Synthesis and Pharmacological Evaluation of Thiopyran Analogues of the Dopamine D_3 Receptor-Selective Agonist (4a*R*,10b*R*)-(+)-*trans*-3,4,4a,10b-Tetrahydro-4-*n*-propyl-2*H*,5*H*-[1]benzopyrano[4,3-*b*]-1,4-oxazin-9-ol (PD 128907)

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Benzopyranoxazine (+)-**7** (PD 128907) is the most dopamine (DA) D_3 receptor-selective agonist presently known. The only structural feature which distinguishes **7** from the analogous nonselective naphthoxazines is an oxygen atom in the 6-position. To extend this series of tricyclic DA agonists we used a classic bioisoster approach and synthesized thiopyran analogues of **7**, which have a sulfur atom in the 6-position. We prepared *trans*-4-*n*-propyl-3,4,4a,10b-tetrahydro-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazin-9-ol (**9**, *trans*-9-OH-PTBTO), its enantiomers ((+)-**9** and (−)-**9**), the racemic *cis*-analogue (**10**), and the racemic *trans*-sulfoxide (**11**) and studied the potency and selectivity for DA receptors of these compounds. As with other rigid DA agonists, the highest affinity for DA receptors resided in one of the enantiomers, in this case the (−)-enantiomer of **9**. On the basis of a single-crystal X-ray analysis of a key intermediate, the absolute configuration of (−)-**9** was found to be 4a*S*,10b*R*, which is homochiral with (+)-(4a*R*,-10b*R*)-**7**. In contrast to (+)-**7** however, (−)-**9** displayed no selectivity for any of the DA receptors. In addition, it has affinity for $5HT_{1A}$ receptors. (±)-*cis*-4-*n*-Propyl-3,4,4a,10b-tetrahydro-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazin-9-ol **(10)**, which was expected to be inactive, displayed affinity and selectivity for the DA D_3 receptor, whereas the sulfoxide **11** displayed some DA D_3 selectivity, but with a lower affinity. Further pharmacological evaluation revealed that (−)-**9** is a very potent full agonist at DA D_2 receptors and a partial agonist at DA D_3 receptors. The *cis*-analogue (±)-**10** displayed the same profile, but with lower potency. These findings were confirmed in vivo: in reserpinized rats (−)-**9** displayed short-acting activation of locomotor activity (DA D_2 agonism) and also lower lip retraction and flat body posture, ($5HT_{1A}$ agonism). Compound (±)-**10** had no effect on locomotor activity. In unilaterally 6-OH-DA lesioned rats, (−)-**9** gave short-acting locomotor activation. Furthermore, in microdialysis studies in rat striatum, (−)-**9** potently decreased DA release, confirming its activation of presynaptic DA D_2 receptors.

Introduction

New molecular biological techniques have, until now, accomplished the identification of five dopamine (DA) receptor subtypes. According to a generally accepted receptor classification, which is mainly based on molecular structural features, these dopamine receptor subtypes are divided into two groups: the DA D_1-like family of receptors, which includes the DA D_1 and D_5 receptor subtypes, and the DA D_2-like family of receptors, which includes the DA D_2, D_3, and D_4 receptor subtypes.[1,2] The selective brain distribution of these receptors, particularly the DA D_3[3−5] and D_4[6] subreceptor types, makes these receptors potential targets for the development of novel psychoactive drugs. New antipsychotics may be developed, because the DA D_3 and D_4 receptors are predominantly located in brain areas where antipsychotics are supposed to exert their action, such as the limbic system. These brain areas are known to be associated with cognition and emotion and are thought to be altered in psychiatric disorders such as psychosis and schizophrenia.

The function of the DA D_3 receptor has been the subject of much research.[7,8] Several studies, many of

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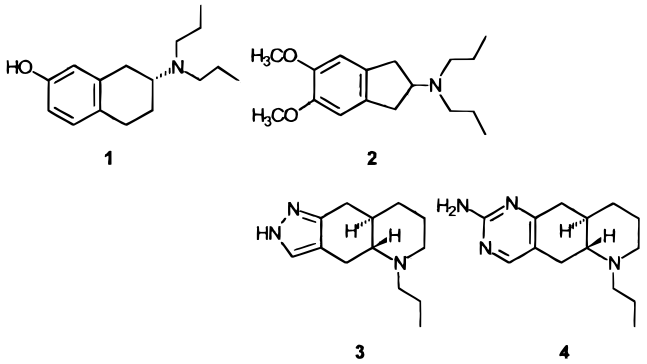


Figure 1. Chemical structures of (*R*)-(+)-7-OH-DPAT (**1**), PNU-990194A (**2**), quinpirole (**3**), and quinelorane (**4**).

which used the DA D_3 preferring ligands described below, have given some insight into this question. The partially DA D_3-selective ligands (*R*)-(+)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin (**1**, (*R*)-(+)-7-OH-DPAT, agonist) and 5,6-dimethoxy-2-(*N*,*N*-di-*n*-propylamino)-indan (**2**, PNU-99194A, antagonist) (Figure 1) were found to inhibit and stimulate locomotor activity, respectively, at doses that do not affect dopamine release. Based on these findings it was speculated that the functional DA D_3 receptor is postsynaptically located where it mediates an inhibitory role on psychomotor function.[9,10] Another study, which employed the partially DA D_3-selective agonists quinpirole (**3**) and quinelorane (**4**), suggested the existence of striatal presynaptic DA D_3 autoreceptors, having an inhibitory effect on DA synthesis.[11]

Furthermore, DA D_3 receptors are suggested to enhance the reinforcing properties of cocaine (presynaptically),[12] to play a role in the subjective effects of cocaine,[13] to mediate hypothermia,[14] and to play a role in memory processes.[15] However, due to the low DA D_3 selectivity (especially for agonists) of the agents used in these studies, a DA D_2 component in their action cannot be excluded and the results have to be interpreted cautiously.

The pharmacological investigation of the DA D_3 and D_4 receptors has generated a need for pharmacological tools that exhibit a high degree of selectivity and affinity for these receptor subtypes. With regard to the DA D_3 receptor subtype, several ligands with a varying degree of selectivity were developed. DA D_3 antagonists include compounds such as **2**,[10] *cis*-(+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(*n*-propylamino)tetralin ((+)-AJ76),[16] *cis*-(+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(di-*n*-propylamino)tetralin ((+)-UH232),[17] (+)-7-(*N*,*N*-dipropylamino)-5,6,7,8-tetrahydronaphtho[2,3-*b*]dihydro-2,3-furan ((+)-S14297),[18] *N*-[(*n*-butyl-2-pyrrolidinyl)methyl]-1-methoxy-4-cyano-naphthalene-2-carboxamide (nafadotride),[19] and (*E*)-1,1′-(2-butene-1,4-diyl)bis[2-[4-[3-(1-piperidinyl)propoxy]-phenyl]-1*H*-benzimidazole] (PD 152255).[20]

Relatively few DA D_3 preferring agonists were developed. The 2-aminotetralin (+)-7-OH-DPAT (**1**) was already known as a DA agonist and was later found to be DA D_3 preferring.[4,21−23] As an extension of the 2-aminotetralin series, rigid tricyclic dopamine agonists were developed. Tricyclic agonists such as the octahydrobenzo[*f*]quinolines[24−30] (e.g. **5**, Figure 2) and the hexahydronaphthoxazines[31,32] (e.g. **6**) display nanomolar affinities for dopamine receptors but are nonselective

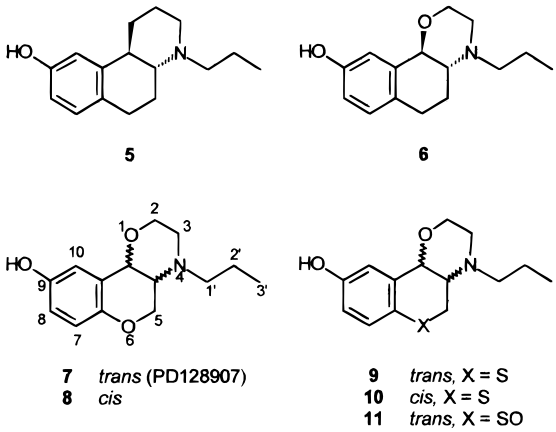


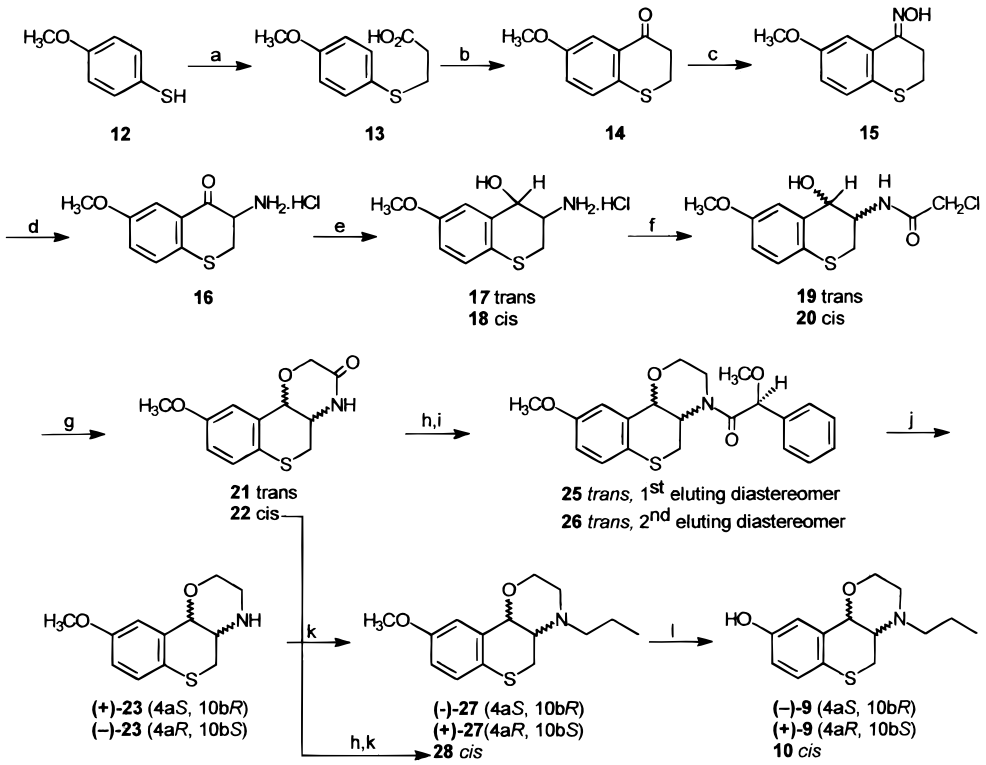
Figure 2. Chemical structures of tricyclic DA agonists: a benzo[*f*]quinoline (**5**), a naphthoxazine (**6**), benzopyranoxazines (**7** and **8**), and benzothiopyranoxazines (*S*-oxide) (**9**−**11**).

