Synthesis and Evaluation of Pharmacological and Pharmacokinetic Properties of Monopropyl Analogs of 5-, 7-, and 8-[[(Trifluoromethyl)sulfonyl]oxy]-2-aminotetralins: Central Dopamine and Serotonin Receptor Activity

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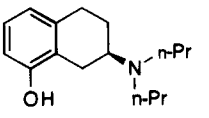
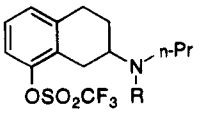
In order to explore further the structure–activity relationships of serotonergic and dopaminergic ligands, a series of enantiopure 5-, 7-, or 8-triflate (-OTf)-substituted 2-(monopropylamino)-tetralins have been synthesized and evaluated in *in vitro* binding and *in vivo* biochemical and behavioral assays in rats. Consequently, the 8-OTf-substituted compound *R*-(+)-**6** was found to be a potent and selective 5-HT_{1A} (5-hydroxytryptamine) receptor agonist inducing a full-blown 5-HT syndrome in normal rats, while the corresponding 5-OTf-substituted compound *S*-(−)-**12** was found to be a preferential dopamine (DA) autoreceptor agonist. A partial 5-HT syndrome was also observed for *S*-(−)-**12**, while the corresponding *R*-(+)-**12** was found to be inactive at the DA and 5-HT receptors both *in vitro* and *in vivo*. Compounds **6** and **12** were found to be major urinary metabolites following oral administration of their dipropyl analogs (**2** and **13**, respectively). Thus **6** was proposed to be the metabolite responsible for the full-blown 5-HT syndrome seen after oral (but not subcutaneous) administration of **2**. Similarly, **12** was proposed to be the metabolite responsible for the partial 5-HT syndrome seen after oral (but not subcutaneous) administration of **13**. The bioavailability of *R*-(+)-**6** (7.6 ± 1.1%) appeared to be slightly lower than that of **2** (11.2 ± 5.2%), although the *in vitro* metabolism of *R*-(+)-**6** appeared to be slower than that of **2**. Therefore first-pass metabolism was not thought to be the reason for the lower bioavailability of *R*-(+)-**6**, as compared to **2**.

Introduction

8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, *R*-(+)-**1**) is a potent, efficacious, centrally active 5-hydroxytryptamine (5-HT) receptor agonist with pronounced selectivity for 5-HT_{1A} receptor sites.[1-3] The oral activity of **1** in the rat is low,[4] mainly due to extensive first-pass elimination *via* O-glucuronidation.[5] The triflate (-OSO_2CF_3, -OTf) derivative of **1** (**2**) has been reported to retain high affinity for this receptor subtype.[6] Recently, this compound was reported to be less potent *in vivo* than its phenolic analog *R*-(+)-**1**, but it possessed higher absolute oral bioavailability (11% vs 2.5%).[7] Interestingly, in the *in vivo* biochemistry assays, compound **2** was found to be more potent after oral (po) than subcutaneous (sc) administration, suggesting the formation of active metabolite(s). The monopropyl analog **6** was found to be the major metabolite (in rat hepatocytes) and was subsequently found to be more potent *in vivo* than compound **2**.[7] These findings may be important contributions to the structure–activity relationship (SAR) studies of compounds with affinity and efficacy at the 5-HT_{1A} receptors. The *N*-substituent studies performed so far have been restricted to structures having a hydrogen, a hydroxy, or a methoxy group in the C8-position of the 2-aminotetralin moiety.[3,8-10] The general trend in these series is that the N,N-dialkylated aminotetralins are more potent than the monoalkylated analogs. This trend is also valid for the 5-substituted aminotetralin dopamine (DA) agonists. However no such data for the 7-substituted aminotetralins have been reported.[11,12]



R-(+)-1



2: R = n-Pr
6: R = H

In order to explore further the SAR of serotonergic and dopaminergic 2-aminotetralins, we have now prepared and pharmacologically evaluated a series of enantiopure 5-, 7-, and 8-OTf-substituted 2-(monopro-

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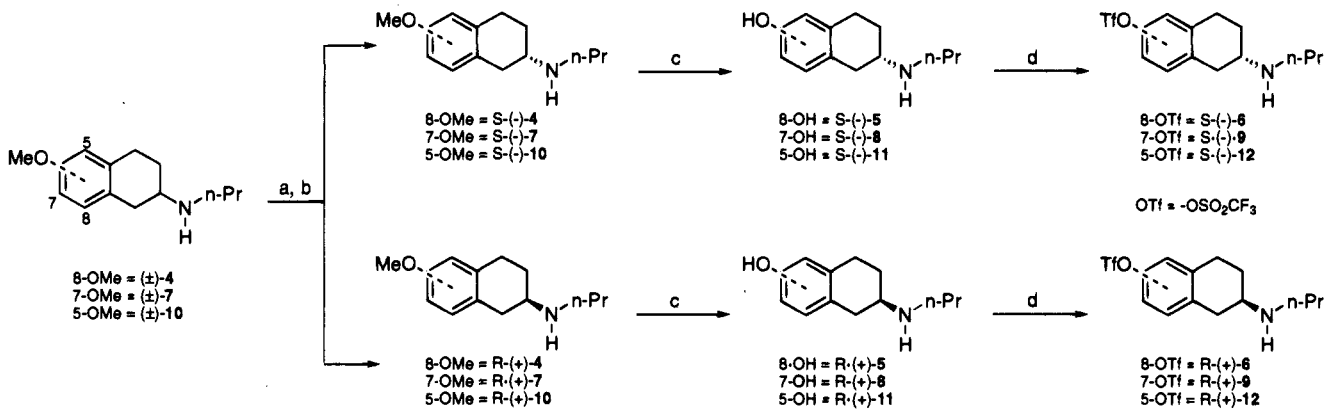
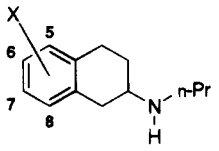
Scheme 1[a]



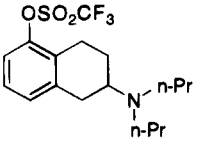
[a] Reagents and conditions: (a) (*R*)-(+)-2-chlocyphos or (*S*)-(−)-2-chlocyphos, 2-propanol or EtOH; (b) NAOH, CH_2Cl_2; (c) 48% HBr, 120 °C; (d) $TBAHSO_4$, NaOH, CH_2Cl_2, Tf_2NPh.

pylamino)tetralins (MPATs), as represented by generic structure **3**. The newly synthezised compounds have been screened in a biological system, involving *in vitro* methods using receptor binding techniques, and *in vivo* by monitoring behavioral activity and central biochemical effects (DA and 5-HT synthesis rates).



3: Generic Formula
X = OSO_2CF_3

Another objective of this study was to gain further insight into the pharmacokinetic properties of these triflate-substituted aminotetralins. This was achieved by measuring the plasma concentrations of *R*-(+)-**6** after po and intravenous (iv) administration to rats. In order to confirm the metabolic findings from the previous *in vitro* studies, urine samples from animals given **2** orally were collected and analyzed for metabolites. We have also studied the *in vivo* metabolism of 5-triflate-2-(dipropylamino)tetralin (**13**) since po administration of this compound produced some 5-HT effects which were not seen after sc administration. We have previously speculated that these effects could emanate from the corresponding primary amine.[7] The *in vitro* metabolism of *R*-(+)-**6** has also been investigated in a rat isolated hepatocyte system.[13]



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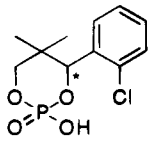
Chemistry

The syntheses of the pure enantiomers of compounds **6**, **9**, and **12** are outlined in Scheme 1, and their physical data are presented in Table 1. The racemic **4**, **7**, and **10** were prepared from the corresponding methoxy-2-tetralones by a reductive amination reaction using propylamine and sodium cyanoborohydride.[14,15] The enantiomers of compound **4** have been synthesized from the resolved 2-(benzylamino)-8-methoxytetralin which has been resolved by using the conventional recrystallization technique.[3] Compounds **7** and **10** have previously been resolved by formation of diastereomeric amides, which then have been separated by conventional column chromatography on SiO_2.[16]

A chiral cyclic phosphoric acid (**14**, 2-chlocyphos) was reported to be an efficient resolving agent for the resolution of amines.[17] It was also stated that compound **10** has been resolved successfully by one crystallization of the diastereomeric salt obtained with **14**. However, no yields or other experimental data were reported.[17]

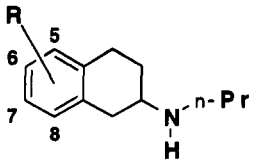


14: 2-chlocyphos

As a part of this study, we have investigated the direct separation of the enantiomers of compounds **4**, **7**, and **10**. This has been achieved efficiently using both enantiomers of **14**. Thus, by means of *R*-(+)-**14** the separation of *R*-(+)-**4** (15%) was achieved in two recrystallizations from 2-propanol, while subsequently *S*-(−)-**4** (15%) was separated from the mother liquor by *S*-(−)-**14**. The enantiomeric excess (>99.5%) was determined by HPLC analysis using a chiral column (Daicel Chiracel OD). In addition, the HCl salts of the corresponding free bases exhibited optical rotations matching those reported in the literature.[3]

