Substituted 3-Phenylpiperidines: New Centrally Acting Dopamine Autoreceptor Antagonists

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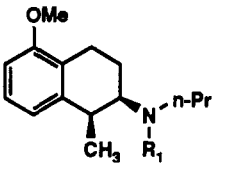
The (+)-and (−)-enantiomer of compounds **4** and **5** were synthesized and tested for central dopamine (DA) receptor stimulating activity, using biochemical and behavioral tests in rats. Based on the available data the (−)-enantiomers of **4** and **5** are characterized as centrally acting DA autoreceptor antagonists with oral activity. They display a similar pharmacological profile as the prototype DA autoreceptor antagonists (+)-**1** and (+)-**2** and show a certain preference for the D_3 DA receptor antagonist binding site.

Introduction

Since the discovery of the pharmacological actions of (+)-**1** (*cis*-(+)-5-methoxy-1-methyl-2-(*n*-propylamino)-tetralin, (+)-AJ76) and (+)-**2** (*cis*-(+)-5-methoxy-1-methyl-2-(di-*n*-propylamino)tetralin, (+)-UH232),[1,2] we have been interested in investigating the SAR and the pharmacological activity of preferential dopamine (DA) autoreceptor antagonists. Such compounds could have a potential utility in the treatment of a variety of central nervous system related disorders, such as schizophrenia, depression, and drug addiction.[3] The two prototype compounds, (+)-**1** and (+)-**2**[1,4] have a preference for DA autoreceptors as judged from biochemical and behavioral data.[2] Unlike classical DA receptor antagonists, which depress locomotor activity and induce catalepsy, these two compounds stimulate locomotor activity, especially at low baseline activity, i.e., in rats habituated to their environment. According to the prevailing hypothesis, the behavioral activation is thought to be due to a preferential antagonism of nerve terminal DA autoreceptors leading to an increased synthesis and release of DA and subsequent increase in locomotor activity. At a very high baseline activity, as the result of pretreatment with direct or indirect agonists such as apomorphine or d-amphetamine, (+)-**1** and especially (+)-**2** counteract the behavioral stimulation. Thus, under these conditions the postsynaptic receptor blockade predominates. Since neither (+)-**1** nor (+)-**2** cause hypokinesia or catalepsy as classical neuroleptics do, they would not be expected to induce EPS in man. However, since the clinical utility may be limited by a relatively low oral bioavailability we have developed a new series of compounds with similar pharmacological profile as (+)-**1** and (+)-**2** but with improved pharmacokinetic properties. Interestingly, (+)-**1** and (+)-**2** have been reported to have greater preference for the dopamine D_3 receptor than all other known dopamine receptor antagonists.[5] However, it is uncertain at this time whether the preferential binding that (+)-**1** and (+)-**2** have for the dopamine D_3 receptor is related to their unique biochemical and behavioral effects.



Compd	R1	config
(+)-**1**	-H	1S2R
(+)-**2**	n-Pr	1S2R

Wikström et al.[6] reported that compound **6** showed weak DA receptor antagonist properties in a biochemical assay. The increase in DOPA observed covaried with an increase in locomotor activity. It was suggested that this compound had antagonist properties preferentially at DA autoreceptors. It was also suggested that the phenethylamine structural element of compound **6** contributed to the D2 receptor affinity, but the lack of a hydrogen bond donor (e.g., a OH group) on the aromatic ring prevented it from having intrinsic activity at these receptors. The cyano substituted compound **5** was tested for DA agonist activity in reserpine pretreated rats but was found to be inactive in that assay.[7] Interestingly, when **5** was tested in non-pretreated rats it was found to possess DA receptor antagonist properties as judged from biochemical observations. In addition, **5** produced behavioral stimulation (see below). Furthermore, the compound displayed good oral availability.

In the present study we describe the synthesis, biological activity, and the pharmacokinetic properties of the enantiomers of the cyano- and triflate-substituted phenylpiperidines.