(Table 1). Within the benzopyranoxazine series[33,34] (+)-**7** ((4a*R*,10b*R*)-(+)-*trans*-3,4,4a,10b-tetrahydro-4-*n*-propyl-2*H*,5*H*-[1]benzopyrano[4,3-*b*]-1,4-oxazin-9-ol) was found to be rather selective for the DA D_3 receptor subtype and to have virtually no affinity for other non-dopamine receptors, although the DA D_3 over D_2 selectivity of (+)-**7** was found to be much smaller when the affinities for these receptors were obtained with an agonist instead of an antagonist radioligand.[35,36] Furthermore, (+)-**7** is presumed to interact selectively with DA D_3 receptors only at low doses.[35] Therefore, agonists exhibiting higher DA D_3 selectivity are still needed to study this receptor subtype in more detail.

By comparing the structures of **5**, **6**, and (+)-**7** (Figure 2) and their affinities (Table 1) it appears that selectivity for the DA D_3 receptor is affected by introducing an oxygen atom in the 6-position of the tricyclic structure, as in (+)-**7**. This selectivity is hard to explain on the basis of geometry, because **5**, **6,** and (+)-**7** are structurally very similar. However, the DA D_3 selectivity of (+)-**7** may be explained by the higher electronegativity of oxygen, as compared to carbon. The oxygen atom at the 6-position of **7** gives the potential for hydrogen-bond formation with the receptor, which may be favorable for binding to the DA D_3 receptor and not so much to the DA D_2 receptor.

In this study we used a classic bioisoster approach and replaced the oxygen in the 6-position of **7** with a sulfur, which gave us benzothiopyranoxazines **9** and **10**. Such bioisosteric replacements of oxygen by sulfur have been applied extensively to study structure−activity relationships of various pharmacologically active agents.[37,38] Often, a relationship is found between the electronegativity of the substituent and the biological activity. For the compounds in this study this replacement will have the following effects: the lower electronegativity of sulfur (as compared to oxygen) will eliminate the ability of the ligand to form a hydrogen bond with the receptor. On the other hand, the introduction of a sulfur will increase the hydrophobic properties around the 6-position of the ligand. The balance between hydrogen bonds and hydrophobic interactions is very important for the tight binding of a drug to a receptor,[39] and this balance may influence the affinity and selectivity of the benzothiopyranoxazines for the DA D_3 receptor as compared to **7**.

Scheme 1[a]



[a] Reagents: (a) 3-bromopropionic acid, NaOH, H_2O; (b) PPA; (c) $NH_2OH{\cdot}HCl$, pyridine, EtOH, reflux; (d) 1. *p*-toluenesulfonyl chloride, pyridine, 2. KOtBu, EtOH, toluene, 3. HCl (concd); (e) 1. $NaBH_4$, CH_2Cl_2/iPrOH (1/1), 35 °C, 2. HCl (aq, 10%); (f) chloroacetyl chloride, EtOAc, H_2O, $NaHCO_3$; (g) NaOH, H_2O, iPrOH; (h) $LiAlH_4$, Et_2O, reflux; (i) (*S*)-(+)-α-methoxyphenylacetic acid chloride, CH_2Cl_2, NaOH (aq, 5%); (j) KOtBu, THF, H_2O; (k) 1-iodopropane, CH_3CN, Cs_2CO_3, reflux; (l) $AlCl_3$, EtSH, 0 °C.

The replacement of oxygen by sulfur may (also) be favorable from a pharmacokinetic point of view: Because sulfides are more lipophilic than their corresponding oxygen compounds, passage through the blood−brain barrier may be enhanced. This is illustrated by a log *D* calculation with the computer program Pallas 1.2.[40] Compound **7** has a calculated log *D* of 1.13, whereas for **9** log *D* = 1.62. (A log *D*-value of 2.5 is regarded optimal for blood−brain penetration of a drug.[41])

We also decided to study the effects of oxidation of benzothiopyranoxazine **9** to its corresponding sulfoxide **11**, thereby increasing the electron density around the 6-position. This restores the ability of the ligand to form a hydrogen bond with the receptor at this position. However, the lipophilicity is reduced quite dramatically (log *D* = −0.15 as predicted with Pallas 1.2[40]).

This study describes the synthesis and pharmacological evaluation of (±)-*trans*-, (+)-*trans*-, (−)-*trans*-, and (±)-*cis*-4-*n*-propyl-3,4,4a,10b-tetrahydro-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazin-9-ol ((±)-**9**, (+)-**9**, (−)-**9**, and (±)-**10,** respectively) and the sulfoxide (±)-*trans*-4-*n*-propyl-3,4,4a,10b-tetrahydro-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazin-9-ol 6-oxide ((±)-**11**). We expected the highest affinity to reside in the isomer which has a trans configuration with respect to the fusion of oxazine to the thiopyran ring, as with the other tricyclic DA agonists. Therefore, we optimized the formation of the trans product, and only the trans isomer was resolved.

Chemistry

The syntheses of **9** (and its enantiomers) and **10** are outlined in Scheme 1 starting from 4-methoxybenzenethiol, **12** (compound **12** is commercially available or can be prepared by chlorosulfonylation of anisole[42] and subsequent reduction of 4-methoxybenzenesulfonyl chloride with zinc and sulfuric acid[43] (not in scheme)). Alkylation with 3-bromopropionic acid gave **13**,[44] which was ring-closed with polyphosphoric acid[45] to benzothiopyranone **14**. Condensation of **14** with hydroxylamine gave oxime **15**, which was reacted with *p*-toluenesulfonyl chloride to the corresponding tosyl oxime and subsequently converted to amino ketone **16** in a Neber rearrangement reaction. The amino ketone **16** was reduced to *trans*- and *cis*-amino alcohols **17** and **18**, respectively, with sodium borohydride, in a 1/1 solvent mixture of methylene chloride and 2-propanol at 35 °C, which gave a trans:cis ratio of 93:7 (determined with GC analysis of the chloroacetamide mixture (**19** and **20**) formed in the next reaction step). Lower reaction temperatures and more polar solvents (methanol, ethanol) gave more of the cis product. Amino alcohols **17** and **18** were difficult to purify and were first acylated with chloroacetyl chloride to afford *trans*-chloroacetamide **19** and *cis*-chloroacetamide **20**. The *trans*-chloroacetamide **19** was obtained pure by recrystallization, whereas the *cis*-chloroacetamide **20** could be purified by column chromatography. *trans*-Chloroacetamide **19** can be distinguished from its cis isomer **20** by comparing the coupling constants of their respective benzylic protons,[33] which are in the diaxial position for the *trans*-chloroacetamide **19**. For the chloroacetamides this difference is quite small (J = 3.7 Hz (δ 4.62 ppm) and J = 2.7 Hz (δ 4.69 ppm) for *trans*- and *cis*-chloroacetamide, respectively). However, for the conformationally more re-

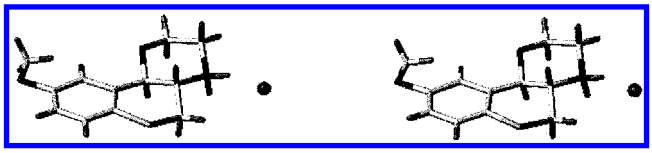


Figure 3. Stereoview of the molecular structure of (+)-**23**·HCl, determined with single-crystal X-ray crystallography.

stricted tricyclic structures this difference is more pronounced (see Experimental Section, compounds **27** and **28**). *trans*- and *cis*-chloroacetamide were separately reacted to final products.

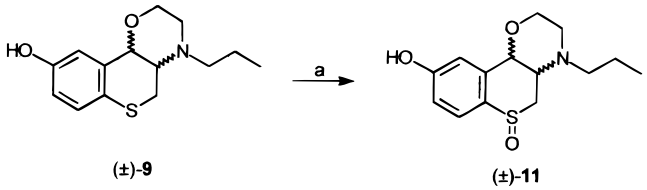
Cyclization of chloroacetamides **19** (trans) and **20** (cis) was achieved with sodium hydroxide in 2-propanol,[33] affording oxazinones **21** (trans) and **22** (cis), respectively, which were reduced with $LiAlH_4$ to give oxazines **23** (trans) and **24** (cis), respectively. The *trans*-oxazine **23** was resolved by coupling with (*S*)-(+)-α-methoxyphenylacetic acid chloride and column chromatographic separation of the thus formed diastereomeric amides **25** (eluting first) and **26** (eluting second).[30] Cleavage of the amides **25** and **26** by potassium *tert*-butoxide and water in THF afforded (+)-*trans*-oxazine (+)-**23** and (−)-*trans*-oxazine (−)-**23**, respectively. The hydrochlorides of these secondary amines yielded crystals suitable for single-crystal X-ray crystallography (Figure 3). On the basis of the X-ray analysis of (+)-**23**, amines (+)-**23** and (−)-**23** could be assigned the absolute configurations 4a*S*,10b*R* and 4a*R*,10b*S*, respectively.

trans-Oxazines **23**, (+)-**23** and (−)-**23**, and the racemic *cis*-oxazine **24** were alkylated with 1-iodopropane in acetonitrile using cesium carbonate as the base, giving the tertiary amines **27**, (−)-**27**, (+)-**27,** and **28**, respectively. Upon propylation, the optical rotation of the enantiomers of **23** changed both sign and amplitude.