Using *R*-(+)-**14**, *S*-(−)-**10** was achieved in one crystallization (43%). *R*-(+)-**10** was achieved from the mother liquor by the addition of *S*-(−)-**14** (35%). *R*-(+)-**7** was obtained first with *S*-(−)-**14** in an analytical scale. Using this pure diastereomeric salt as seeding crystals, *R*-(+)-**7** was achieved in 34% yield after two crystallizations on a large scale. The choice of seeding crystals is important. It is possible to influence the precipitation by adding one of the pure diastereomeric salts. From the mother liquor, *S*-(−)-**7** was obtained in 37% yield by using *R*-(+)-**14**. The enantiomeric excess of the amines **7** and **10** could be determined through ^{31}P NMR spectroscopy forming the adduct of the amines with the

Table 1. Physical data of some Novel 2-Aminotetralin Derivatives



no.	R	mp, °C	yield, %	formula[a]	$[\alpha]^{25}_D$,[b] deg
R-(+)-**4**	8-OMe	base	15	$C_{14}H_{21}NO$	+76.4
S-(−)-**4**	8-OMe	base	15	$C_{14}H_{21}NO$	−77.6[d]
R-(+)-**5**	8-OH	283–286	78	$C_{13}H_{19}NO$·HBr	+63.5
S-(−)-**5**	8-OH	273–277	76	$C_{13}H_{19}NO$·HBr	−64.5
R-(+)-**6**	8-OSO_2CF_3	238–240	68	$C_{14}H_{18}F_3NO_3S$·HCl	+61.5
S-(−)-**6**	8-OSO_2CF_3	235–238	66	$C_{14}H_{18}F_3NO_3S$·HCl	−61.6
R-(+)-**7**	7-OMe	257–260	34	$C_{14}H_{21}NO$·HCl	+75.2[e]
S-(−)-**7**	7-OMe	255–257	37	$C_{14}H_{21}NO$·HCl	−75.1[f]
R-(+)-**8**	7-OH	215–224	79	$C_{13}H_{19}NO$·HBr	+57.8
R-(+)-**9**	7-OSO_2CF_3	206–208	34	$C_{14}H_{18}F_3NO_3S$·HCl	+50.6
S-(−)-**10**	5-OMe	279–283	43	$C_{14}H_{21}NO$·HCl	−65.2[g]
R-(+)-**10**	5-OMe	274–278	35	$C_{14}H_{21}NO$·HCl	+72.4[h]
S-(−)-**11**	5-OH	249–251	45	$C_{13}H_{19}NO$·HBr	−58.4[i]
R-(+)-**11**	5-OH	246–248	89	$C_{13}H_{19}NO$·HBr	+57.4[j]
S-(−)-**12**	5-OSO_2CF_3	232–233	59	$C_{14}H_{18}F_3NO_3S$·HCl	−62.4
R-(+)-**12**	5-OSO_2CF_3	239–241	45	$C_{14}H_{18}F_3NO_3S$·HCl	+73.3[k]

[a] Empirical formula; errors for elemental analysis of all compounds analyzed were within 0.4% of theory for C, H, N. [b] CH_3OH, *c* 1.0. [c] Lit.[3] $[\alpha]_D$ +78.3° (*c* 1.05). [d] Lit.[3] $[\alpha]_D$ −77.0° (*c* 1.03). [e] Lit.[16] $[\alpha]_D$ +70.2° (*c* 1.0). [f] Lit.[16] $[\alpha]_D$ −72.6° (*c* 1.0). [g] Lit.[16] $[\alpha]_D$ -63° (*c* 1.0). [h] *c* 0.42 (lit.[16] $[\alpha]_D$ +68.2° (*c* 1.0). [i] *c* 0.76. [j] *c* 0.272. [k] *c* 0.172.

Table 2. Affinities at Central D_2, D_3, 5-HT_{1A}, and 5-HT_{1D} ($\alpha + \beta$)-Sites *in Vitro*

	$K_i \pm$ SEM, nM[a]				
no.	D_2[b,c] [^{3}H]spiperone	D_2[b,c] [^{3}H]U86170	D_3[b,c] [^{3}H]spiperone	5-HT_{1A}[b,c] [^{3}H]-8-OH-DPAT	5-HT_{1D}[b,c] [^{3}H]-5-HT
R-(+)-**1**	>413	90 ± 4	179 ± 19	0.5 ± 0.02	164 ± 30 α 638 ± 75 β
(±)-**2**	>413	62 ± 7	273 ± 81	0.8 ± 0.1[d]	12 ± 4 α 127 ± 26 β
(±)-**6**	>413	108 ± 12	691 ± 71	2.8 ± 0.4	15 ± 1 α 169 ± 17 β
R-(+)-**6**	311 ± 18	69 ± 4	524 ± 108	1.3 ± 0.3	6.7 ± 0.5 α 138 ± 22 β
S-(−)-**6**	>413	225 ± 14	1570 ± 301	13 ± 7	157 ± 15 α 1255 ± 344 β
R-(+)-**9**	NT[e]	50 ± 5	359 ± 61	222 ± 33	125 ± 11 α 340 ± 62 β
S-(−)-**12**	115 ± 29	8.7 ± 1	15 ± 2	10 ± 0.5	63 ± 3 α 130 ± 10 β
R-(+)-**12**	NT	>3800	120	>1000	>1500 α >1500 β

[a] K_i values for displacement of the dopamine D_2 receptor antagonist spiperone, the dopamine D_2 receptor agonist U86170, the dopamine D_3 receptor antagonist spiperone, the 5-HT_{1A} receptor agonist 8-OH-DPAT, and the 5-$HT_{1D\alpha/\beta}$ agonist 5-HT. [b] Data from cloned mammalian receptors expressed in CHO-k1 cells. [c] Values were obtained with 11 drug concentrations in which each value was determined in duplicate. [d] Have been tested earlier for 5-HT_{1A} affinity in homogenized rat brain tissue and displayed a 10-fold lower affinity compared to cloned mammalian receptors (ref 7). [e] NT means not tested.

acid chloride of *S*-(−)-**14** and was greater than 99.5%.[17] The decoupled ^{31}P NMR spectrum showed a 0.09 ppm difference between the diastereomeric amides. This acid chloride was readily prepared by the reaction of phosphorus pentacloride with *S*-(−)-**14**.

The enantiomerically pure phenols **5**, **8**, and **11** were prepared from the corresponding methoxy compounds through demethylation in 48% aqueous HBr. Recently the synthesis of racemic **6** from the corresponding phenol using triflic anhydride in the presence of triethylamine was reported.[7] This method was not suitable since a mixture of O- and N-triflated products (the triflate group is substituted on the amine site) was obtained. However, using phase-transfer conditions, it was possible to direct the triflation to the oxygen. Thus, using *N*-phenyltrifluoromethanesulfonimide and tetrabutylammonium hydrogen sulfate in dichloromethane in the presence of 10% sodium hydroxide solution, it was possible to obtain enantiomerically pure **6** and **12** in 59–68% yield. However, the yield of compound **9** was only 34%.

Pharmacology

***In Vivo* Biochemistry.** The *in vivo* biochemical test utilizes the well-established phenomenon of receptor-mediated feedback inhibition of the presynaptic neuron.[18] The synthesis rate of the catecholamines dopamine and norepinephrine (NE) is decreased by agonists and increased by antagonists via action at dopaminergic and α-adrenergic receptors, respectively. Similarly, the synthesis rate of 5-HT is inhibited by 5-HT_{1A} receptor agonists.[19–21] 5-Hydroxytryptophan accumulation (5-HTP), following decarboxylase inhibition by (3-hydroxybenzyl)hydrazine (NSD 1015), was used as an indicator of the 5-HT synthesis rate in the three brain areas (Table 3). In addition, the DOPA accumulation was