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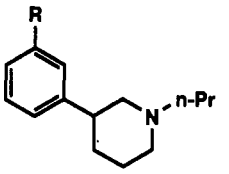


Compd	R	confign
(-)-3	-OH	S
(+)-3	-OH	R
(-)-4	$-OSO_2CF_3$	S
(+)-4	$-OSO_2CF_3$	R
(±)-5	-CN	Racemate
(-)-5	-CN	S
(+)-5	-CN	R
(±)-6	$-CF_3$	Racemate

Chemistry

The preparation of the enantiomers of 4 and 5 is outlined in Scheme I. The synthesis of racemic 5 has been described elsewhere.[7] The synthetic route for the compound is tedious, and the yield is poor. Since the enantiomers of 3 (3-(3-(hydroxyphenyl)-*N*-*n*-propyl)piperidine, 3-PPP) were available to us, we sought to devise a process for the conversion of these phenols into the corresponding cyano analogs. The synthesis of aryl cyanides from aryl triflates and potassium cyanide by means of a palladium or nickel catalyst has been described earlier.[8-10] In an attempt to convert *R*-(+)- and *S*-(-)-4 to the corresponding nitriles this approach was unsuccessful, and we turned to use Stille's aryl triflate methodology.[11] A modification of the reaction according to Kosugi et. al.[12] gave the cyano derivatives *R*-(+)- and *S*-(-)-5 in satisfactory yields (Scheme I). The reaction mechanism of the palldium-mediated cyanation of aryl triflates seems to be dissimilar to that of a so-called cross-coupling reaction[13] and a likely mechanism is shown in Scheme II. The formation of PhCN in the reaction of the equimolar ternary mixture of PhI, Bu_3SnCN, and $Pd(PPh_3)_4$ is considered to involve process B but not A. However, process B cannot construct a catalytic cycle because of deposition of metallic palladium. An advantage with this cyanation procedure of aryl triflates (via palladium mediated coupling) is that the optical purities and absolute configurations of the enantiomers of 3 are retained in the modified analogues. *R*-(+)- and *S*-(-)-4 were prepared from the readily available enantiomers of 3 by treatment with triflic anhydride in the presence of triethylamine.

Pharmacology

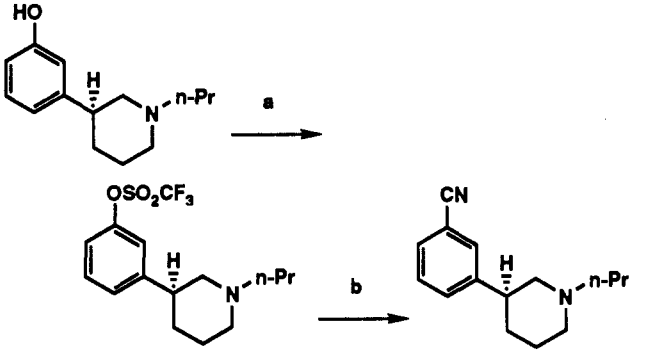
The compounds were tested for their *in vitro* binding affinity to rat striatal dopamine (DA) D_2 receptors ([^{3}H]spiperone and [^{3}H]N-0437), cloned mammalian receptors expressed in CHO-K1 cells ([^{3}H]U-86170 and [^{3}H]spiperone for D2 DA receptors and [^{3}H]spiperone for D_3 and D_4 DA receptors). The compounds were also tested for their affinity for σ-receptors in homogenates or membranes from brain minus cerebellum using 2 nM [^{3}H]DTG (1,3-di-*o*-tolylguanidine) as ligand (Table V).[14]