To be able to assign all hydrogen atoms in the ^1H NMR spectrum of the *trans*-benzothiopyranoxazine **27**, a COSY experiment was performed. Interestingly, the two methylene hydrogen atoms (of the propyl group) attached to the oxazine nitrogen (hydrogens H1′) show a high degree of nonequivalency resulting in two separate signals (2 multiplets, at $\delta = 2.35$ and 2.78 ppm). These hydrogens are nonequivalent because they are diastereotopic. However, for the *cis*-benzothiopyranoxazine **28** this nonequivalency is much less pronounced, and no separate signals are observed. This phenomenon has been described previously for *trans*-*N*-propylhexahydronaphthoxazine (**6**)[46] and also for *trans*-*N*-benzyloctahydrobenzo[*f*]quinolines, for which the benzylic protons are nonequivalent.[25,26]

Finally, demethylation of **27**, (−)-**27**, (+)-**27,** and **28** was achieved cleanly by applying aluminum chloride in EtSH, giving the final products (±)-**9**, (−)-**9**, (+)-**9,** and (±)-**10**, respectively. The sulfoxide **11** was prepared by oxidizing the (±)-*trans*-sulfide (±)-**9** with $NaIO_4$ in $MeOH/H_2O$ (Scheme 2).

Scheme 2[a]



[a] Reagents: (a) $NaIO_4$, MeOH, H_2O.

Pharmacology

Receptor Binding. Sulfides (±)-**9**, (−)-**9**, (+)-**9,** and (±)-**10** and the sulfoxide **11** were tested for their in vitro binding affinity for human dopamine (DA) D_{2L}, D_3, or $D_{4.2}$ receptors, expressed in Chinese hamster ovary (CHO) K-1 cells. In the antagonist binding studies, the affinity of the compounds was determined by their ability to displace [^{3}H]spiperone from D_{2L}, D_3, or $D_{4.2}$ DA receptors.[35] In the agonist binding studies, the affinity for the D_{2L} DA receptor was determined using [^{3}H]NPA ([^{3}H]-*N*-propylnorapomorphine) as the radioligand. The affinity data obtained with [^{3}H]NPA are comparable to those obtained with [^{3}H]N-0437, the agonist radioligand which was used for the reference compound **7**. In vitro affinity for rat hippocampal serotonin $5HT_{1A}$ and cortical $5HT_2$ receptors was determined with [^{3}H]-8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT) and [^{3}H]ketanserin as radioligands, respectively.[35] Receptor affinities are presented in Table 1.

Intrinsic Activity. The intrinsic activity (IA) of compounds (−)-**9** and (±)-**10** was determined with a functional test, the mitogenesis assay.[47,48] In CHO-L6 cells transfected with the rat DA D_{2L} or D_3 receptor, [^{3}H]-thymidine uptake was determined as a measure of agonism at these receptors (Table 2). The IA was compared with that of quinpirole, which is a full agonist at both receptors (IA = 100%). In the same cells the ability of (−)-**9** and (±)-**10** to inhibit quinpirole-stimulated [^{3}H]thymidine uptake was determined, which is a measure of antagonism (Table 2).

Locomotor Activity and Behavior in Reserpinized Rats. In vivo, postsynaptic agonistic effects of compounds (−)-**9** and (±)-**10** were determined in rats, which had been treated with reserpine 18 h beforehand. The reversal of the reserpine-induced akinesia is a measure of DA D_2 agonism. In addition, (stereotypic) behavior was observed, such as sniffing (a marker of DA D_2 agonism), flat body posture and lower lip retraction (markers of agonism at serotonin $5HT_{1A}$ receptors) and yawning (a putative marker of activation of DA D_3 receptors[21,49]). The time course of locomotor activity is depicted in Figure 4; a summary of behavior is presented in Table 3.

Contralateral Turning in 6-OH-DA Lesioned Rats. Compound (−)-**9** was further evaluated in rats unilaterally lesioned with 6-OH-DA (Figure 5). In this model, the DA neurons of one side (left or right) of the nigrostriatal DA system are selectively and completely degenerated by intracerebral injection of the neurotoxin 6-OH-DA. This causes a postsynaptic supersensitivity to develop on the lesioned side.[50] Upon systemic administration of a DA agonist, the rat will start to turn contralaterally, i.e., toward the nonlesioned side.[51] The evoked turning behavior is a measure of the DA (D_1 and/or D_2) agonist properties of a compound.

Microdialysis in Rat Striatum. The effects of (−)-**9** on in vivo DA release in rat striatum were assessed with microdialysis methods (Figure 6).

Results and Discussion

Receptor Binding. As has been discussed previously, for DA agonists DA D_2 affinity, and consequently DA D_3 selectivity, is most reliably measured using an

Table 1. DA (human D_{2L}, D_3, and D_4) and Serotonin (rat $5HT_{1A}$ and $5HT_2$) Receptor Affinities of the 9-OH-benzothiopyranoxazines and Several Known Tricyclic DA Agonists

	K_i[a] (nM)				ratio	K_i[a] (nM)	
compd	D_{2L} [^{3}H]NPA	D_{2L} [^{3}H]spip	D_3 [^{3}H]spip	D_4 [^{3}H]spip	D_{2A}/D_3	$5HT_{1A}$ [^{3}H]8-OH-DPAT	$5HT_2$ [^{3}H]ketanserin
			9-OH-benzothiopyranoxazines				
(±)-**9**	ND[b]	533	1.56	44.4		ND	ND
(−)-**9**	1.14	ND	2.87	17.6	0.4	30	>5000
(+)-**9**	854	ND	3003	>3300		>1000	>5000
(±)-**10**	74	ND	3.58	193	21	NA[c]	NA
(±)-**11**	197	ND	29	IC_{50} > 10000	6.8	NA	NA
			Other Tricyclic DA Agonists				
6	ND	6.24[d]	0.21[d]	ND			
(±)-**7**[e]	ND	>10000	78	ND			
(+)-**7**	42[f,g]	1180[h]	1.4[g]	169[g]	30	1670[g]	NA
(−)-**7**	ND	>10000	>10000	ND			
(±)-**8**	ND	>10000	>10000	ND			

[a] K_i and IC_{50} values are means of three separate experiments, the results of which did not vary by more than 25%. [b] ND, not determined. [c] NA, not active. [d] Unpublished results. [e] Compound **7** is PD 128907. [f] Determined with the agonist radioligand [^{3}H]N-0437. [g] Ref 35. [h] Ref 36.

Table 2. Agonist and Antagonist Effects of Selected Compounds as Measured in the Mitogenesis Assay at the Rat D_{2L} and D_3 Receptors[a]

	[^{3}H]thymidine uptake, EC_{50}, nM (IA[b])		inhib of quinpirole (30 nM)-stimulated [^{3}H]thymidine uptake	
compd	DA D_{2L}	DA D_3	DA D_{2L}	DA D_3
(−)-**9**	0.013 ± 0.004 (80% ± 7)	0.007 ± 0.001 (45% ± 7)	NA[c]	A[d] ≥0.1 nM
(±)-**10**	122 ± 39 (87% ± 12)	36 ± 16 (57% ± 8)	NA	NA
(+)-**7**[e]	5.7 ± 1.2 (81% ± 1.2)	0.36 ± 0.12 (54% ± 2)	NA	NA
quinpirole	2.2 (100%)	1.7 (100%)	NA	NA

[a] All values are means of three determinations ± SEM. [b] IA, intrinsic activity. [c] NA, not active. [d] A, active. [e] (+)-PD 128907.

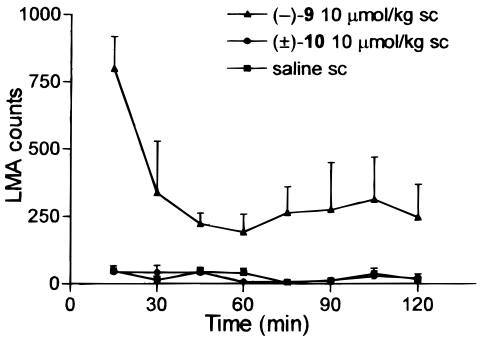


Figure 4. Locomotor activity (LMA). Each point is the mean ± SEM of four determinations (saline, $n = 8$).

Table 3. Behavior in Reserpinized Rats[a]

	behavior			
compd	FB	LL	Sni	Ya
saline	0/8	0/8	0/8	0
(−)-**9**	4/4	3/4	4/4	0
(±)-**10**	0/4	0/4	0/4	11 ± 3

[a] Flat body posture (FB), lower lip retraction (LL), and sniffing (Sni): shown is the number of rats displaying the indicated behavior during the first 30 min after injection. Yawning (Ya): shown is the mean ± SEM of total yawns/rat during the first 30 min after injection.

agonist radioligand.[22,35] Accordingly, the calculated DA D_3 selectivity for (+)-**7** was substantially reduced when DA D_2 affinity was measured with [^{3}H]N-0437 (agonist radioligand) instead of [^{3}H]spiperone (antagonist radioligand).[35] To obtain a proper selectivity ratio for the newly synthesized benzothiopyranoxazines **9** and **10** and the sulfoxide **11**, also for these compounds the DA D_2 affinity was measured with an agonist radioligand, in this case [^{3}H]NPA. (See Table 1, the affinity of racemic *trans*-**9** was in a preliminary binding assay only determined with [^{3}H]spiperone.)