Table 3. Effects on Rat Brain DA and 5-HT Synthesis Rates (DOPA and 5-HTP Accumulation) *in Vivo* in Reserpine-Pretreated and Nonpretreated Rats

	reserpine-pretreated rats,[a] ED_{50}, μmol/kg[b] (pED_{50})						nonpretreated rats % of ctrl[c]			
	DOPA acc[d]			5-HTP acc[e]			DOPA acc		5-HTP acc	
compd	limb	stri	hem	limb	stri	hem	limb	stri	limb	stri
(+)-**1** sc	P[f] (50)	P (50)	P (50)	0.036[g]	0.047[g]	0.05[g]	94 ± 6[h]	80 ± 6** [h,i]	50 ± 3*** [h,j]	48 ± 4*** [h]
2 sc	P (50)	P (50)	P (50)	8.3 (5.08 ± 0.32)	26.9[k] (4.57 ± 0.24)	13.8[k] (4.86 ± 0.19)	NT[l]	NT	NT	NT
(+)-**6** sc	I[m] (12.5)	I (12.5)	I (12.5)	0.50 (6.30±0.28)	0.62 (6.21±0.65)	0.93 (6.03±0.20)	114 ± 10[n]	102 ± 5[n]	39 ± 4*** [n]	44 ± 3*** [n]
(+)-**6** po	NT	NT	NT	NT	NT	NT	91 ± 5[n]	86 ± 3[n]	63 ± 6*** [n]	62 ± 4*** [n]
(−)-**6** sc	I (50)	P (50)	I (50)	24.0[k] (4.62 ± 0.42)	24.0[k] (4.62 ± 0.45)	18.2[k] (4.74 ± 0.21)	103 ± 3°	81 ± 4** °	52 ± 3*** °	53 ± 3*** °
(+)-**9** sc	I (50)	I (50)	I (50)	P (50)	P (50)	P (50)	NT	NT	NT	NT
(−)-**12** sc	0.83[k] (6.08 ± 0.18)	1.1[k] (5.96 ± 0.16)	P (3.1)	P (12.5)	P (12.5)	P (12.5)	48 ± 3*** [n]	53 ± 1*** [n]	59 ± 3*** [n]	52 ± 3*** [n]
(+)-**12** sc	NT	NT	NT	NT	NT	NT	78 ± 2** [n]	98 ± 1[n]	101 ± 6[n]	83 ± 6[n]

[a] The animals received reserpine 18 h before the test drug, 60 min and NSD 1015 30 min before decaptitation. Shown are the values producing a half-maximal decrease in the accumulation of DOPA and 5-HTP in the limbic, striatal and hemispheral brain areas. [b] ED_{50} values were calculated by fitting a sigmoidal curve to the dose responses according to the method described in ref 38. Given are ED_{50} in μmol/kg and in parentheses pED_{50} with confidence limits 95%. [c] The values are percent of control, means ± SEM (n = 16 and 4 in the control and tested groups, respectively). [d] DOPA accumulation (3,4-dihydroxyphenylalanine). [e] 5-HTP accumulation (5-hydroxytryptophan). [f] P means partial response at the highest dose tested (given in parentheses as μmol/kg). [g] Data taken from ref 3. [h] Dose 0.25 μmol/kg. [i] $^{**}p < 0.01$ vs saline-treated controls. [j] $^{***}p < 0.005$ vs saline-treated controls. [k] Dose−response curve not fully covered. In these cases either the slope or the maximal decrease value was fixed in order to obtain an ED_{50} with confidence limits. [l] NT means not tested. [m] I means inactive at the highest dose tested (given in parentheses as μmol/kg). [n] Dose 25 μmol/kg. ° Dose 50 μmol/kg.

Table 4. Locomotor Activity in Reserpinized and Nonpretreated Rats

compd	counts/30 min,[a] mean ± SEM (dose)[e]	LMA,[b] % of ctrl (dose)	5-HT syndrome[c]		DA syndrome[d]
			normal[f]	reserpine	reserpine
ctrls	125 ± 30	100	0/25	0/12	0/12
R-(+)-**1** sc	NT[g]	64 ± 7** [h] (0.25)[i]	4/4 (0.25)	4/4 (0.25)	0/4 (0.25)
R-(+)-**6** sc	672 ± 136*** [j] (1.6)	79 ± 9 (25)	4/4 (25)	4/4 (1.6)	0/4 (1.6)
R-(+)-**6** po	NT	58 ± 8 (25)	1/4[m] (25)	NT	NT
S-(−)-**6** sc	1492 ± 436*** (50)	61 ± 6** (50)	0/4 (50)	4/4[k] (50)	0/4 (50)
R-(+)-**9** sc	2153 ± 650*** (50)	NT	NT	4/4[l] (50)	4/4 (50)
S-(−)-**12** sc	785 ± 123** (12.5)	18 ± 6*** (25)	0/4 (25)	4/4[m] (12.5)	4/4[n] (12.5)
R-(+)-**12** sc	NT	130 ± 15 (25)	0/4 (25)	NT	NT

[a] Reserpine-pretreated rats. [b] Locomotor activity in nonpretreated rats. [c] Shown is the number of rats displaying the 5-HT syndrome (flat body posture, reciprocal forepaw treading "pianoplaying", straub tail). The gross behavior was observed during the motility recordings. [d] Shown is the number of rats displaying the DA syndrome (sniffing, stilts, licking, grooming). The gross behavior was observed during the motility recordings. [e] μmol/kg. [f] Nonpretreated rats. [g] NT means not tested. [h] $^{**}p < 0.01$. [i] Dose μmol/kg. [j] $^{***}p < 0.005$. [k] Rats only exhibited flat forebody posture and piano playing. [l] Rats only exhibit flat body posture. [m] Rats only exhibited flat forebody posture. [n] Rats also exhibited jerks.

used as an indicator of the DA synthesis rate in the DA-rich areas (i.e., the limbic system and corpus striatum)[22] and the NE synthesis rate in the NE-rich hemispheres (mainly cortex). For this study we used reserpine-pretreated rats (5 mg/kg sc, 18 h). This model is designed to detect directly acting agonists (with various degrees of intrinsic activity) at central monoamine receptors through both biochemical and behavioral effects. Compounds *R*-(+)-**6** and *S*-(−)-**12** were also evaluated in nonpretreated rats in order to study their *in vivo* efficacy as well as their potential antagonistic effects.

Locomotor Activity and Gross Behavioral Observations. Postsynaptic agonistic effects of the test compounds were assessed by the increase in locomotor activity (reversal of reserpine-induced hypokinesia). 5-HT_{1A} agonists induce the 5-HT behavioral syndrome (flat body posture and forepaw treading),[23] while postsynaptically acting dopamine agonists induce locomotor activity and stereotyped behavior (rearing and sniffing). Motor activity recordings were carried out as previously described with the use of motility meters (Table 4).[11] The gross behavior of the animals was observed through semitransparent glass mirrors.

***In Vitro* Binding.** The compounds were evaluated for their in *vitro* binding affinity at 5-HT_{1A} receptors using [^{3}H]-8-OH-DPAT, at 5-HT_{1D} (α + β)-receptors using [^{3}H]-5-OH-tryptamine, at dopamine D_2 receptors using either the antagonist [^{3}H]spiperone or the agonist [^{3}H]U86170, and at dopamine D_3 receptors using [^{3}H]-spiperone (Table 2). In further attempts to establish the pharmacological profile of the compounds, attempting to find clues to their target(s) in the brain, the compounds were tested in an extended battery of central nervous system (CNS) *in vitro* radioligand receptor binding assays according to standard methodology. The compounds tested displayed less than 50% inhibition at 1 μmol for dopamine D_1 ([^{3}H]SCH23390), dopamine D_4 ([^{3}H]spiperone), α_1- ([^{3}H]prazosin), α_2-([^{3}H]clonidine), and β-adrenoreceptors ([^{3}H]dihydroalprenolol), 5-HT_2 ([^{3}H]ketanserin), acetylcholine muscarinic ([^{3}H]oxotremorine), benzodiazepine ([^{3}H]flunitrazepam), and opiate ([^{3}H]etorphine)-labeled sites.

Oral Bioavailability. The absolute oral bioavailabilities of the test compounds were determined by measuring their plasma concentrations after both oral and intravenous administration. Blood samples were collected at various time intervals up to 12 h after drug administration. The doses were 25 μmol/kg (po, n = 5) and 5 μmol/kg (iv, n = 3) for compound *R*-(+)-**6**. The test compound was administered orally by gavage to animals that had been fasted for 18 h (Table 5).

Table 5. Pharmacokinetic Data for Compounds **1**, **2**, and *R*-(+)-**6** in the Rat

compd	AUC ratio po/iv, %[a]	half-life, min[b]	clearance, mL/min kg
1	2.4 ± 0.9[c]	72	NT
2	11.2 ± 5.2[d]	90	57
R-(+)-**6**	7.6 ± 1.1	130	75

[a] Blood samples were taken from rats having arterial catheters at various time intervals between 5 min and 12 h. The absolute oral bioavailability was estimated by comparing the areas under the curves (AUC) in graphs where the drug concentrations were plotted against time (n = 4 for both iv and po administration). The doses used were 20 (po) and 1 (iv) μmol/kg for compound 1, 40 (po) and 5 (iv) μmol/kg for compound **2**, and 25 (po) and 5 (iv) μmol/kg for compound *R*-(+)-**6**. [b] The half-lives were estimated graphically from the elimination phase of the blood–concentration curves after oral administration. [c] Data taken from ref 4. [d] Data taken from ref 7.

***In Vitro* Metabolism.** The metabolism of *R*-(+)-**6** was studied following incubation with suspensions of rat isolated hepatocytes.[13] The metabolic profiles were examined by thermospray (TSP) LC/MS with or without β-glucuronidase/sulfatase treatment of incubates. Structural information on metabolites was obtained by the MS/MS daughter ions analysis.