Table I. Physical Data

compd	yield, %	mp,[a] °C	$[\alpha]^{22}_D$ (*c* 1.0, MeOH)	formula	anal. (±0.4%)
4-(-)	92	156-58	-6.6	$C_{15}H_{20}F_3NO_3S \times HCl$	CHN
4-(+)	90	154-58	+6.6	$C_{15}H_{20}F_3NO_3S \times HCl$	CHN
5[b]		180-81	racemate	$C_{15}H_{20}N_2 \times HCl$	CHN
5-(-)	52	198-99	-10.6	$C_{15}H_{20}N_2 \times HCl$	CHN
5-(+)	56	196-98	+10.1	$C_{15}H_{20}N_2 \times HCl$	CHN

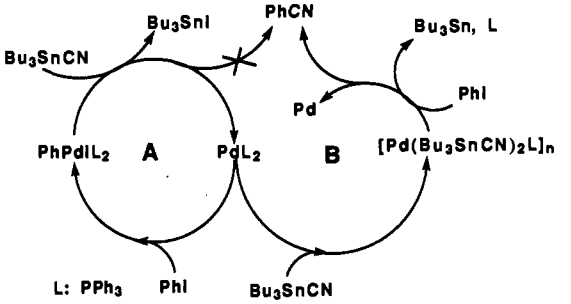
[a] Recrystallization solvents were EtOH/isopropyl ether for all compounds. [b] All data taken from ref 7.

Scheme I. Preparation of (+)- and (-)-4 and -5[a]



[a] Reagents: a = NEt_3, $(CF_3SO_2)_2O$; b = $Pd(PPh_3)_4$, *n*-Bu_3SnCN.

Scheme II. Suggested Mechanism by Kosugi et al. (ref 12) for the Palladium-Mediated Cyanation of PhI



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 CNS *in vitro* radioligand receptor-binding assays according to standard methodology. The compounds tested displayed less than 50% inhibition at 1 mmol for dopamine D_1 ([^{3}H]SCH23390), α_1- ([^{3}H]prazosin), α_2- ([^{3}H]clonidine), β-adrenoreceptors ([^{3}H]dihydroalprenolol), 5-HT_{1A} ([^{3}H]-8-OH-DPAT), 5-HT_{1D} ($\alpha + \beta$, cloned cells, [^{3}H]-5-OH-tryptamin),[15] 5-HT_2 ([^{3}H]ketanserin), acetylcholine muscarinic ([^{3}H]oxotremorine), benzodiazepine ([^{3}H]flunitrazepam), and to opiate ([^{3}H]-etorphine)-labeled sites. After initial *in vitro* screening the compounds were tested for central DA and 5-HT receptor agonist and/or antagonist activity *in vivo* by the use of biochemical and behavioral methods.[16-22]

The compounds were biochemically investigated for their ability to control the synthesis, release and metabolism of monoamines (DA, 5-HT, and NA) in reserpine pretreated, nonpretreated rats which had or had not been habituated to their environment (Tables II-IV). The behavioral effects of the compounds tested were also monitored. The concept of these biochemical and behavioral screening methods have been described earlier.[21] Experimental data obtained with the prototype preferential DA autoreceptor antagonists (+)-1 and (+)-2 and