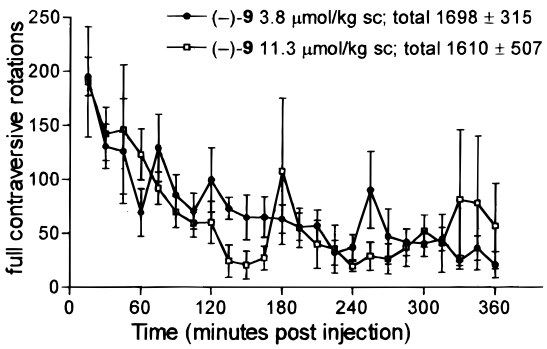


Figure 5. Effect on turning behavior of (−)-**9** in unilaterally 6-OH-DA lesioned rats. Each point is the mean ± SEM of five (3.8 μmol/kg) or three (11.3 μmol/kg) rats. The total number of full contraversive rotations ± SEM is indicated at the top.

In this series of benzothiopyranoxazines, the trans compound **9** (as compared to its cis analogue **10**) has the highest affinity for dopamine receptors, like in the series of the before mentioned benzo[*f*]quinolines,[25,28,30] naphthoxazines,[31] and benzopyranoxazines (Table 1). Concluding from the X-ray analysis (Figure 3) of its intermediary secondary amine (+)-**23**, the configuration of the chiral centers in the (−)-*trans*-benzothiopyranoxazine (−)-**9** is 4a*S*,10b*R*. Although (−)-**9** has a negative optical rotation, it is homochiral (i.e., has an identical spatial orientation at its chiral centers) with (4a*R*,10b*R*)-(+)-**7**, the active enantiomer of the *trans*-benzopyranoxazines. (The designation 4a*S* for (−)-**9** is the result of a change in substituent priority, as compared to (+)-**7**, upon introduction of a sulfur.) Of both trans enantiomers, (−)-**9** has the highest affinity for dopamine receptors (Table 1). Thus, with regard to the stereochemistry of the *trans*-9-OH-benzothiopyranoxazines the structure−affinity relationship is analogous to that of the before mentioned benzo[*f*]quino-

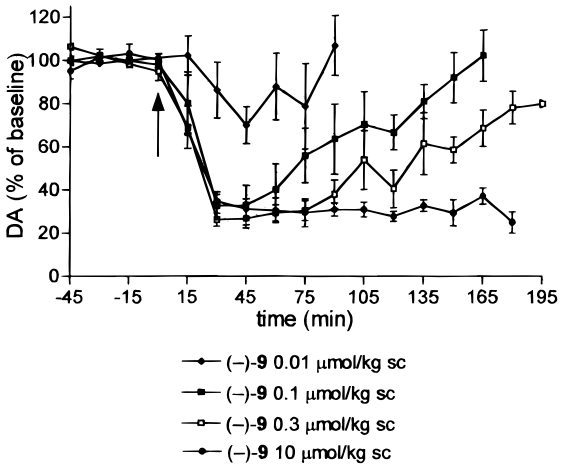


Figure 6. Effect of (−)-**9** on DA release in rat striatum. Each point is the mean ± SEM of three determinations. The arrow indicates the time of injection. DA decreases were significant ($p < 0.05$) for 0.1, 0.3, and 10 μmol/kg.

lines, naphthoxazines, and benzopyranoxazines. In each of these tricyclic dopamine agonists the (+)-trans compound (which for all three compound classes is homochiral with (−)-**9**) is the enantiomer with the highest affinity for DA receptors.[30,31,34]

Compound (−)-**9** binds to the DA D_3 receptor with similar affinity as to the DA D_2 receptor (Table 1), and it also shows good affinity ($K_i = 17.6$ nM) for the DA D_4 receptor. With this combined DA D_2 and DA D_3 affinity, (−)-**9** shows more similarity with the corresponding *trans*-benzo[*f*]quinoline **5** and *trans*-naphthoxazine **6**, which are also nonselective, than with (+)-**7**. This similarity may be explained by the charge distribution around the sulfur atom, because the electronegativity of sulfur is closer to carbon than to oxygen. (The electronegativity values for carbon, oxygen, and sulfur are 2.75, 3.65 and 2.96, respectively, according to the Sanderson scale.[52]) Apparently, with the introduction of more hydrophobic properties around the 6-position, the affinity for DA D_2-like receptors is enhanced, but with the loss of a hydrogen-bonding site the selectivity for the DA D_3 receptor is lost.

To our surprise, during the locomotor activity and microdialysis experiments with (−)-**9**, we observed the $5HT_{1A}$ behavioral syndrome (flat body posture, lower lip retraction, see Figure 4). Therefore, we decided to determine binding to serotonin ($5HT_{1A}$ and $5HT_2$) receptors as well. Unlike (+)-**7**, compound (−)-**9** displayed moderate affinity for rat hippocampal $5HT_{1A}$ receptors ($K_i = 30$ nM). $5HT_{1A}$ receptor binding has been reported for 10-hydroxy-substituted octahydrobenzo[*f*]quinolines.[28] However, for 9-hydroxy-substituted tricyclic DA agonists, affinity for $5HT_{1A}$ receptors has not been reported previously.

In contrast with the corresponding *cis*-benzopyranoxazine **8**, which has no affinity for DA D_2 or D_3 receptors, the difference in affinity between *trans*-**9** and its cis analogue **10** is much less pronounced (Table 1). On the contrary, **10** shows a remarkable affinity and selectivity for the DA D_3 receptor, which is unexpected for a cis tricyclic dopamine agonist structurally related to the benzo[*f*]quinolines, naphthoxazines, and benzopyranoxazines. One of the enantiomers of **10** may display an even higher selectivity for the DA D_3 receptor. However, by optimizing the trans:cis ratio, we did not obtain enough of the cis secondary amine **24** to resolve this compound.

The affinity for dopamine receptors of the sulfoxide compound **11** was substantially reduced (Table 1), as compared to compound **9**. However, the affinity for the DA D_3 receptor was much less reduced than the affinity for the DA D_2 receptor, suggesting that an electronegative (hydrogen-bonding) site in the 6-position of the molecule may be allowed by the DA D_3, but not by the DA D_2 receptor. The lower lipophilicity of compound **11** (log $D = -0.15$, as predicted with Pallas 1.2[40]), or the electron-withdrawing effect of the sulfoxide group on the aromatic nucleus and the resulting higher acidity of the phenolic hydroxy group, may contribute to its lower affinity.

Intrinsic Activity. The functional assay revealed that (−)-**9** is very potent ($EC_{50} = 0.013$ nM, Table 2) and a full agonist (IA = 80%) at DA D_{2L} receptors. This was confirmed by its inability to inhibit quinpirole stimulated [^{3}H]thymidine uptake. At DA D_3 receptors (−)-**9** displayed partial agonism, unlike (+)-**7**, which is reported to be a full agonist at DA D_2 and D_3 receptors.[35]

The cis analogue (±)-**10** displayed full agonism at DA D_2 and partial agonism at D_3 receptors, but with lower potency, reflecting its affinity for these receptors. No inhibition of quinpirole stimulated [^{3}H]thymidine uptake was detected for this compound, supporting its agonism.

Locomotor Activity and Behavior in Reserpinized Rats. The in vivo effects in reserpinized rats confirmed the receptor binding profile of (−)-**9** (Figure 4). This compound shows actions at both DA and $5HT_{1A}$ receptors: activation of locomotor activity and sniffing behavior (reflecting postsynaptic DA D_2 (and/or D_1) agonism), besides lower lip retraction and flat body posture (reflecting $5HT_{1A}$ agonism), were observed. The onset of these effects can be observed almost immediately after injection, which reflects a good absorption, but the maximum effect only lasts for about 30 min.

Compound (±)-**10** had no effect on locomotor activity in reserpinized rats, probably due to its relatively low DA D_2 affinity ($K_i = 74$ nM). Behaviorally, (±)-**10** appeared to increase yawning, which may reflect its preference for DA D_3 receptors.[21,49] However, this is controversial, and yawning may be mediated by a number of other neurotransmitters and neuropeptides (for a review see ref 53). Yawning elicited by (+)-PD 128970 ((+)-**7**) has been observed at lower doses (for example, 0.35 μmol/kg (+)-**7** causes 12 ± 2 yawns/rat, monitored during 20 min).[54]

Contralateral Turning in 6-OH-DA Lesioned Rats. Also in rats with a unilateral lesion of the nigrostriatal system, (−)-**9** turned out to be a potent agonist at DA receptors, as is obvious from the evoked rotation behavior (Figure 5). The time course of the rotation was similar to the time course of the effects of (−)-**9** observed in reserpinized rats: an immediate onset of action after injection but a short duration of the maximum effect.

Microdialysis in Rat Striatum. A decrease of DA release was observed after administration of (−)-**9**, which was to be expected for a DA D_2 agonist. At 0.3

μmol/kg (−)-**9** showed a DA reduction of 75% (which is almost the maximum effect which can be observed with microdialysis, a reduction of 80%), which is more potent than (+)-**7**, which displays a 55% DA reduction at the same dose.[35] Furthermore, the reduction of DA release lasted longer for (−)-**9**, which may reflect its higher lipophilicity, as compared to (+)-**7**. As was also observed with the experiments with reserpinized rats, the rats were activated and showed lower lip retraction and flat body posture.

Concluding from the results described above, it is clear that the pharmacological profile of the thiopyran analogue (−)-**9** is quite different from that of (+)-**7**. Compound (−)-**9** displays a mixed receptor binding profile, having affinity for DA D_2 and D_3 and $5HT_{1A}$ receptors. A small change in the structure of a tricyclic DA ligand turns out to have a large effect on its receptor binding profile. Although sulfur is often used as a bioisosteric replacement of oxygen, within this series of tricyclic DA agonists, sulfur substitution on the 6-position has a dramatic effect.

In contrast with (−)-**9**, the cis analogue (±)-**10** displays DA D_3 (over D_2) selectivity. Study of the enantiomers of **10** could certainly be worthwhile, as one of the enantiomers may display twice the affinity for DA D_3 receptors, as compared to the racemate. The selectivity for DA D_3 receptors of one of the enantiomers of **10** may even be higher, because the DA D_2 affinity which is measured for the racemate may reside fully in the other enantiomer.