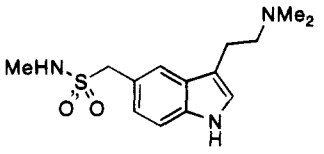
Results and Discussion

Pharmacological Effects. On the basis of the data described in Tables 2–4, it may be concluded that the enantiomers of compound **6** are 5-HT_{1A} receptor agonists similar in profile to *R*-(+)-**1**. The *R*-(+)-enantiomer of **6** displayed potent and selective interaction with 5-HT_{1A} receptors and was approximately 10 times less potent than *R*-(+)-1 (reserpinized animals, Table 3). Interestingly, in nonpretreated rats, a high dose (25 μmol/kg sc) of *R*-(+)-**6** induced a full-blown 5-HT_{1A} behavioral syndrome along with a maximal decrease in 5-HTP accumulation, indicative of a 5-HT_{1A} agonist with full intrinsic activity (Tables 3 and 4).

In contrast, the (−)-enantiomer of compound **6** was found to be a weak 5-HT_{1A} agonist (>40 times less potent *in vivo*, as compared to *R*-(+)-**6**) and did not induce a 5-HT_{1A} behavioral syndrome in nonpretreated rats (Tables 3 and 4). In addition, *S*-(−)-**6**, but not *R*-(+)-**6**, induced a decrease in striatal DOPA levels (50 μmol/kg sc).

The low potency and intrinsic activity of *S*-(−)-**6** at the 5-HT_{1A} receptors, along with fairly high affinity *in vitro* (13 nM, Table 2), prompted us to investigate any possible antagonistic properties of *S*-(−)-**6**. This compound was tested for the ability to antagonize the behavioral actions induced by *R*-(+)-**1**. Interestingly, the forepaw treading of *R*-(+)-**1** (1 μmol/kg sc) in nonpretreated rats was nearly completely blocked by *S*-(−)-**6** (50 μmol/kg, $p < 0.05$), while the flat body posture was not affected at all. No antagonism on the biochemical effects of *R*-(+)-**1** was observed. These data suggest that *S*-(−)-**6** is a partial 5-HT_{1A} receptor agonist.

Recently, the 5-HT_{1D} receptor subtype was discovered.[24] The clinical usefulness of selective ligands for 5-HT_{1D} is still unclear. However, sumatriptan (**15**) is a selective 5-HT_{1D} agonist with mainly peripheral effects and has clinical efficacy in the acute treatment of migrane.[25–27] Centrally acting 5-HT_{1D} antagonists may be useful as therapeutic agents in the treatment of depression or anxiety.[28] On the basis of *in vitro* data (Table 2), it is noteworthy that the affinity for the human cloned 5-$HT_{1D\alpha}$ receptor is improved by substituting the phenolic group in *R*-(+)-**1** with a triflate group (**2**). A comparision between compounds **2** and **6** indicates that compounds with mono- and dipropyl substitution on the nitrogen display similar affinity to the 5-$HT_{1D\alpha}$ receptors and that the affinity seems to reside in the *R*-(+)-enantiomer. However, compounds **2** and **6** display a weak selectivity for 5-HT_{1A} over 5-$HT_{1D\alpha}$ receptors.

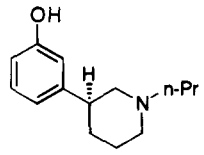


15: Sumatriptan

The corresponding 7-triflate *R*-(+)-**9** was suprisingly found to be biochemically inactive with respect to DA turnover *in vivo* (Table 3). However, a typical dopamine behavioral syndrome, including a large increase in locomotor activity, was observed in reserpine-pretreated rats (50 μmol/kg, Table 4). *R*-(+)-**9** also induced one component of the 5-HT syndrome (flat body posture) concomitant with a partial decrease in 5-HTP accumulation in striatal and cortical brain regions. The mechanisms underlying this behavioral response remain to be elucidated. In the *in vitro* binding assay, *R*-(+)-**9** displayed fairly low affinities for 5-HT_{1A}/5-HT_{1D} and DA D_2/D_3 receptors (Table 2).

As shown in Table 2, the 5-triflate-substituted compound *S*-(−)-**12** was suprisingly found to be nonselective with respect to DA D_2 and 5-HT_{1A} receptors. It also displayed high affinity for the DA D_3 receptor subtype. Although exhibiting similar affinities for DA D_2 and 5-HT_{1A} receptors *in vitro*, *S*-(−)-**12** was found to display full *in vivo* intrinsic activity at the DA autoreceptors (maximal reduction in DOPA accumulation), with an ED_{50} of 1 μmol/kg (striatum), while only a partial decrease in 5-HTP accumulation was observed in reserpine-pretreated rats at 12.5 μmol/kg sc (Table 3). On the basis of behavioral data from the former assay, *S*-(−)-**12** induced the DA syndrome in combination with "jerks" (jumping behavior), which is indicative of a preference for presynaptic DA receptors.[29] A partial 5-HT syndrome (flat forebody posture) was also observed. In nonpretreated rats, *S*-(−)-**12** induced hypomotility (LMA = 18 ± 6% of saline controls) concomitant with a maximal decrease in DOPA accumulation (25 μmol/kg, Tables 3 and 4). Thus, compound *S*-(−)-**12** seems to be a preferential DA autoreceptor agonist with a similar pharmacological profile as the putative partial agonist (−)-3PPP (**16**).[30,31] However, the increase in LMA in reserpine-pretreated rats after administration of *S*-(−)-**12** might be an indication of higher intrinsic activity, as compared to **16**.

Interestingly, the corresponding *R*-enantiomer *R*-(+)-**12** was found to be inactive at DA and 5-HT receptors in *in vivo*, as judged from both biochemical and behavioral data in nonpretreated rats (Tables 3 and 4). The *in vitro* data further support the inactivity. Compound *R*-(+)-**12** displays affinity neither for DA nor 5-HT receptors (Table 2). From a SAR point of view, the discrepancies between *S*-(−)-**12** and *R*-(+)-**12** in the



16: (-)-3PPP

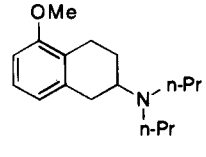
activity at the DA receptors may reveal some suggestions of the receptor topography. Recently it was reported that a pure fraction of *R*-(+)-5-OH-DPAT and (*R*)-(+)-2-(*N*-propyl-*N*-2-thienylamino)-5-hydroxytetralin (N-0437) displayed weak antagonistic properties at the DA receptors.[32,33] Accordingly, introduction of a bulky group such as the triflate in the aromatic ring seems to be detrimental for affinity and antagonistic activity at DA receptors in the *R*-enantiomer series of 5-substituted aminotetralins. In addition, it is also worth noting that substitution with a triflate group in the 8-position of *R*-(+)-8-OH-MPAT yields a compound that displays full intrinsic activity at 5-HT_{1A} receptors. However, a similar substitution in *S*-(−)-5-OH-MPAT results in a partial DA agonist. Thus, it seems as if the DA receptors are more sensitive to changes of the aromatic substituents than the 5-HT_{1A} receptors, which can accept substituents with different physical properties.[34]

Metabolism and Pharmacokinetics. The results discussed above for *R*-(+)-**6** are in sharp contrast to the results obtained for racemic **2**, which we have reported to be a weak 5-HT_{1A} agonist in both biochemical and behavioral assays in reserpinized rats. This contrast is intriguing, since compounds **2** and *R*-(+)-**6** displayed similar affinities for the 5-HT_{1A} site *in vitro* (Table 2). In homogenized rat brain tissue the affinity (K_i) for compound **2** at 5-HT_{1A} sites was found to be 9.8 ± 2.3 nM while for racemic **6** it was 3.8 ± 0.2 nM (see ref 7). Recently, Liu et al. reported both enantiomers of **2** to be inactive *in vivo* following sc administration.[34] They proposed that the inability of the enantiomers of **2** to produce central effects might be due to extensive metabolism, yielding inactive metabolites, or to the inability of these enantiomers to cross the blood brain barrier. However, since we have demonstrated that the major metabolite of **2** *in vitro* is the monopropyl analog **6**, and further that **6** is more potent *in vivo* than **2**,[7] it seems unlikely that metabolic removal of **2** could account for its weak *in vivo* activity as reported by Liu et al. (who studied this compound by the sc route only).[34] Indeed, **2** was more active after oral compared to subcutaneous administration, which supports the notion of first-pass metabolism to a pharmacologically more active species. Urine from rats dosed orally with compound **2** was analyzed for metabolites by thermospray LC/MS. Figure 1 shows a mass chromatogram of rat urine following an oral dose of **2** (50 μmol/kg), and Figure 2 shows the proposed metabolic pathway. The data show that N-dealkylation of **2** to yield **6** was a major pathway as well as further metabolism to the primary amine. Oxidation, was also important, and the major metabolite appeared to be the product of N-dealkylation and dioxidation, although the relative responses of the metabolites are unknown, making quantification speculative. MS/MS evidence was consistent with the proposed structures, and although the positions of the oxidations were not clear, propyl side-chain hydroxylation could be ruled out, as well as N-oxidation. No changes were observed following treatment of urine with β-glucuronidase, indicating the absence of significant amounts of conjugated metabolites. The *in vivo* metabolism data are supportive of the conclusions drawn from the *in vitro* investigations.