Table II. Effects on *in Vivo* DA Synthesis in Rat Brain

	nonpretreated rats[o] 5-HTP increase	nonpretreated rats DOPA acc					reserpine pretreated rats[p] DOPA acc	
compd	limbic (μmol/kg s.c.)	hemisphere (μmol/kg s.c.)	limbic (μmol/kg s.c.)		striatum (μmol/kg s.c.)		limbic	striatum
	100[s]	100[s]	25[s]	100[s]	25[s]	100[s]	100[s]	100[s]
(+)-4	86 ± 14	86 ± 4[q]	93 ± 4	82 ± 5[q]	92 ± 5	80 ± 5[q]	75 ± 8[q]	80 ± 7[q]
(−)-4	102 ± 4	108 ± 7	139 ± 6[q]	168 ± 13[q]	221 ± 25[q]	264 ± 13[i,q]	94 ± 5	89 ± 4
(±)-5	99 ± 3[a]	103 ± 9	NT[r]	198 ± 10[a,q]	NT	281 ± 11[a,q]	NT[r]	NT[r]
(+)-5	71 ± 2[b]	94 ± 5[b]	109 ± 7	94 ± 3[b]	84 ± 7[q]	78 ± 4[b,q]	93 ± 5[a]	81 ± 5[a,q]
(−)-5	148 ± 10[q]	158 ± 8[q]	204 ± 14[q]	219 ± 15[q]	285 ± 3[q]	268 ± 16[k,q]	136 ± 8[a]	105 ± 12[a]
(+)-1	NT[r]	NT[r]	223[c,d,q]	253[a,d,q]	330[c,d,q]	400[a,d,m,q]	108 ± 11[d,a]	102 ± 8[d,a]
(+)-2	NT[r]	NT[r]	180[c,e,q]	245[a,e,q]	270[c,e,q]	370[a,e,n,q]	116 ± 4[d,a]	109 ± 4[d,a]
(−)-3	89 ± 5[g]	87 ± 10[g]	91 ± 1.3[f]	NT	147 ± 3.4[f,q]	NT[r]	30[f,g,q]	NT[r]
(+)-3	NT[r]	NT[r]	37 ± 2.5[f,q]	NT	43 ± 1.9[f,q]	NT[r]	18[f,g,q]	NT[r]

[a] 52 μmol/kg s.c. [b] 200 μmol/kg s.c. [c] 13 μmol/kg s.c. [d] Data taken from ref 2. [e] Data taken from ref 35. [f] Data taken from ref 22. [g] 27 μmol/kg s.c. [h] ED_{50} = 30 μmol/kg. [i] ED_{50} = 15 μmol/kg. [j] ED_{50} = 20 μmol/kg. [k] ED_{50} = 10 μmol/kg. [l] ED_{50} = 15 μmol/kg. [m] ED_{50} = 10 μmol/kg. [n] ED_{50} = 13 μmol/kg. [o] Animals were injected with test drug 65 min and NSD 1015 (100 mg/kg ip) 30 min before death. Controls received corresponding saline injections. Values are expressed as percent of saline controls. [p] Animals were injected with reserpine (5 mg/kg ip) 18 h, test drug 60 min and NSD 1015 (100 mg/kg ip) 30 min before death. Controls received corresponding saline injections. Values are expressed as percent of saline controls. [q] $p < 0.05$. [r] NT means not tested. [s] Dose.

Table III. Effects of Tested Compounds on Rat Locomotor Activity

	nonpretreated habituated rats locomotor activity: percent of saline controls, mean ± SEM		nonhabituated rats locomotor activity: percent of saline controls, mean ± SEM		reserpine pretreated rats locomotor activity: accumulated counts/30 min means ± SEM (μmol/kg)
compd	25 μmol/kg[m]	100 μmol/kg[m]	25 μmol/kg[m]	100 μmol/kg[m]	
(+)-4	30 ± 11[k]	34 ± 14[k]	115 ± 19	95 ± 19	2 ± 1(100)
(−)-4	128 ± 33	445 ± 131[k]	128 ± 26	129 ± 22	25 ± 12(100)
(±)-5	NT[l]	878 ± 43[k]	167 ± 19[d,k]	NT[l]	NT
(+)-5	NT[l]	559 ± 122[k]	104 ± 16	118 ± 24[e]	6 ± 2(50)
(−)-5	418 ± 91[k]	719 ± 45[k]	154 ± 20[k]	165 ± 8[k]	12 ± 4(50)
(+)-1	300[a]	350[a]	155 ± 24[b,c,k]	159 ± 23[b,d,k]	11 ± 4(50)[a]
(+)-2	220[a]	220[a]	140 ± 10[b,c,k]	70 ± 13[b,d]	29 ± 8(50)[a]
(−)-3	NT[l]	208 ± 27[k]	74[h,i,k]	40[f,g]	12 ± 2(213)[h]
(+)-3	NT[l]	NT[l]	75[h,j,k]	230[f,g,k]	78 ± 14(13)[h,k]

[a] Data taken from ref 2. [b] Data taken from ref 35. [c] At 13 μmol/kg s.c. [d] At 52 μmol/kg s.c. [e] At 200 μmol/kg s.c. [f] Data taken from ref 22. [g] At 213 μmol/kg s.c. [h] Data taken from ref 44. [i] 0.8 μmol/kg s.c. [j] 1.6 μmol/kg s.c. [k] $p < 0.05$. [l] NT means not tested. [m] Dose.