Finally, the DA D_3 preference of the sulfoxide (±)-**11** suggests that an electronegative (hydrogen-bonding) site on the 6-position of the tricyclic system is allowed by the DA D_3 but not by the DA D_2 receptor. However, other factors, such as the influence of the sulfoxide on the nitrogen or aromatic hydroxy pK_a value, or on the aromatic ring, may play a role.

Experimental Section

Chemistry, General. Melting points were determined in open glass capillaries on an Electrothermal digital melting-point apparatus and are uncorrected. Optical rotations were determined at 589 nm, at 20 °C on a Perkin-Elmer 241 polarimeter. ^{1}H NMR spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-24 B spectrometer, at 200 MHz on a Varian Gemini 200 spectrometer, or at 500 MHz on a Varian Unity 500 spectrometer. Chemical shifts are given in δ units (ppm) and are relative to TMS for 60 MHz spectra or relative to the solvent for 200 and 500 MHz spectra. Coupling constants are given in hertz (Hz). The spectra recorded were consistent with the proposed structures. IR spectra were obtained on an ATI-Mattson spectrophotometer, and only the important absorptions are given. Electronic ionization (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, and were within 0.4% of the theoretical values, except where noted. Compounds that were obtained as an oil, or as a solid in a very small amount, were analyzed by high-resolution mass spectrometry (HRMS), performed on a JEOL MSroute JMS-600H by the Department of Chemistry, University of Groningen.

If necessary, compounds were purified with medium-pressure chromatography (MPLC) on silica, starting with an apolar eluent (usually 100% hexane) and gradually increasing its polarity (usually by adding ethyl acetate), finishing with an eluent mixture that gave a R_f value of approximately 0.3 on TLC.

Single-crystal X-ray analysis was performed by S. Sundell at the Department of Medical Biochemistry, University of Göteborg.

***β*-(4-Methoxyphenylmercapto)propionic Acid (13).** A solution of sodium carbonate (19.1 g, 0.18 mol) and 3-bromopropionic acid (27.5 g, 0.18 mol) in 90 mL of ice-cold water was added to a solution of sodium hydroxide (7.2 g, 0.18 mol) and 4-methoxybenzenethiol **12** (24.8 g, 0.18 mol) in 90 mL of water. This mixture was refluxed for 1.5 h, and after cooling to room temperature it was washed once with ethyl acetate (100 mL). The water layer was then acidified with 10% hydrochloric acid, and the product was extracted with ethyl acetate (2 × 100 mL). These combined organic layers were then washed once with brine, dried over $MgSO_4$, and concentrated under reduced pressure, which yielded an off-white crystalline mass (25.9 g, 68%): mp 71 °C; EIMS *m/e* 212 (M^+); IR (KBr, cm^{-1}) 2839, 1701, 1595, 1496; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 2.4 (t, 2H), 2.8 (t, 2H), 3.7 (s, 3H), 6.6 (d, 2H), 7.15 (d, 2H), 9.8 (s, 1H). Anal. ($C_{10}H_{12}O_3S\cdot{}^1/_4H_2O$) C,H.

6-Methoxy-3,4-dihydro-*2H*-[1]benzothiopyran-4-one (14). Polyphosphoric acid (70 g) was heated to 90 °C and mechanically stirred. Melted (ca. 80 °C) *β*-(4-methoxyphenylmercapto)propionic acid (**13**) (25.0 g, 0.12 mol) was added in one portion, and the mixture was stirred for 3 min. Then another amount of polyphosphoric acid (120 g), which was heated to 90 °C in advance, was added in one portion, and the mixture was stirred for 4 min. After cooling to 60 °C, ice (180 g) was added and the reaction mixture was stirred until the polyphosphoric acid had hydrolyzed (ca. 10 min). Subsequently, the product was extracted with ethyl acetate (4 × 100 mL). The combined ethyl acetate layers were washed once with water, once with NaOH (5%, aq), then again with water until neutral pH. After washing the combined organic layers once with brine they were dried over $MgSO_4$ and concentrated under reduced pressure. The resulting brown oil was purified by distillation under reduced pressure which yielded **14** as a yellow oil (15.5 g, 68%): bp 109 °C (0.03 mmHg); IR (neat, cm^{-1}) 2833, 1674, 1598, 1471; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.94−3.00 (m, 2H), 3.19−3.25 (m, 2H), 3.83 (s, 3H), 7.01 (dd, $J_1 = 8.7$, $J_2 = 2.9$, 1H), 7.19 (d, $J = 8.7$, 1H), 7.61 (d, $J = 2.9$, 1H); ^{13}C NMR δ 26.8, 39.7, 55.5, 111.3, 122.3, 128.9, 131.6, 133.6, 157.4, 194.1; HRMS calcd (obsd) for $C_{10}H_{10}O_2S$ 194.0401 (194.0400).

6-Methoxy-3,4-dihydro-*2H*-[1]benzothiopyran 4-oxime (15). A solution of 6-methoxy-3,4-dihydro-*2H*-[1]benzothiopyran-4-one (**14**) (12.9 g, 66.5 mmol), hydroxylamine hydrochloride (9.9 g, 142 mmol) and pyridine (9.8 mL) in 100 mL of ethanol was heated to reflux under a nitrogen atmosphere for 35 min. After cooling to room temperature, about 2/3 of the ethanol was evaporated under reduced pressure. The resulting liquid was dissolved in 150 mL of 3% HCl, and the product was extracted with ethyl acetate (2 × 150 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure which yielded **15** as a white crystalline solid (13.7 g, 98%): mp 121 °C; EIMS *m/e* 209 (M^+); IR (KBr, cm^{-1}) 2830, 1593, 1481; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.91−2.98 (m, 2H), 3.13−3.19 (m, 2H), 3.82 (s, 3H), 6.86 (dd, $J_1 = 8.6$, $J_2 = 2.9$, 1H), 7.18 (d, $J = 8.6$, 1H), 7.46 (d, $J = 2.9$, 1H); ^{13}C NMR δ 26.1, 26.4, 55.4, 109.7, 117.2, 129.4, 153.8, 157.5, 172.0, 192.5. Anal. ($C_{10}H_{11}NO_2S$) C,H,N.

3-Amino-6-methoxy-3,4-dihydro-*2H*-[1]benzothiopyran-4-one Hydrochloride (16). A solution of **15** (13.7 g, 65.2 mmol) in 50 mL of pyridine was cooled to 0 °C, and treated with *p*-toluenesulfonyl chloride (54.0 g, 283 mmol) which was added slowly in 5 g portions. After complete addition, the reaction mixture was stirred 1 h at 0 °C and 2 h at room temperature. Ice (320 mL) was added, and after the ice had melted the mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed once with 1 N HCl, once with brine, and dried over $MgSO_4$. Concentration under reduced pressure yielded the tosyloxime as a brown powder (26.4 g, ca. 100%), which was used without further purification.

A suspension of the tosyl oxime (23.8 g, ca. 65 mmol) in 260 mL of toluene was added slowly to a cooled (0 °C) solution of potassium *tert*-butoxide (12.6 g, 113 mmol) in 90 mL of ethanol. The reaction mixture was stirred at 0 °C for 1 h, then stirred at room temperature for 1.5 h, and finally stirred at 35 °C for 30 min to complete te reaction. The precipitate (potassium tosylate) was removed by filtration, and concentrated hydrochloric acid (26.5 mL) was slowly poured to the filtrate, which turned dark purple-red. Ether was added to promote crystallization of the product, which was filtered off as a yellow powder and dried in an desiccator (12.8 g, 80%): mp 174 °C dec; IR (KBr, cm^{-1}) 2830, 1685, 1560, 1476; ^1H NMR (D_2O, 200 MHz) δ 3.39 (dd, $J_1 = 12.8$, $J_2 = 4.7$, 1H), 3.59−3.74 (m, 1H), 3.85 (s, 3H), 4.68−4.77 (m, 1H), 7.16 (m, 1H), 7.29 (d, $J = 10.3$, 1H), 7.51−7.53 (m, 1H); ^{13}C NMR δ 31.1, 45.0, 71.8, 72.1, 128.8, 139.6, 145.3, 145.5, 149.5, 173.3; HRMS calcd (obsd) for $C_{10}H_{11}NO_2S$ 209.0510 (209.0512).

***trans*- and *cis*-3-Amino-6-methoxy-3,4-dihydro-*2H*-[1]-benzothiopyran-4-ol Hydrochloride (17 and 18).** Amino ketone **16** (8.5 g, 34.7 mmol) was dissolved in a mixture of 135 mL of dichloromethane and 135 mL of 2-propanol. The solution was heated to 35 °C, and sodium borohydride (4 g, 105 mmol) was added at a rate of 0.5 g per 10 min. After complete addition, the reaction mixture was stirred 1 h at 35 °C and allowed to cool to room temperature. The reaction mixture was quenched by slowly adding 10% HCl until acidic pH, and the solvents were evaporated to dryness under reduced pressure. The remaining solids were dissolved in 250 mL of hot ethanol, the insoluble inorganic salts were removed by filtration, and the filtrate was concentrated under reduced pressure, which yielded a mixture of **17** and **18** as a red-brown foam (6.28 g, 73%): mp 160−180 °C dec; IR (KBr, cm^{-1}) 2928, 1560; ^1H NMR (D_2O, 200 MHz, chemical shifts of major diastereomer (trans, **17**) are given) δ 3.25 (dd, $J_1 = 13.7$, $J_2 = 6.8$, 1H), 3.50 (dd, $J_1 = 13.7$, $J_2 = 3.4$, 1H), 3.87 (s, 3H), 3.89−3.98 (m, 1H), 4.87 (d, $J = 6.4$, 1H), 6.97 (dd, $J_1 = 8.6$, $J_2 = 3.0$, 1H) 7.14 (d, $J = 3.0$, 1H), 7.19 (d, $J = 8.6$, 1H); ^{13}C NMR δ 41.8, 66.6, 72.0, 83.8, 131.6, 132.3, 138.5, 144.2, 149.3, 173.4; HRMS calcd (obsd) for $C_{10}H_{13}NO_2S$ 211.0667 (211.0687).