R-(+)-**6** elicted a full-blown 5-HT syndrome when administered subcutaneously, but not orally, to normal rats (Table 4). When *R*-(+)-**6** was incubated with rat isolated hepatocytes, the major metabolite was the primary amine resulting from N-dealkylation. Minor oxidized metabolites were also observed. It is therefore likely that *R*-(+)-**6** is metabolized by hepatic N-dealkylation when administered orally. This lends further support to the proposal that it is *R*-(+)-**6** which is the active species following oral administration of **2**.

Table 5 shows the pharmacokinetic data for compounds **2** and *R*-(+)-**6**. The oral bioavailability of *R*-(+)-**6** appeared to be slightly lower than that of **2** which reflects the slightly higher clearance value obtained for *R*-(+)-**6**. In a semiquantitative assay, the metabolism of *R*-(+)-**6** *in vitro* was comparatively slower than that of **2**. These data would predict that *R*-(+)-**6** would undergo less extensive first-pass metabolism *in vivo* resulting in a higher oral bioavailability. Thus other factors such as absorption or possibly inhibition of metabolism by a metabolite are important in determining the bioavailability of these compounds. However, the authors recognize the need to measure levels of *R*-(+)-**6** and **2** in both plasma and brain in order to further substantiate the hypothesis. In addition, such data would rationalize earlier results by relating *in vivo* biochemical effects to levels of drug and metabolite.

We have previously reported that oral administration of **13** and 5-OMe-2-(di-*n*-propylamino)tetralin (**17**) resulted in behavioral and biochemical effects indicative of mixed DA/5-HT_{1A} agonist actions.[7] Such effects were not seen following subcutaneous administration, suggesting that active metabolites of **13** and **17** are formed after oral dosing. It has been reported that 5-methoxy-2-aminotetralin (the primary amine analog of **17**) produces 5-HT_{1A} effects in behavioral and biochemical assays.[35] We therefore speculated that the active metabolites of **13** and **17** were the corresponding primary amines. Thermospray LC/MS analysis of urine from rats dosed orally with **13** revealed N-dealkylation to be the major metabolic route with the production of both the secondary amine (racemic **12**) and the primary amine. Pharmacological data presented here (Tables 2−4) suggest that the secondary amine metabolite (**12**) may be at least partly responsible for the effects of **13** after oral dosing. The corresponding primary amine has not yet been synthesized and remains to be tested.



17

Experimental Section

General. ^1H and ^{13}C NMR spectra were recorded at 200 and 50.3 MHz, respectively, on a Varian Gemini 200 spectrometer. $CDCl_3$ was employed as the solvent unless otherwise

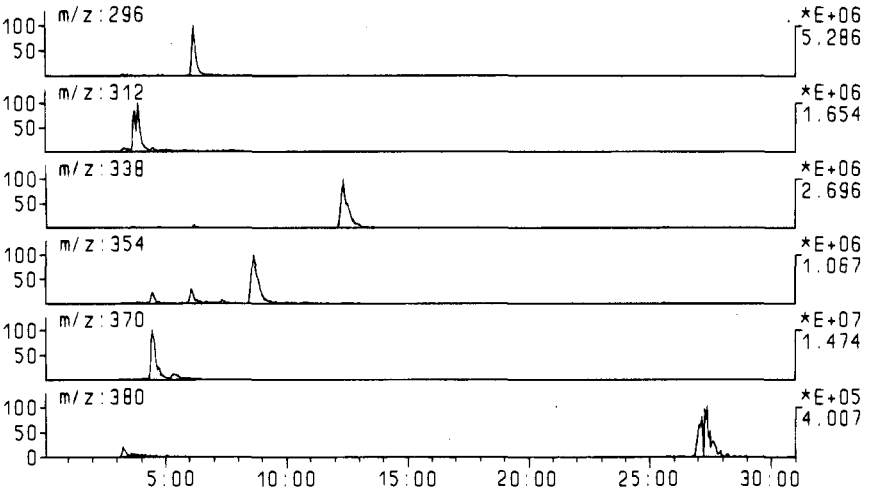


Figure 1. Thermospray LC/MS chromathogram of rat urine collected 0–24 h following a single po administration of racemic **2** (50 μmol/kg). Units on right-hand vertical axis are ion current in exponential notation; those on the left are arbitrary. Horizontal axis is time (min); m/z represents $[M + H]^+$ ions for metabolites (see Figure 2).

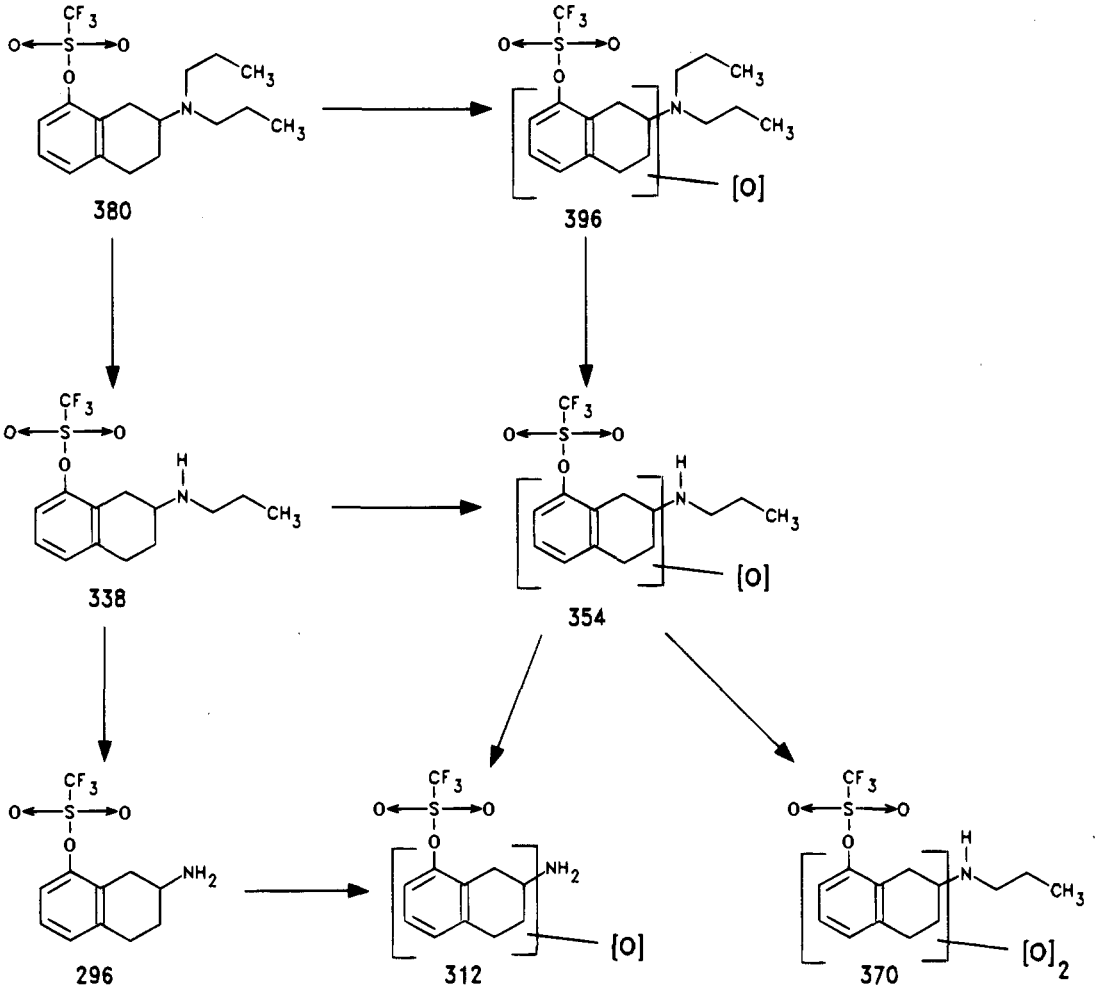


Figure 2. Proposed metabolic pathway of **2** in the rat. Numbers below structures are the $[M + H]^+$ ion for each metabolite (see Figure 1).

stated. Chemical shifts are given in units (ppm) and are relative to TMS or deuterated solvent. J values are reported in hertz (Hz). IR spectra were obtained on a ATI-Mattson spectrometer. Elemental analyses were performed in the Micro Analytical Department at the University of Groningen. The chemical ionization (CI) mass spectra were obtained on a Finnegan 3300 system. Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. Specific optical rotations were measured in methanol (c 1.0) at 25 °C on a Perkin Elmer 241 polarimeter. The amine products were converted into their corresponding HCl salts by dissolving the free base in an ethanolic HCl solution. The solvent was removed and azeotroped with absolute ethanol or toluene *in vacuo* followed by recrystallization from an appropriate solvent.

Materials. All monomethoxylated aminotetralins were prepared according to the literature procedures.[14,15] Chemicals used were commercially available (Aldrich) and used without further purification. (*R*)-(+)- and (*S*)-(−)-4-(2-chlorophenyl)-5,5-dimethyl-2-hydroxy-1,3,2-dioxaphosphorane 2-oxide (2-

chlocyphos)[17] were obtained from Syncom BV, The Netherlands.