Table IV. Maximal Effects of Tested Compounds on DA Release and Metabolism in the Brain Dialysis Model After 50 μmol/kg s.c.

	striatum		accumbens	
	DA	DOPAC	DA	DOPAC
(−)-4	208 ± 13[b,c]	162 ± 4[c]	153 ± 11[c]	152 ± 12[c]
(−)-5	295 ± 56[c]	299 ± 21[c]	294 ± 47[c]	168 ± 13[c]
(+)-1[a]	285 ± 33[c]	315 ± 26[c]	313 ± 32[c]	235 ± 11[c]
(+)-2[a]	209 ± 5[c]	307 ± 36[c]	231 ± 9[c]	207 ± 20[c]
(−)-3	190 ± 11[c]	163 ± 11[c]	98 ± 9	115 ± 13
(+)-3	28 ± 1[c]	42 ± 3[c]	NT[d]	NT[d]

[a] Data were taken from ref 33. [b] Values are expressed as percent of saline controls. [c] $p < 0.05$. [d] NT means not tested.

the DA receptor agonists (+)- and (−)-3 (Preclamol) are included for comparative purposes. The pharmacokinetic properties *in vivo* were evaluated by estimating the oral bioavailability and plasma half life ($t_{1/2}$, Table VI).

Results and Discussion

Biochemical Effects of the S-(−)-Enantiomers. It was recently speculated by Yevich et al.[23] that compounds which lack strong affinity for DA receptors, having *in vivo* behavioral activity, might represent a major advance in the therapy of psychoses due to the minimal side effect liability (EPS).

In vitro binding (Table V) data demonstrate that the compounds tested possess, albeit weak, affinity for the DA receptor sites identified by the dopamine agonists [^{3}H]N-0437 and [^{3}H]U-86170 or the dopamine antagonist [^{3}H]spiperone. The affinities for *S*-(−)-4 and *S*-(−)-5 are generally higher at the high affinity agonist (HiAg) binding site (defined by [^{3}H]N-0437, [^{3}H]U-86170) at both rat striatal DA receptors and cloned mammalian DA D_2 and D_3 receptors than at the antagonist, i.e., low agonist (LowAg), binding site (defined by [^{3}H]spiperone in the presence of GTP). Interestingly, higher affinity at the HiAg site than the LowAg is indicative of intrinsic activity (IA) at DA receptors as discussed by Lahti et al.[24] However, despite this binding profile the *in vivo* biochemical assays do not reveal any IA at these receptors for *S*-(−)-4 and *S*-(−)-5. In contrast to *S*-(−)-4 and *S*-(−)-5, *S*-(−)-3 displays a clear-cut agonist profile in both the binding assay and the *in vivo* biochemistry assays. The rank order of binding potency in the DA receptor binding assays is (+)-2 > *S*-(−)-4 > (+)-1 > *S*-(−)-5.

Hutchison et al.[25] have screened DA agonists in a spiperone binding assay. They found that a weak affinity in this binding assay indicates a preference for the presynaptic DA receptors and high affinity indicate preference for the postsynaptic DA receptors. If this reasoning can be applied to antagonists derived from agonist structures such as (+)- and (−)-3 and *S*-(−)-5-OMe-2-(di-*n*-propylamino)tetralin the rank order above might suggest that the nitrile analogs *S*-(−)-5 and (+)-1, due to their lower spiperone affinity, are the more presynaptically preferring compounds in this series.