***trans*- and *cis*-3-Chloroacetamido-6-methoxy-3,4-dihydro-*2H*-[1]benzothiopyran-4-ol (19 and 20).** A mixture of amino alcohols **17**·HCl and **18**·HCl (6.0 g, 24.3 mmol) was dissolved in a well-stirred two-layer system of $NaHCO_3$ (5.7 g, 67 mmol) in 70 mL of water and 170 mL of ethyl acetate. The mixture was cooled to 0 °C and a solution of chloroacetyl chloride (2 mL, 25.4 mmol) in 50 mL of ethyl acetate was added dropwise. After the addition was completed the mixture was stirred for 30 min on ice and was allowed to warm to room temperature. The layers were separated and the aqueous layer was extracted once with ethyl acetate (100 mL). The combined organic layers were washed once with brine, dried over $MgSO_4$ and concentrated until crystallization started. The solution was put aside overnight, and the light-green crystalline solid was obtained by suction filtration, which yielded most of the *trans*-chloroacetamide **19** (4.78 g). The mother liquor contained a mixture of both diastereomers **19** and **20**, which were separated by MPLC on silica (initial eluent 100% hexane, final eluent hexane:ethyl acetate 1:1) yielding *cis*-chloroacetamide **20** as a light-brown oil (0.39 g, 6% yield, $R_f = 0.26$ in hexane:ethyl acetate 1:1), and the rest of the *trans*-chloroacetamide **19** (0.30 g, 78% total yield, $R_f = 0.21$ in hexane:ethyl acetate 1:1).

***trans*-3-Chloroacetamido-6-methoxy-3,4-dihydro-*2H*-[1]benzothiopyran-4-ol (19)**: mp 178−180 °C; EIMS *m/e* 287 (M^+); ^1H NMR ($CDCl_3$, 200 MHz) δ 2.83 (dd, $J_1 = 12.9$, $J_2 = 5.1$, 1H), 3.65 (dd, $J_1 = 13.3$, $J_2 = 2.3$, 1H), 3.79 (s, 3H), 4.01 (s, 2H), 4.48−4.60 (m, 1H), 4.62 (d, $J = 3.7$, 1H), 6.84 (dd, $J_1 = 8.7$, $J_2 = 2.8$, 1H), 6.90 (d, $J = 2.9$, 1H), 7.10 (d, $J = 8.6$, 1H), 7.24 (bd, 1H); ^{13}C NMR (CD_3OD, 125.7 MHz) δ 27.0, 42.5, 49.4, 55.2, 69.0, 115.7, 116.8, 123.4, 127.7, 135.2, 158.2, 168.3. Anal. ($C_{12}H_{14}NO_3SCl$) C,H,N.

***cis*-3-Chloroacetamido-6-methoxy-3,4-dihydro-*2H*-[1]-benzothiopyran-4-ol (20)**: ^1H NMR ($CDCl_3$, 200 MHz) δ 3.00 (dd, $J_1 = 10.9$, $J_2 = 3.4$, 1H), 3.13 (t, $J = 10.9$, 1H), 3.76 (s, 3H), 4.04 (s, 2H), 4.41−4.56 (m, 1H), 4.69 (d, $J = 2.7$, 1H), 6.79 (dd, $J_1 = 8.8$, $J_2 = 2.9$, 1H), 6.94 (d, $J = 2.9$, 1H), 7.04 (d, $J = 8.8$, 1H), 7.33 (bd, 1H); ^{13}C NMR δ 25.9, 42.3, 48.7, 55.2, 68.7, 115.4, 115.6, 122.1, 127.2, 134.5, 157.2, 166.5; HRMS calcd (obsd) for $C_{12}H_{14}NO_3SCl$ 287.0383 (287.0409).

(±)-*trans*-3,4,4a,10b-Tetrahydro-9-methoxy-2*H*,5*H*-[1]-benzothiopyrano[4,3-*b*]-1,4-oxazin-3-one (21). *trans*-Chloroacetamide **19** (4.9 g, 17.1 mmol) was dissolved in 320 mL of hot 2-propanol. After the solution had cooled to room temperature, a solution of sodium hydroxide (1.4 g, 35.8 mmol) in 2.7 mL of water was added. The reaction was followed on TLC and was complete after stirring for 2.5 h. The reaction mixture was neutralized with 4 N HCl and concentrated under reduced pressure. The remaining white solid, which contained both product and sodium chloride, was transferred to a Soxhlet thimble and extracted overnight in a Soxhlet apparatus with 500 mL of ethyl acetate. The ethyl acetate was concentrated to 100 mL, and lactam **21** was filtered off as very fine white crystals (2.9 g, 68% yield): mp 240 °C; EIMS *m/e* 251 (M^+); ^1H NMR ($(CD_3)_2SO$, 200 MHz) δ 2.99 (dd, $J_1 = 12.2$, $J_2 = 3.9$, 1H), 3.15 (t, $J = 12.0$, 1H), 3.26−3.48 (m, 1H), 3.69 (s, 3H), 4.27 (d, $J = 3.2$, 2H), 4.56 (d, $J = 9.8$, 1H), 6.79 (dd, $J_1 = 8.6$, $J_2 = 2.7$, 1H), 6.98−7.02 (m, 2H), 8.46 (s, 1H); ^{13}C NMR δ 28.1, 51.7, 55.4, 67.8, 74.4, 111.1, 115.0, 122.0, 126.7, 133.5, 157.0, 167.7. Anal. ($C_{12}H_{13}NO_3S$) C,H,N.

(±)-*cis*-3,4,4a,10b-Tetrahydro-9-methoxy-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazin-3-one (22). *cis*-Chloroacetamide **20** (0.32 g, 1.11 mmol) was dissolved in 21 mL of 2-propanol. A solution of sodium hydroxide (0.09 g, 2.3 mmol) in 0.18 mL of water was added, and the reaction was stirred for 19 h. The reaction mixture was neutralized with 4 N HCl and concentrated under reduced pressure. The remaining solids were dissolved in water (30 mL), and the product was extracted with ethyl acetate (2 × 30 mL). The combined organic layers were extracted with brine, dried over Na_2SO_4, and evaporated to dryness which yielded the *cis*-lactam **22** as a light-yellow solid (0.24 g, 86%). An analytical sample was obtained by recrystallization from ethyl acetate: mp 215−217 °C; EIMS *m/e* 251 (M^+); ^1H NMR ($CDCl_3$, 500 MHz) δ 2.80 (dd, $J_1 = 12.7$, $J_2 = 1.4$, 1H), 3.37 (t, $J = 12.7$, 1H), 3.80 (s, 3H), 3.90−3.93 (m, 1H), 4.37 (dd, $J_1 = 30.7$, $J_2 = 16.7$, 2H), 4.68 (d, $J = 3.0$, 1H), 6.85 (dd, $J_1 = 8.6$, $J_2 = 2.7$, 1H), 6.92 (d, $J = 2.7$, 1H), 7.07−7.08 (m, 2H); ^{13}C NMR δ 28.0, 51.2, 55.3, 67.6, 71.0, 116.6, 116.8, 123.3, 127.5, 131.0, 157.3, 168.5. Anal. ($C_{12}H_{13}NO_3S$) C,H,N.

(±)-*trans*-3,4,4a,10b-Tetrahydro-9-methoxy-2*H*,5*H*-[1]-benzothiopyrano[4,3-*b*]-1,4-oxazine (23). $LiAlH_4$ (1.1 g, 28.9 mmol) was suspended in 22 mL of dry ether. A suspension of the *trans*-lactam **21** (2.8 g, 11.3 mmol) in 220 mL of dry ether was added in 30 mL portions to the $LiAlH_4$ suspension. When the addition was completed, the reaction mixture was heated to reflux for 2 h. After cooling to room temperature and further cooling on ice, the reaction was quenched by the cautious addition of 1.1 mL of water, 1.1 mL of 4 N NaOH and 3.3 mL of water (in that order). The mixture was heated to reflux until all precipitates had turned white (15 min), cooled to room temperature, and filtered over Celite. The yellow filtrate was dried over Na_2SO_4 and concentrated under reduced pressure, which yielded **23** as a yellow oil which solidified upon cooling (2.6 g, 97%): mp 74−76 °C; EIMS *m/e* 237 (M^+); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.95 (br s, 1H), 2.68 (d, $J = 2.7$, 1H), 2.88−3.12 (m, 4H), 3.70−3.84 (m, 1H), 3.75 (s, 3H), 4.08 (dd, $J_1 = 11.0$, $J_2 = 3.7$, 1H), 4.20 (d, $J = 8.5$, 1H), 6.72 (dd, $J_1 = 8.5$, $J_2 = 2.9$, 1H), 6.97 (d, $J = 8.5$, 1H), 7.13 (d, $J = 2.7$, 1H); ^{13}C NMR δ 29.6, 45.8, 55.18, 55.21, 67.8, 78.1, 110.9, 114.6, 122.0, 126.6, 134.7, 157.3. Anal. ($C_{12}H_{15}NO_2S \cdot {}^1/_4H_2O$) C,H,N.

(±)-*cis*-3,4,4a,10b-Tetrahydro-9-methoxy-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazine (24). The procedure described above for the preparation of **23** was used for the reduction of the *cis*-lactam **22** (0.2 g, 0.80 mmol) to **24**, which was obtained as a light-yellow oil (0.18 g, 95%): ^1H NMR ($CDCl_3$, 200 MHz) δ 2.98−3.08 (m, 3H), 3.25 (dd, $J_1 = 12.9$, $J_2 = 7.3$, 1H), 3.45−3.48 (m, 1H), 3.59−3.64 (m, 2H), 3.78 (s, 3H),

4.63 (d, J = 3.7, 1H), 6.76 (m, 1H), 7.00−7.07 (m, 2H); ^{13}C NMR δ 29.2, 43.0, 49.8, 55.2, 63.4, 72.0, 114.6, 115.3, 124.0, 127.1, 133.5, 157.6; HRMS calcd (obsd) for $C_{12}H_{15}NO_2S$ 237.0823 (237.0829).

