 65% (Fig. 5).

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 (**8**), 5-(*N*,*N*-di-*n*-propylamino)tetrahydrobenzo[*b*]thiophene (**9**) and 5-OH-DPAT (**7**) were calculated from Figs. 2b–4, and are shown in Table 2. For compounds **8** and **9** 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% (unpublished data).

3.2. Locomotor activity in reserpinized rats

Both compounds **8** and **9** induced a significant increase in locomotor activity in reserpinized rats (Fig. 6). The basal level of locomotor activity of reserpinized 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 **8**, 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 effect was 750 counts/15 min, which returned to basal levels after 90 min. The effects of compound **9** in the same doses were less pronounced than those for compound **8**. In a dose of 10 μmol/kg compound **9** induced an increase in locomotor activity to 300 counts/15 min and in a dose of 30 μmol/kg the effect was 650 counts/15 min The latter returned to basal levels after 105 min.

3.3. Behavior in reserpinized rats

Table 3 shows that s.c. administration of compound **8** induced both dopamine receptor stereotyped behavior (sniffing, licking and rearing) and a 5-HT behavioral syn-

Table 3
Behavior after s.c. administration of the tetrahydrobenzo[*b*]thiophenes (**8** and **9**) represented as the number of animals that showed the behavior of the total number of animals used in the experiment

Compound	Dose (μmol/kg s.c.)	Sniffing	Licking	Rearing	Flat body posture	Lower lip retraction
8	10	4/4	0/4	0/4	2/4	2/4
8	30	4/4	2/4	3/4	4/4	4/4
9	10	4/4	4/4	0/4	0/4	0/4
9	30	4/4	4/4	4/4	0/4	0/4

drome (flat body posture and lower lip retraction) in the reserpinized rats. Administration of compound **9** induced dopamine receptor but no serotonin receptor induced behavior.

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 **8, 9** and **11** but not **10** 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 (Westerink et al., 1990). Compound **11** was less effective compared to compounds **8** and **9** in decreasing dopamine release in the striatum and compound **10** was without effect, which is in line with the differences of the four compounds in binding affinities found for the dopamine D_2 and D_3 receptor. All compounds were less potent than 5-OH-DPAT, again in agreement with the higher affinity at the dopamine D_2 and D_3 receptors of the latter compound. The role of the dopamine D_3 receptor as an autoreceptor is still under debate (Waters et al., 1993; Koeltzow et al., 1998), while it is generally accepted that the dopamine D_2 receptor functions as an autoreceptor (L'hirondel et al., 1998).

The affinities of compounds **8** and **9** for the dopamine D_2 and D_3 receptors are 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 **11** was more effective compared to compound **10** is most likely due to a better fit into the receptor of the *n*-propyl substituent of compound **11** than the hydrogen of compound **10**.

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 (Grol et al., 1985; Katerinopoulos and Schuster, 1987). 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 (unpublished data). For 5-OH-DPAT this has been formerly published by Malmberg et al. (1994). 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 **8** and **9** in a minimised conformation are 5.4 and 6.0 Å, respectively (unpublished data). This might explain why compound **8** displays dopamine receptor activity beside its serotonin receptor activity different than 8-OH-DPAT.

The relative oral bioavailability of compounds **8, 9** 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 **8** and **9** 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 2). Thus, the structural changes did influence the oral bioavailability in a positive manner. For hydroxylated aminotetralins glucuronidation is the main route of metabolism (Swart et al., 1991). The thiophene ring is not a target for glucuronidation, which most likely explains the higher relative oral bioavailability of compounds **8** and **9**, as compared to 5-OH-DPAT.

The effects of compounds **8** and **9** on postsynaptic dopamine receptors were determined using a locomotor activity measure and looking at the behavioral characteristics after administration of the drugs. Compounds **8** and **9** induced a significant increase in locomotor activity in reserpinized rats, which again confirms that these compounds are dopamine receptor agonists. The behavioral scoring (Table 3) showed that compound **8** induced dopamine receptor stereotyped behavior (sniffing, licking and rearing), as well as the 5-HT behavioral syndrome (flat body posture and lower lip retraction). Compound **9**, on the other hand, only induced dopamine receptor activity. Thus, the behavioral models confirm that both compounds are active at postsynaptic dopamine receptors. In the microdialysis experiments and in the locomotor activity experiments compound **8** was in low doses more potent than compound **9**. The behavioral 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 **8** attenuated the dopamine receptor activity of this compound which was not the case for compound **9**.

Bioisosteres are groups or molecules which have chemical and physical similarities producing broadly similar biological effects (Thornber, 1979). The substitution of –CH=CH– by –S– in aromatic rings has been one of the most successful applications of classical isosterism (Burger, 1991). Since the dopamine D_2 and D_3 receptor binding affinities of compounds **8** and **9** are comparable to DPAT (**5**), 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 **8** and **9** should have been the same. However, compound **9** did not induce the 5-HT behavioral syndrome in reserpinized rats, whereas compounds **8** and **5** both possess serotonin and dopamine receptor properties (Yu et al., 1996). Thus, these compounds are not bioisosteres of a benzene moiety. When the thiophene

moiety is considered as a bioisostere for a phenol it is clear that there are similarities between compounds **8** and **9** 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 analogs. 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 **10** and **11** were synthesised as possible bioisosteres for PHNO (**12**) or one of its analogs. After changing the structure of the hexahydronaphthoxazines (**12–14**) to the hexahydrothianaphthoxazines (**10,11**) the position of the sulfur atom would suggest that the thianaphthoxazines are bioisosteres for *trans*-7-hydroxy-4-(*n*-propyl)-2,3,4a,5,6,10b-hexahydro-*4H*-naphth[1,2*b*]-[1,4]oxazine (**13**) 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 neglectable dopamine receptor affinity (Feenstra, 1984). On the other hand, in the series of the hexahydronaphthoxazines (**12–14**) only the 9-hydroxy analog possesses potent dopamine receptor activity (Dijkstra et al., 1985), while in the series of the benzo[*f*]quinolines (**15,16**) both the 7- and 9-hydroxy isomers are potent dopamine receptor ligands (Wikström et al., 1985; Liljefors and Wikström, 1986). Also, this study shows that the structural changes of compounds **8** and **9** result in a higher dopamine receptor activity of compound **8** compared to compound **9**, which was unexpected based on the ranking of the monohydroxy 2-aminotetralins.

Still, the binding data indicate that indeed compounds **10** and **11** are ligands with low dopamine receptor affinity. Despite this low affinity for the dopamine D_2 and D_3 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 (Wong et al., 1993). The pharmacological data, however, show that the dopamine receptor activity of compounds **10** and **11** resembles the low dopamine receptor efficacy of compound **13** or its nonhydroxylated analog **14** (Dijkstra et al., 1985). Given the small difference in binding affinities between compound **13** and its nonhydroxylated analog **14** (Dijkstra et al., 1985) 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 these hexahydrothianaphthoxazines.

Because of the diminished activity of compounds **8** and **9**, 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 bioavailability as do our compounds **8** and **9**, but with a higher affinity and activity at the dopamine D_2 and D_3 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 thianaphthoxazines it was not possible to discriminate between bioisosterism for a phenyl or a phenol moiety. The tetrahydrobenzo[*b*]thiophenes (**8,9**) possess higher relative oral bioavailabilities than 5-OH-DPAT.

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