Resolution of (±)-8-Methoxy-2-(*n*-propylamino)tetralin (4). A mixture of racemic amine **4**[14] (11.1 g, 50.7 mmol) and (*R*)-(+)-2-chlocyphos[17] (14.0 g, 50.7 mmol) in absolute ethanol (200 mL) was refluxed until all material was dissolved, after which the solvent was removed in vacuo giving an off-white solid. The salt (24.1 g, 48.7 mmol) was recrystallized from 2-propanol yielding 5.16 g (10.4 mmol, 21%) of white crystals with $[\alpha]_D$ +53.1°. A second recrystallization gave 3.74 g (7.56 mmol, 16%) salt with $[\alpha]_D$ +60.1°. This salt (3.65 g, 7.37 mmol) was converted to the free base by stirring in 10% KOH (50 mL), extracting with ether, and drying over Na_2SO_4. Evaporation of the solvent in vacuo yielded *R*-(+)-**4** (1.58 g, 15%) as a colorless oil with $[\alpha]_D$ +76.4° (lit.[3] $[\alpha]^{22}_D$ +78.3° (*c* 1.05)). The ee >99.5% was determined by HPLC analysis using a chiral column (Daicel Chiralcel OD, 250 mm, i.d. 4.6 mm, flow rate 0.5 mL/min, eluent hexane/ethanol/diethylamine, 99:1:0.1).

The residual salt (11.2 g, 22.7 mmol) was converted to the free base as described above using 10% KOH (100 mL). Repeating the above procedure with the enriched (−)-enantiomer of **4** (4.57 g, 21.7 mmol) with (*S*)-(−)-chlocyphos[17] (5.99 g, 21.7 mmol) gave *S*-(−)-**4** (1.65 g, 15%) as a colorless oil with $[\alpha]_D$ −77.6 (lit.[3] $[\alpha]^{22}_D$ −77.0° (*c* 1.03)). The ee >99.5% was determined by HPLC analysis using a chiral column (Daicel Chiralcel OD, 250 mm, i.d. 4.6 mm, flow rate 0.5 mL/min, eluent hexane/ethanol/diethylamine, 99:1:0.1).

(*R*)-(+)-8-Hydroxy-2-(*n*-propylamino)tetralin Hydrobromide (*R*-(+)-5). *R*-(+)-**4**·HCl (1.74 g, 6.82 mmol) was refluxed in 48% HBr (50 mL; freshly distilled) for 2 h under a N_2 atmosphere. The reaction mixture was allowed to cool to room temperature and evaporated to dryness giving 1.88 g (97%) of a pale-brown solid, of which 445 mg was recrystallized from ethanol/ether for purification (374 mg, 78%): mp 283–286 °C; IR (KBr) 3275 cm^{-1}; ^1H NMR (CD_3OD) 1.07 (t, J = 7.69, 3H), 1.70–1.91 (m, 3H), 2.33 (m, 1H), 2.60 (dd, J_1 = 10.25, J_2 = 16.23, 1H), 2.91 (m, 2H), 3.08 (m, 2H), 3.26–3.37 (m, 1H), 3.43–3.58 (m, 1H), 6.61 (d, J = 7.7, 1H), 6.62 (d, J = 8.12, 1H) 6.97 (dd, J_1 = 7.69, J_2 = 8.12, 1H); ^{13}C NMR (CD_3OD) 11.0, 20.7, 26.6, 27.2, 28.3, 47.5, 55.8, 112.6, 120.0, 120.3, 127.8, 136.9, 156.0; MS (CI with NH_3) *m/e* 206 (M + 1). Anal. Calcd for $C_{13}H_{19}NO$·HBr: C, H, N. $[\alpha]_D$ +63.5° (HBr).

(*S*)-(−)-8-Hydroxy-2-(*n*-propylamino)tetralin Hydrobromide (*S*-(−)-5). Demethylation of *S*-(−)-4 (1.92 g, 7.53 mmol) was performed according to procedure as described for *R*-(+)-**5** as above giving *S*-(−)-**5**·HBr in a quantitative yield. Part of the salt (1.06 g) was recrystallized from ethanol/ether yielding 0.80 g (76%) of off-white crystals: mp 273–277 °C (HBr). Anal. Calcd for $C_{13}H_{19}NO$·HBr: C, H, N. $[\alpha]_D$ −64.5 (HBr).

(*R*)-(+)-8-[[(Trifluoromethyl)sulfonyl]oxy]-2-(*n*-propylamino)tetralin (*R*-(+)-6). A mixture of *R*-(+)-**5** (200 mg, 0.70 mmol), *N*-phenyltrifluoromethanesulfonimide (376 mg, 1.05 mmol), and tetrabutylammonium hydrogen sulfate (24 mg, 10 mol %) in dichloromethane (8 mL) and 10% NaOH (3 mL, w/v) was stirred at room temperature for 24 h. The reaction was quenched with 5% HCl solution (v/v) until pH 1 and the mixture diluted with H_2O (25 mL) and washed with ether (50 mL). The ether layer was extracted with H_2O and 5% HCl solution (20 mL). The combined aquous layers were basified with solid Na_2CO_3 until pH 9–12 and extracted with ether (3 × 30 mL), after which the organic phase was washed with brine and dried over Na_2SO_4. Evaporation in vacuo yielded a colorless oil, which was converted to the HCl salt and recrystallized from methanol/ether (177 mg, 68%): mp 238–240°C (HCl); IR (KBr) 1217 cm^{-1}; ^1H NMR (CD_3OD) 0.96 (t, J = 7.5, 3H), 1.35 (br s, NH), 1.55 (m, 2H), 1.63 (m, 1H), 2.05 (m, 1H), 2.53 (dd, J_1 = 8.55, J_2 = 16.24, 1H), 2.69 (t, J = 7.5, 2H), 2.83–3.04 (m, 3H), 3.12 (dd, J_1 = 4.71, J_2 = 16.24, 1H), 7.05–7.18 (m, 3H); ^{13}C NMR (CD_3OD) 11.8, 23.4, 27.8, 28.6, 30.7, 49.0, 52.5, 118.3, 118.6 (q, J = 321, CF_3), 126.7, 128.6, 128.7, 139.9, 148.4; MS (CI with NH_3) *m/e* 338 (M + 1). Anal. Calcd for $C_{14}H_{18}NO_3SF_3$·HCl: C, H, N, F, S. $[\alpha]_D$ +61.5° (HCl).

(*S*)-(−)-8-[[(Trifluoromethyl)sulfonyl]oxy]-2-(*n*-propylamino)tetralin (*S*-(−)-6). Triflation of *S*-(−)-**5** (880 mg, 3.08 mmol) was performed according to the procedure given for the synthesis of *R*-(+)-**6** above, giving an oil after extractive workup. Conversion to the HCl salt and subsequent recrystallization from methanol/ether gave 760 mg (66%) of white crystals: mp 235–238°C (HCl). Anal. Calcd for $C_{14}H_{18}NO_3SF_3$·HCl: C, H, N, F, S; $[\alpha]_D$ −61.6° (HCl).

Resolution of (±)-7-Methoxy-2-(*N*-*n*-propylamino)tetralin (7). A mixture of 6.36 g (29 mmol) of racemic 7-methoxy-2-(*N*-*n*-propylamino)tetralin and 8.0 g (29 mmol) of (−)-chlocyphos was dissolved in 2-propanol (450 mL). The solution was allowed to cool to room temperature while stirring. During the first hour, optically pure seeding crystals (*R*-(+)-**7**/(−)-chlocyphos; obtained from an analytical sample) were added. After 4 h a white solid with a rotation of $[\alpha]_D$ +0.5° (*c* 1.0, MeOH) was filtered off. A second recrystallization from 2-propanol gave 6 g (42%) of salt with $[\alpha]_D$ +14.6° (*c* 1.0, MeOH). This salt was dissolved in water (50 mL) and made alkaline with 2 N NaOH, and the aqueous layer was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The free amine was converted to the HCl salt, and recrystallization from ethanol/ether afforded 2.5 g of pure *R*-(+)-**7**·HCl (33.7%), having an $[\alpha]_D$ +75.2° (*c* 1.0, MeOH) (lit.[16] $[\alpha]_D$ +70.2° (*c* 1.06)). The ee >99.5% was determined by decoupled ^{31}P NMR using the adduct of the acid chloride of chlocyphos (Δ δ 0.09 difference between the diastereomeric amides) according to ref 17.

The filtrates from the two crystallizations were combined, concentrated, made alkaline, and extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and concentrated to yield 4.1 g (>50%) of enriched of *S*-(−)-**7**. This amine, (+)-chlocyphos (5 g, 18 mmol), and 2-propanol (200 mL) were heated to a clear solution. The heating mantle was removed, and the solution was allowed to cool to room temperature with stirring. After 6 h the salt was filtered off and washed with ether to give 6.4 g (12.9 mmol) with $[\alpha]_D$ −14.3° (*c* 1.0, MeOH). This salt was converted into the HCl salt, and recrystallization from ethanol yielded 2.8 g (37.7%) of *S*-(−)-**7** with an $[\alpha]_D$ −75.1° (*c* 1.0, MeOH) (lit.[16] $[\alpha]_D$ −72.6° (*c* 0.8)).