(±)-*trans*-3,4,4a,10b-Tetrahydro-4-[(*S*)-methoxyphenylacetyl]-9-methoxy-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazine, Diastereomeric Amides 25 and 26.[30] (*S*)-(+)-α-Methoxyphenylacetic acid (0.91 g, 5.5 mmol) and thionyl chloride (7.6 mL, 104 mmol) were dissolved in 26 mL of methylene chloride. The mixture was stirred and heated to reflux for 1 h, then the excess thionyl chloride and the solvent were evaporated. The resulting acid chloride (a clear oil) was redissolved in 25 mL of methylene chloride and added dropwise to a vigorously stirred solution of amine **23** (1.2 g, 5.0 mmol) in 25 mL of methylene chloride, layered with 50 mL of 5% NaOH (aq). After 20 min all starting amine was consumed, and the layers were separated. The organic layer was washed once with water, dried over Na_2SO_4 and concentrated under reduced pressure which yielded a yellow oil (1.9 g, 99%). The two diastereomeric amides were separated by careful and repeated MPLC on silica with diisopropyl ether. The combined fractions of the diastereomeric amide that eluted first (**25**) (R_f = 0.30, 100% diisopropyl ether) yielded a light-yellow oil (0.70 g, 36%) of 96% purity according to GLC and crystallized overnight: mp 128 °C. Anal. ($C_{21}H_{23}NO_4S$) C,H,N.

The diastereomeric amide that eluted second (**26**) (R_f = 0.21, 100% diisopropyl ether) was obtained as a light-yellow oil (0.62 g, 32%) of 96% purity according to GLC: HRMS calcd (obsd) for $C_{21}H_{23}NO_4S$ 385.1348 (385.1349).

(+)-(4a*S*,10b*R*)-3,4,4a,10b-Tetrahydro-9-methoxy-2*H*,5*H*-[1]benzothiopyrano-[4,3-*b*]-1,4-oxazine ((+)-23). The diastereomeric amide that eluted first (**25**) (0.60 g, 1.56 mmol) was dissolved in dry THF (53 mL). Potassium *tert*-butoxide (1.12 g, 9.75 mmol) and water (0.083 mL, 4.5 mmol) were added, and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was partitioned between ether and water, and the water layer was extracted once more with ether. The combined organic layers were extracted with 5% HCl (3 × 50 mL), the water phase was alkalinized (10% Na_2CO_3) and extracted with ether (3 × 50 mL). The combined organic layers were washed once with brine, dried over Na_2SO_4 and concentrated under reduced pressure, which yielded (+)-(**23**) as a light-yellow solid (0.34 g, 92%). A sample was converted to the hydrochloride and recrystallized from 100% ethanol, yielding off-white crystals: mp 238−242 °C; [α] = +51.6° (c = 1.36, MeOH). All spectral data for (+)-**23** were identical with those described for **23**. Anal. ($C_{12}H_{15}NO_2S$·HCl) C,H,N.

(−)-(4a*R*,10b*S*)-3,4,4a,10b-Tetrahydro-9-methoxy-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazine ((−)-23). The diastereomeric amide that eluted second (**26**) (0.60 g, 1.56 mmol) was converted to (−)-**23** (0.29 g, 78%) as described above for (+)-**23**. A sample was converted to hydrochloride, yielding off-white crystals: mp 234−236 °C; [α] = −48.3° (c = 4.62, MeOH). All spectral data for (−)-**23** were identical with those described for **23**. Anal. ($C_{12}H_{15}NO_2S$·HCl) C,H,N.

(±)-*trans*-3,4,4a,10b-Tetrahydro-9-methoxy-4-propyl-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazine (27). Amine **23** (0.72 g, 3.04 mmol) and cesium carbonate (2.95 g, 9.1 mmol) were suspended in dry acetonitrile (19 mL). 1-Iodopropane (0.83 mL, 8.3 mmol) was added and the mixture was heated to reflux overnight. The reaction mixture was concentrated under reduced pressure, water was added to the residue and the product was extracted with ether (2 × 100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure, which yielded a yellow oil. The product was purified with MPLC on silica (initial eluent 100% hexane, final eluent hexane:ethyl acetate = 4:1) yielding **27** as a light-yellow oil (0.65 g, 77%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.92 (t, J = 7.3, 3H), 1.49−1.57 (m, 2H), 2.32−2.37 (m, 1H), 2.48−2.54 (m, 1H), 2.55−2.59 (m, 1H), 2.75−2.81 (m, 1H), 2.88−2.95 (m, 2H), 3.15 (dd, J_1 = 12.4, J_2 = 4.0, 1H), 3.79 (s, 3H), 3.86−3.91 (m, 1H), 4.08−4.11 (m, 1H), 4.33 (d, J = 9.2, 1H), 6.72−6.75 (m, 1H), 6.99 (d, J = 8.6, 1H), 7.16 (dd, J_1 = 2.8, J_2 = 0.9, 1H); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 11.7, 18.6, 27.3, 51.4, 55.2, 55.4, 60.5, 66.8, 77.3, 110.8, 114.5, 121.7, 126.5, 135.6, 157.3; HRMS calcd (obsd) for $C_{15}H_{21}NO_2S$ 279.1293 (279.1286).

(−)-(4a*S*,10b*R*)-3,4,4a,10b-Tetrahydro-9-methoxy-4-propyl-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazine ((−)-27). Compound (+)-**23**·HCl (0.20 g, 0.73 mmol) was converted to (−)-**27** (0.20 g, 98%), as described above for **27**. A sample was converted to hydrochloride and recrystallized from 100% ethanol, yielding white crystals: mp 205−210 °C; [α] = −3.1° (c = 2.61, MeOH). All spectral data for (−)-**27** were identical with those described for **27**. Anal. ($C_{15}H_{21}NO_2S$·HCl) C,H,N.

(+)-(4a*R*,10b*S*)-3,4,4a,10b-Tetrahydro-9-methoxy-4-propyl-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazine ((+)-27). Compound (−)-**23**·HCl (0.24 g, 0.87 mmol) was converted to (+)-**27** (0.23 g, 95%), as described for **27**. A sample was converted to hydrochloride and recrystallized from 2-propanol, yielding pink crystals: mp 207−212 °C; [α] = +3.3° (c = 2.73, MeOH). All spectral data for (+)-**27** were identical with those described for **27**. Anal. ($C_{15}H_{21}NO_2S$·HCl) C,H,N.

(±)-*cis*-3,4,4a,10b-Tetrahydro-9-methoxy-4-propyl-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazine (28). Amine **24**·HCl (0.16 g, 0.59 mmol) was converted to **28** as described above for **27** and was obtained as a light-yellow oil (0.08 g, 49%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.94 (t, J = 7.3, 3H), 1.44−1.62 (m, 2H), 2.47−2.72 (m, 5H), 3.28 (dd, J_1 = 11.5, J_2 = 2.0, 1H), 3.49 (t, J = 11.7, 1H), 3.77 (s, 3H), 3.91−3.97 (m, 2H), 4.45 (s, 1H), 6.76 (dd, J_1 = 8.5, J_2 = 2.7, 1H), 6.85 (d, J = 2.4, 1H), 7.01 (d, J = 8.5, 1H); ^{13}C NMR δ 11.5, 18.6, 19.9, 46.4, 55.2, 55.9, 57.3, 67.5, 75.0, 115.9, 116.8, 123.9, 126.9, 133.8, 156.9; HRMS calcd (obsd) for $C_{12}H_{15}NO_2S$ 279.1293 (279.1282).

(±)-*trans*-4-*n*-Propyl-3,4,4a,10b-tetrahydro-2*H*,5*H*-[1]-benzothiopyrano[4,3-*b*]-1,4-oxazin-9-ol (9). Amine **27** (0.35 g, 1.25 mmol) was dissolved in 3.5 mL of ethanethiol, and the solution was cooled on ice. Aluminum chloride (0.50 g, 3.8 mmol) was added as 3 portions of 0.17 g, with an interval of 30 min. After all the aluminum chloride was added, the reaction mixture was stirred on ice for 1 h. The reaction was quenched with water, alkalinized (10% $NaHCO_3$) and extracted with ethyl acetate, adding sodium chloride to enhance layer separation. The combined organic layers were washed once with brine, dried over Na_2SO_4 and concentrated to give **9** as a light-yellow solid (0.26 g, 78%). A sample was converted to hydrochloride and recrystallized from ethanol 100%, yielding off-white crystals: mp 263−267 °C dec; EIMS m/e 265 (M^+); IR (KBr, cm^{-1}) 3099, 2553, 1474, 1324, 1105; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.91 (t, J = 7.3, 3H), 1.49−1.57 (m, 2H), 2.33−2.39 (m, 1H), 2.48−2.53 (m, 1H), 2.56−2.60 (m, 1H), 2.76−2.82 (m, 1H), 2.88−2.96 (m, 2H), 3.12 (dd, J_1 = 12.5, J_2 = 4.2, 1H), 3.86−3.91 (m, 1H), 4.05 (dd, J_1 = 11.2, J_2 = 2.4, 1H), 4.33 (d, J = 9.3, 1H), 6.64 (dd, J_1 = 8.3, J_2 = 2.4, 1H), 6.93 (d, J = 8.3, 1H), 7.06 (d, J = 2.4, 1H); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 11.8, 18.6, 27.3, 51.5, 55.5, 60.8, 66.8, 77.2, 113.4, 115.3, 121.5, 126.8, 135.6, 153.4. Anal. ($C_{14}H_{19}NO_2S$·HCl) C,H,N.