In contrast to classical DA antagonists the prototype autoreceptor antagonists (+)-1 and (+)-2 have a certain preference for the D3 receptor.[5,26] This preference is confirmed in this study comparing D2 and D3 spiperone binding affinity (Table V). Interestingly, *S*-(−)-4 and *S*-(−)-5 that show the same pharmacological profile as (+)-1

Table V. Pharmacological Data: Binding

compd	K_i (nM)						
	D2-spip[a,d]	D2-N0437[a,e]	D2-U86170[b,f]	D2-spip[b,f]	D3-spip[b,f]	D4-spip[b,f]	σ-DTG[c,e,g]
(−)-4	410 ± 115	135 ± 30	40 ± 2	180 ± 22	68 ± 3	>933	32
(+)-4	8300 ± 3300	5600 ± 1000	>1684	>2000	>1623	>933	6.0
(−)-5	1140 ± 280	396 ± 18	119 ± 33	889 ± 181	249 ± 39	>933	2200
(+)-5	12600 ± 2500	4900 ± 800	1724 ± 285	>2000	>1425	>933	520
(+)-1	530[h]	300[i]	145 ± 13	158 ± 29	26 ± 2	117 ± 10	NT[j]
(+)-2	70[h]	100[i]	13 ± 8	18 ± 3	4 ± 0.3	48 ± 2	NT[j]
(−)-3	100[h]	30[i]	8.5 ± 1.1	137 ± 19	132 ± 16	130 ± 33	940
(+)-3	700[h]	70[i]	32 ± 4	776 ± 230	217 ± 21	>1000	430
haloperidol	0.2[h]	0.7[i]	0.7 ± 0.8	0.3 ± 0.03	6.4 ± 1.5	2.2 ± 0.4	17

[a] Data from rat striatal membrane. [b] Data from cloned mammalian receptors expressed in CHO-K1 cells. [c] Binding of 2 nM [^{3}H]DTG (1,3-di-o-tolylguanidine) to σ receptors in membranes from brain minus cerebellum. [d] Values are the mean of six drug concentrations (10^{-4}–10^{-9} M) in which each value was determined in triplicate. [e] Values are the mean of seven drug concentrations (10^{-4}–10^{-10} M) in which each value was determined in triplicate. [f] Values are the mean of 11 drug concentrations in which each value was determined in duplicate. [g] The result is reported as IC_{50} values. [h] Values are the mean of six drug concentrations (10^{-4}–10^{-9} M) in which each value was determined in single determination. [i] Values are the mean of seven drug concentrations (10^{-4}–10^{-10} M) in which each value was determined in single determination. [j] NT means not tested.

Table VI. Pharmacokinetic Data

compd	AUC ratio po/iv[a] (%)	half-life[b] (min)	compd	AUC ratio po/iv[a] (%)	half-life[b] (min)
(−)-3	3.4 ± 1.5[c]		(−)-4	75 ± 19	210
(+)-1	1.6 ± 0.3		(−)-5	28 ± 4	
(+)-2	3.7 ± 1.6		(±)-5	74 ± 19	

[a] Blood samples were taken from rats with arterial catheters at various time intervals between 5 min and 12 h. The absolute oral availability 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). [b] The half-lives were estimated graphically from the elimination phase of the blood-concentration curves after per oral administration. [c] Data from man.

and (+)-2 also have a similar preference for the D3 site. The significance of this observation in relation to the *in vivo* pharmacological profile is difficult to assess. However, more compounds with this profile have to be discovered and evaluated to adress the possible involvement and function of the D3 receptor in the pharmacology of the DA autoreceptor antagonists.