(*R*)-(+)-7-Hydroxy-2-(*n*-propylamino)tetralin Hydrobromide (*R*-(+)-8). Demethylation of *R*-(+)-**7** (537 mg, 2.11 mmol) was performed according to the procedure as described for *R*-(+)-**5** as above, giving *R*-(+)-**8** as a pale-brown solid. The title compound was purified by recrystallization from ethanol/ether (478 mg, 79%): mp 215–224 °C; IR (KBr) 3315 cm^{-1}; ^1H NMR (CD_3OD) 1.07 (t, J = 7.41, 3H), 1.70–2.00 (m, 3H), 2.36 (m, 1H), 2.91–3.16 (m, 5H), 3.30–3.42 (m, 1H), 3.51–3.65 (m, 1H), 7.13–7.33 (m, 3H); ^{13}C NMR (CD_3OD) 11.0, 20.6, 26.4, 27.6, 32.5, 47.6, 54.8, 120.3, 122.5, 131.5, 136.0, 136.9, 148.9; MS (CI with NH_3) *m/e* 206 (M + 1). Anal. Calcd for $C_{13}H_{19}NO$·HBr: C, H, N. $[\alpha]_D$ +57.8° (HBr).

(*R*)-(+)-7-[[(Trifluoromethyl)sulfonyl]oxy]-2-(*n*-propylamino)tetralin (*R*-(+)-9). Triflation of *R*-(+)-**8** (302 mg, 1.06 mmol) was performed according to the procedure given for the synthesis of *R*-(+)-**6** above giving a colorless oil after extractive workup. Conversion to the HCl salt and subsequent recrystallization from methanol/ether gave 107 mg (34%) of *R*-(+)-**9** as a white crystalline material: mp 206–208 °C (HCl); IR (KBr) 1211 cm^{-1}; ^1H NMR (CD_3OD) δ 1.00 (t, J = 7.31, 3H), 1.54–1.77 (m, 3H), 2.13–2.27 (m, 1H), 2.64–3.23 (m, 7H), 7.06–7.26 (m, 3H); ^{13}C NMR (CD_3OD) δ 11.6, 22.7, 28.1, 28.5, 35.2, 48.9, 54.2, 119.6, 119.8 (q, J = 319, CF_3), 122.3, 131.3, 137.7, 138.1, 148.7; MS (CI with NH_3) *m/e* 338 (M + 1). Anal. Calcd for $C_{14}H_{18}NO_3SF_3$·HCl: C, H, N. $[\alpha]_D$ +50.6° (HCl).

Resolution of (±)-5-Methoxy-2-(*N*-*n*-propylamino)tetralin (10). A mixture of 6.23 g (28.4 mmol) of 5-methoxy-2-(*N*-*n*-propylamino)tetralin[14] ((±)-**10**) and 7.8 g (28.4 mmol) of (+)-chlocyphos was dissolved in ethanol (30 mL) and H_2O (10 mL). The solution was allowed to cool to room temperature with stirring. After 6 h product was collected and washed with ethanol/ether and ether, which afforded a salt (43%; $[\alpha]_D$ −1.5° (*c* 1.0, MeOH)). The salt was stirred for 1 h with 1 g of sodium hydroxide in 50 mL of H_2O, and then chloroform was added, and the mixture was stirred for an additional 30 min. The layers were separated, and the aqueous phase was extracted with chloroform. The chloroform layers where washed with

water, dried over Na_2SO_4, and evaporated to give an oil. Treatment with dry HCl–ether and recrystallization from ethanol/ether gave 3.1 g (43%) of *S*-(−)-**10**·HCl: mp 279–283 °C (HCl); $[\alpha]_D = -65.2°$ (*c* 1.0, MeOH) (lit.[16] $[\alpha]_D$ −63.0° (*c* 1.0)). The ee >99.5% was determined by decoupled ^{31}P NMR using the adduct of the acid chloride of chlocyphos ($\Delta\ \delta$ 0.09 difference between the diastereomeric amides) according to ref 17.

The filtrate from the crystallization was concentrated, made alkaline, and extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and concentrated to yield enriched *R*-(+)-**10**. Treatment of the amine with (−)-chlocyphos afforded 2.5 g (35%) of optically pure *R*-(+)-**10**, having an $[\alpha]^{30}_D$ +72.4° (*c* 0.428, MeOH) (lit.[16] $[\alpha]_D$ +69.7° (*c* 1.0))). The ee >99.5% was determined by ^{31}P NMR according to the previously described method.[17]

(*S*)-(−)-5-Hydroxy-2-(*n*-propylamino)tetralin Hydrobromide (*S*-(−)-11). Demethylation of *S*-(−)-**10**·HCl (0.5 g, 1.96 mmol) was performed according to the procedure described for the preparation *R*-(+)-**5** as above giving the title compound as a pale-brown solid. Recrystallization from ethanol/ether gave white crystals (350 mg, 62.4%): mp 249–251°C; IR (KBr) 3327 cm^{-1}; ^1H NMR (CD_3OD) 1.07 (t, J = 7.45, 3H), 1.69–1.92 (m, 3H), 2.30–2.43 (m, 1H), 2.56–2.74 (m, 1H), 2.82–3.35 (m, 5H), 3.41–3.55 (m, 1H), 6.63 (d, J = 7.69, 2H), 6.97 (t, J = 7.69, 1H); ^{13}C NMR (CD_3OD) 11.0, 20.7, 22.4, 26.5, 32.9, 47.4, 55.5, 113.2, 120.8, 122.7, 127.6, 134.1, 155.9; MS (CI with NH_3) *m/e* 206 (M + 1). Anal. Calcd for $C_{13}H_{19}NO$·HBr: C, H, N. $[\alpha]_D$ −58.4° (*c* 0.76, MeOH).

(*R*)-(+)-5-Hydroxy-2-(*n*-propylamino)tetralin Hydrobromide (*R*-(+)-11). Demethylation of *R*-(+)-**10** (160 mg, 0.63 mmol) was performed according to the procedure described for the preparation of *R*-(+)-**5** as above giving *R*-(+)-**11**·HBr as a salt (160 mg, 89%). The salt was recrystallized from ethanol/ether: mp 246–248 °C (HBr). Anal. Calcd for $C_{13}H_{19}NO$·HBr: C, H, N. $[\alpha]_D^{27}$ +57.4° (HBr, *c* 0.272, MeOH).

(*S*)-(−)-5-[[(Trifluoromethyl)sulfonyl]oxy]-2-(*n*-propylamino)tetralin (*S*-(−)-12). Triflation of *S*-(−)-**11** (246 mg, 0.86 mmol) was performed according to the procedure given for the synthesis of *R*-(+)-**6** above, giving 248 mg of an oil after extractive workup. This product was purified on a silica gel column, eluting with CH_2CL_2:MeOH (1:1). Conversion to the HCl salt and subsequent recrystallization from methanol/ether gave white crystals (190 mg, 59%): mp 232–233°C (HCl); IR (KBr) 1213 cm^{-1}; ^1H NMR (CD_3OD) δ 1.00 (t, J = 7.5, 3H), 1.54–1.73 (m, 3H), 2.19–2.32 (m, 1H), 2.65–2.86 (m, 4H), 2.97–32.26 (m, 3H), 7.12–7.28 (m, 3H); ^{13}C NMR (CD_3OD) δ 11.0, 20.6, 23.1, 25.7, 32.4, 47.8, 54.6, 119.7 (q, J = 319, CF_3), 120.2, 128.7, 129.2, 130.4, 137.1, 148.9; MS (CI with NH_3) *m/e* 338 (M + 1). Anal. Calcd for $C_{14}H_{18}NO_3SF_3$·HCl: C, H, N. $[\alpha]_D$ −62.4° (HCl).

(*R*)-(+)-5-[[(Trifluoromethyl)sulfonyl]oxy]-2-(*n*-propylamino)tetralin (*R*-(+)-12). Triflation of *R*-(+)-**11** (85 mg, 0.3 mmol) was performed according to the procedure given for the synthesis of *R*-(+)-**6** above. Conversion to the HCl salt and subsequent recrystallization from ethanol/ether yielded white crystals (50 mg, 45%): mp 239–241 °C (HCl). Anal. Calcd for $C_{14}H_{18}NO_3SF_3$·HCl: C, H, N, F, S. $[\alpha]_D^{27}$ +73.3° (HCl, *c* 0.172, MeOH).

Pharmacology. Animals. Animals used in the biochemical and motor activity experiments were male rats of the Sprague–Dawley strain (ALAB, Sollentuna, Sweden), weighing 200–300 g. The rats were housed five per cage with free access to water and food. At least 1 week was allowed from arrival until the animals were used in the experiments. The animals treated orally with the test compound were starved 18 h before the experiment.