(−)-*trans*-(4a*S*,10b*R*)-3,4,4a,10b-Tetrahydro-4-propyl-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazin-9-ol ((−)-9). Amine (−)-**27** (0.20 g, 0.72 mmol) was converted to (−)-**9** (0.18 g, 94%) as described above for **9**. The product was converted to hydrochloride and recrystallized from 100% ethanol yielding pink crystals: mp 250−260 °C dec; [α] = −8.8° (c = 2.49, MeOH). All spectral data for (−)-**9** were identical with those described for **9**. Anal. ($C_{14}H_{19}NO_2S$·HCl) C,H,N.

(+)-*trans*-(4a*R*,10b*S*)-3,4,4a,10b-Tetrahydro-4-propyl-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazin-9-ol ((+)-9). Amine (+)-**27** (0.21 g, 0.72 mmol) was converted to (+)-**9** (0.19 g, 96%) as described above for **9**. The product was converted to hydrochloride and recrystallized from 100% ethanol yielding pink crystals: mp 251−255 °C dec; [α] = +10.1° (c = 2.76, MeOH). All spectral data for (+)-**9** were identical with those described for **9**. Anal. ($C_{14}H_{19}NO_2S$·HCl) C,H,N.

(±)-*cis*-3,4,4a,10b-Tetrahydro-4-propyl-2*H*,5*H*-[1]benzothiopyrano[4,3-*b*]-1,4-oxazin-9-ol (10). Amine **28** (0.09 g, 0.32 mmol) was converted to **10** (0.080 g, 94%) as described above for **9**. The product was converted to hydrochloride and

recrystallized from 100% ethanol yielding a pink solid: mp 189−192 °C dec; EIMS *m/e* 265 (M^+); IR (KBr, cm^{-1}) 2579, 2498, 2411, 1480, 1276; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.95 (t, J = 7.2, 3H), 1.52−1.60 (m, 2H), 2.52−2.64 (m, 3H), 2.67−2.76 (m, 2H), 3.29−3.33 (m, 1H), 3.48 (t, J = 11.9, 1H), 3.91−3.98 (m, 2H), 4.44 (s, 1H), 6.68 (dd, J_1 = 8.6, J_2 = 2.6, 1H), 6.76 (d, J = 2.6, 1H), 6.96 (d, J = 8.6, 1H); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 12.4, 19.5, 20.7, 47.3, 56.8, 58.1, 68.1, 75.5, 117.6, 119.8, 124.4, 127.8, 134.5, 153.7. Anal. ($C_{14}H_{19}NO_2S\cdot HCl\cdot^1/_4H_2O$) C,H,N.

(±)-*trans*-4-*n*-Propyl-3,4,4a,10b-tetrahydro-2*H*,5*H*-[1]-benzothiopyrano[4,3-*b*]-1,4-oxazin-9-ol *S*-Oxide (11). $NaIO_4$ (0.083 g, 0.39 mmol) was dissolved in 2 mL of a 1/1 mixture of water and methanol, and the solution was cooled to 0 °C on ice. A suspension of sulfide **9** (0.073 g, 0.28 mmol) in 1 mL of methanol, was added dropwise, and the reaction mixture was stirred on ice for 2 h. The reaction was monitored on TLC. To complete the oxidation extra $NaIO_4$ (0.038 g, 0.18 mmol) was added and the mixture was stirred for another 0.5 h. The reaction mixture was extracted with chloroform (3 × 5 mL), and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The remaining brown oil was purified with MPLC on silica (initial eluent hexane:ethyl acetate = 1:1, final eluent 100% ethyl acetate) yielding **11** as an off-white oil (0.023 g, 29%). The product was converted to hydrochloride and recrystallized from 2-propanol/isopropyl ether to yield white crystals: mp 236−240 °C dec; IR (KBr, cm^{-1}) 2972, 1596, 1475, 1115, 1023; ^{1}H NMR ($CDCl_3$, 500 MHz, hydrochloride) δ 1.08 (t, J = 7.4, 3H), 1.80−1.90 (m, 2H), 3.19−3.25 (m, 1H), 3.33−3.38 (m, 1H), 3.45−3.55 (m, 2H), 3.73 (d, J = 12.7, 1H), 3.97−4.06 (m, 1H), 4.08−4.17 (m, 1H), 4.24 (t, J = 12.5, 1H),4.37 (d, J = 10.6, 1H), 4.90 (d, J = 9.5, 1H), 6.98 (dd, J_1 = 8.7, J_2 = 2.4, 1H), 7.21 (dd, J_1 = 2.7, J_2 = 1.1, 1H), 7.62 (d, J = 8.5, 1H); HRMS calcd (obsd) for $C_{14}H_{19}NO_3S$ 281.1086 (281.1100).

Pharmacology, General. All experimental drugs tested were in the hydrochloride form.

Receptor Binding. The receptor binding studies were performed at Parke Davis Pharmaceutical Research Division, by T. A. Pugsley. Binding to cloned human DA D_2, D_3, and D_4 receptors was carried out as described in ref 22. Binding to rat hippocampal $5HT_{1A}$ and cortical $5HT_2$ receptor binding was carried out as described in ref 35.

Intrinsic Activity. The intrinsic activity determinations were performed at Pharmacia & Upjohn, CNS Diseases Research, by M. E. Lajiness, as described in refs 47, 48.

Contralateral Turning in 6-OH-DA Lesioned Rats. Contralateral turning experiments were performed at Parke Davis Pharmaceutical Research Division, by L. Meltzer, essentially according to the original reference by Ungerstedt and Arbuthnott.[51] Briefly, rats were lesioned in the right medial forebrain bundle (P4.8 mm, L1.1 mm, V−8.2 mm from bregma) with 8 μg/4 μL 6-hydroxydopamine HBr in saline with ascorbic acid 1 mg/mL added. After 3 weeks recovery, completeness of lesion was assessed with apomorphine 50 μg/kg sc. Only animals rotating more than 100 turns/h were used in subsequent experiments.

Rats were removed from home cages in morning, weighed, dosed and placed into harnesses in rotorat apparatus. Rats sat in stainless steel, flat-bottomed, hemispheric bowls and were connected via the harness and a flexible spring tether to an automated data collection system. Data are presented as full rotations in contraversive directions. Rats were used once weekly.

Locomotor Activity and Behavior in Reserpinized Rats. Locomotor activity experiments were performed at the Department of Medicinal Chemistry, University of Groningen, by L. A. van Vliet and N. Rodenhuis. Male Wistar rats (from Harlan, Zeist, The Netherlands) weighing 180−200 g were used. The rats were housed in PMMA 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%; 8 a.m. to 8 p.m., 8 p.m. to 8 a.m. light−dark periods). The animals were housed at least 1 week after arrival prior to use in the experiments. Experimental drugs were first dissolved in a few drops of ethanol 96% and slightly warmed, then saline was added. The administered volume was 1 mL/kg.

Rats were reserpinized 18−24 h prior to the experiments with 10 mg/kg sc reserpine (RBI). Reserpine was dissolved in 10% glacial acetic acid, 80% ultrapure water and 10% sucrose (MERCK). On the day of the experiments the animals were placed alone in PMMA boxes to acclimate. After the rats were acclimated the compounds were administered subcutaneously in the neck region, after which the locomotor activity counting was started. Locomotor activity was measured using AUTOMEX II (Columbus Instruments, Columbus, OH) activity monitors. Observations of behavior were made during the first 30 min of the locomotor activity measurements.

Microdialysis in Rat Striatum. Microdialysis experiments were performed at the Department of Medicinal Chemistry, University of Groningen, by N. Rodenhuis. Male Wistar rats (from Harlan, Zeist, The Netherlands) weighing 280−320 g were used and housed as described for the locomotor activity experiments.

On-line brain microdialysis in freely moving animals was essentially performed as described previously by Westerink.[55] Briefly, rats were anesthetized with chloral hydrate (400 mg/kg ip) and 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 in a horizontal position. The skull was exposed and burr holes were drilled. An Y-shaped cannula was used for the experiments, with an exposed tip length of 3 mm. The dialysis tube (i.d.: 0.22 mm; o.d.: 0.31 mm) was prepared from polyacrylonitrile methalys sulfonate copolymer (AN 69, Hospal, Bologna, Italy). The dialysis membrane was implanted in the striatum with coordinates which were calculated relative to bregma: A1, L±3, D−6, according to Paxinos and Watson (1982). The dura was removed with a sharp needle. Two anchor screws were positioned in different bone plates nearby. Before insertion into the brain the dialysis probes were perfused with successively ultrapure water, methanol, ultrapure water and Ringer solution (1.2 mM Ca). The dialysis probe was positioned in the burr hole under stereotaxic guidance. The probe was cemented in this position with phosphatine dental cement (Associated Dental Products Ltd., Kemdent Works, Purdon, Swinden, Wiltshire SN5 9HT, U.K.).

The experiments were performed in conscious rats 17−56 h after implantation of the cannula. The striatum was perfused with a Ringer solution (147 mM NaCl, 4 mM KCl, 1.2 mM $CaCl_2$, 1.1 mM $MgCl_2$) at 2 μL/min (CMA/102 microdialysis pump). 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.

Dopamine, DOPAC, and 5-HIAA were quantitated by HPLC with electrochemical detection. An HPLC pump (LKB, Pharmacia) was used in conjugation with an EC detector (Antec, Leiden) working at 625 mV versus Ag/AgCl reference electrode. The analytical column was a Supelco Supelcosil LC-18 column (15 cm, 4.6 mm, 3 μm). The mobile phase consisted of a mixture of 4.1 g/L sodium acetate (Merck), 85 mg/L octanesulfonic acid (Aldrich), 50 mg/L EDTA (Merck), 8.5% methanol (Labscan) and ultrapure water (pH = 4.1 with glacial acetic acid).

Statistics. The microdialysis data were analyzed using Friedman repeated measures analysis of variance on ranks with as post hoc test Dunnett's method.

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Supporting Information Available: X-ray analysis, crystal data, and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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