Materials. All substances to be tested were dissolved in physiological (0.9%, w/v) saline immediately before use, occasionally with the addition of a few drops of glacial acetic acid and/or moderate heating in order to obtain complete dissolution. Reserpine was dissolved in a few drops of glacial acetic acid and made up to volume with 5.5% glucose (w/v). Dose volumes were 5 or 10 mL/kg, and all solutions had neutral pH values (except for the solutions of reserpine).

Biochemistry. The biochemical experiments and the determinations of DOPA and 5-HTP by means of HPLC with electrochemical detection were performed as previously described.[36,37] ED_{50} values were calculated by fitting a sigmoidal curve to the dose responses according to Stjernlöf et al.[38] The ED_{50} values are given in μmol/kg. From these graphs, the doses of the drug yielding a half-maximal decrease (ED_{50} value) of the 5-HTP (the maximal effects, expressed as percent of controls, were as follows: limbic system, striatum, and the hemispheres = 50%) and the DOPA (the maximal effects, expressed as percent of controls, were as follows: limbic system = 35%, striatum = 20%, and the hemispheres = 50%) levels were estimated separately (Table 2). Control values for the 5-HTP data were (ng/g, mean ± SEM, n = 4) as follows: limbic system = 160 ± 20, striatum = 120 ± 10, and the hemispheres = 100 ± 10. The DOPA levels were (ng/g, mean ± SEM, n = 10) as follows: limbic system = 810 ± 60, striatum = 3700 ± 200, and the hemispheres = 170 ± 10.

Motor Activity. Reserpine-Pretreated Animals. The motility measurements were carried out using a set of eight photocell animal motility meters (Digiscan activity monitor RXYZM(16)TAO; Omnitech Electronics, Inc., Columbus, OH). The motility meters were kept in sound- and light-proof boxes equipped with semitransparent mirrors, allowing observations of the animals during the course of the experiments. Eighteen hours prior to the motility testing (carried out between 9 a.m. and 1 p.m.), the rats were subcutaneously injected in the neck region with reserpine (5 mg/kg). The different test compounds were also administered subcutaneously in the neck region (n = 4). Immediately after drug administration, the rats were put into the motility meters (1 rat/cage). The activity was then followed and recorded for the subsequent 30 min (reserpine control values = 125 ± 30 counts/30 min, mean ± SEM, n = 28 (Table 4)).

Nonpretreated Animals. The motor activity was measured as described for reserpine-pretreated animals. The different test compounds were administered subcutaneously in the neck region or orally *via* gavage. Immediately after drug administration, the rats were placed in the test cages (1 rat/cage) and put into the motility meters. Motor activity was then followed and recorded for the subsequent 30 min (Table 4). Observations of gross behavior were made throughout the activity sessions through semitransparent mirrors. Control levels were 4247 ± 399 counts/30 min (mean ± SEM, n = 20).

Receptor Binding. The compounds were evaluated for their *in vitro* binding affinity at 5-HT_{1A} receptors using [^{3}H]-8-OH-DPAT (147 Ci/mmol, 1 nM), at 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors using [^{3}H]-5-hydroxytryptamine (69 Ci/mmol, 1.7 nM), at D_2 dopamine receptors using [^{3}H]U86170 (62 Ci/mmol, 1 nM)[39] or [^{3}H]spiperone (123 Ci/mmol, 0.3 nM), and at D_3 dopamine receptors using [^{3}H]spiperone (123 Ci/mmol, 0.5 nM). All receptors were mammalian clones expressed in CHO-k1 cells.[40–43] Buffers (all at pH 7.4) were 50 mM TRIS, 10 mM $MgCl_2$ for 5-HT_{1A}, 50 mM TRIS, 10 mM $MgCl_2$, 1 mM EDTA, 0.1% ascorbic acid for 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$, 20 mM HEPES, 10 mM $MgSO_4$ for DA D_2, and 20 mM HEPES, 10 mM $MgCl_2$, 150 mM NaCl, 1 mM EDTA for DA D_3 receptors. Incubation of the 0.9 mL binding mixtures was for 1 h at room temperature. Reactions were stopped by vacuum filtration. Scintillation counting was performed with a 1205 betaplate (Wallac) using MeltiLex B/HS (Wallac) as scintillant. IC_{50} values were estimated by a nonlinear least squares fitting to competition curves employing 11 drug concentrations in duplicate. Inhibition constants (K_i) were calculated with the Cheng and Prushoff equation.[44] The data in Table 2 are giving in nM ± SEM.

Estimation of Oral Bioavailability. Blood levels of *R*-(+)-**6** were measured by means of gas chromatography (Hewlett Packard)–mass spectrometry (VG Trio II). The doses were 25 μmol/kg (po) and 5 μmol/kg (iv) for compound *R*-(+)-**6**. The drug was administered orally to the animals *via* gavage, and blood samples (150 μL) were collected from arterial catheters. The experiment was started 24 h after the operation, and blood samples were collected at various time intervals up to 12 h after drug injection. The samples were diluted with 1 mL of water followed by the addition of 50 μL

of the internal standard (S)-(−)-3-[3-[[(trifluoromethyl)sulfonyl])oxy]phenyl]-N-ethylpiperidine[45] having a concentration of 10 pmol/μL. The pH was adjusted to 11.0 by the addition of saturated Na_2CO_3 solution. After mixing, the samples were extracted with 4 mL of dichloromethane by shaking for 30 min. The organic layer was transferred to a smaller tube and evaporated to dryness under a stream of nitrogen. The residue was redissolved in 40 μL of toluene for GC−MS analysis. A standard curve over the range 2−1000 pmol/mL was prepared by adding appropriate amounts of R-(+)-**6** standard to blank blood samples. A set of control samples at two different concentrations (made up in bulk and kept frozen) were included in each assay. GC was performed on a cross-linked PS 264 capillary column (15 m × 0.25 mm), and a 2 μL sample was injected in the splitless mode. The GC temperature was held at 90°C for 1 min following injection and then increased by 30 °C/min to the final temperature of 290 °C. Each sample was run in duplicate. The absolute oral bioavailability of the compounds was assessed by comparing the areas under the curves (AUC), for po ($5 = 4$) and iv ($n = 3$) administration, from plots of concentration in blood versus time. Rats treated orally with drug were starved 18 h before the experiment.

Urine Samples. Urine samples were collected for 24 h after oral dosing of compounds **2** and **13** (50 μmol/kg). The samples were analyzed for metabolites with LC/MS thermospray as described below.

Hepatocytes. Hepatocytes were isolated from male Sprague−Dawley rats (Charles River, Margate, U.K.; bodyweight ≈ 200 g) by *in situ* collagenase perfusion based on the method of Seglen.[46] Cell viability was determined by trypan blue dye exclusion (typically 85−90%). Isolated hepatocytes were suspended at 5×10^6 viable cells mL^{-1} in 10 mM phosphate-buffered saline (pH 7.4 at 4 °C; prepared from tablets obtained from Sigma Chemical Co., Poole, U.K.) containing 1% (w/v) glucose.

Incubation of Hepatocytes with Compound R-(+)-6. A 5 mL aliquot of the hepatocyte suspension was dispensed into a poly(carbonate) 25 mL conical flask. Following preincubation of the flask for 5 min at 37 °C in a shaking water bath (≈120 strokes/min), 100 μL of a solution of compound R-(+)-**6** (1 mg/mL equiv in dimethyl sulfoxide) was added and the incubation continued for a further 120 min. After 30 and 120 min, samples (2 mL) were withdrawn from the flask into 2 mL of 0.1 M sodium acetate buffer (pH 5.0 at 37 °C) and ultrasonicated for 5 min. The samples were then stored at −20 °C overnight. A sample of hepatocyte suspension without drug was processed as above to act as a blank.

Incubation of Samples with β-Glucuronidase. Following thawing, samples were divided into equal aliquots and incubated with or without 50 μL of a β-glucuronidase/sulfatase preparation (type H3, from *Helix pomatia*; Sigma). Protein was precipitated with acetonitrile (2 mL) followed by centrifugation at 12000*g* for 5 min. Removal of acetonitrile *in vacuo* yielded an aqueous residue (≈1 mL).

Mass Spectrometry. Samples were analyzed by thermospray (TSP) LC/MS using a Finnigan MAT TSQ 70 mass spectrometer coupled *via* a TSP II interface (both Finnigan MAT, Paradise, Hemel Hempstead, U.K.) to a reversed phase HPLC system. The spectrometer was operated in Q3 MS mode to collect the TSP/LC/MS data and in TSP/LC/MS/MS mode to obtain the collisionally induced dissociation (CID).

HPLC Conditions: Injector, manual U6K (Millipore); pump, Waters 600MS (Millipore, Watford, U.K.); guard column, Brownlee RP8 15 × 3.2 mm (Anachem, Luton, U.K.); column, Zorbax Rx C8 250 × 4.6 mm (Hichrom, Reading, U.K.); mobile phase, 33% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid in Milli-Q water (Millipore); flowrate, 1.5 mL/min; injection volume, 100 μL, equivalent to 100 μL of the original incubate.

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