During the past years an increased interest for compounds with affinity for σ-receptors have evolved.[27,28] Though much work has been done to elucidate the functional role of these sites, the significance of these receptors has been questioned (for review see Walker et al.[27]). Nevertheless, it has been speculated that σ-receptor ligands might have potentially antipsychotic properties.[29] Since (+)- and (−)-3[30] frequently have been used as ligands for σ-receptors, the arylpiperidine skeleton seems to have the structural elements which determines affinity to these binding sites. Table V shows that the substituent on the aromatic ring has a significant influence on the affinity for the σ-DTG binding site. The nitrile analog, *S*-(−)-5, shows no affinity, while the triflate substituent on *S*-(−)-4 dramatically increased the affinity for the DTG site as compared to –OH substitution (*S*-(−)-3). The same trend in substituent effect is seen with the *R*-(+) enantiomers.

Tables II and III show that racemic 5 displays a similar pharmacological profile to (+)-1 and (+)-2. The (−) enantiomers of 4 and 5 appear to be the active enantiomers. In nonpretreated animals, *S*-(−)-4 and *S*-(−)-5 dose-dependently increased the DA synthesis rate (measured as DOPA formation in striatal, limbic, and the remaining cortical brain regions). *S*-(−)-5 maximally increased DOPA accumulation to 290% of control values in the striatum (data not shown). The efficacy of *S*-(−)-5 is in the same range as that of haloperidol (increase to 210% in the limbic and to 310% in the striatal brain regions[31]). However, this is slightly less than what is observed for (+)-2 and (+)-1 (370–400% of controls in the striatum, Table II). The ED_{50} values in this assay for *S*-(−)-4, *S*-(−)-5, (+)-2, and (+)-1 are in the same range (15, 10, 13, and 10 μmol/kg s.c., respectively).

Similar results were obtained in nonpretreated rats habituated to their environment. *S*-(−)-4 and *S*-(−)-5 dose-dependently increased the DOPAC values (217% and 325%, respectively, in the striatum at 100 μmol/kg s.c.).

The compounds tested all increased the release of DA, as determined in the *in vivo* brain dialysis experiments (Table IV). At 50 μmol/kg s.c. (+)-1 and *S*-(−)-5 showed a maximal increase to about 300% of control values on DA and the major metabolite DOPAC in both striatum and accumbens. The maximal increase in DA release for these two compounds is considerably higher than for (+)-2 and *S*-(−)-4 and, e.g., haloperidol.[32-34] The mechanism underlying this is not clear but might indicate a better "efficacy" for these compounds to interact with the presynaptic DA release controlling autoreceptors.

Neither *S*-(−)-4 nor *S*-(−)-5 influenced the DOPA accumulation or locomotor activity in reserpine pretreated animals (Tables II and III). These data indicate a lack of direct agonistic effects at central dopamine receptors for compounds *S*-(−)-4 and *S*-(−)-5. In contrast, *S*-(−)-3 induced a nearly maximal decrease (to 30% of control values) in DOPA formation in the striatal brain regions displaying almost full intrinsic activity in this assay.[22]

S-(−)-5 was the only compound tested which had a slight effect on 5-HTP formation indicating that this compound may have weak activity at central 5-HT_1 receptors. However, these effects were not dose-dependent. Rather than direct receptor mediated effects this suggests indirect effects on the 5-HT system. *S*-(−)-5 also slightly increased the DOPA accumulation in cortical brain regions showing possible interactions with noradrenergic neurons.

Behavior

In nonpretreated rats *S*-(−)-5, like (+)-1, produced a dose-dependent increase (+60%) in locomotor activity, while *S*-(−)-4 had no effect in this assay. (+)-2 increased LMA at doses <100 μmol/kg s.c. but induced a decrease at doses >100 mmol as compared to controls.[35] The ability of (+)-1, *S*-(−)-4 and *S*-(−)-5 to dose-dependently increase DOPA accumulation (discussed above) without producing any reduction in locomotor activity as normally observed for DA antagonists suggests that *S*-(−)-5 like (+)-1 are

more efficacious antagonists at DA autoreceptors than at postsynaptic DA receptors. According to the prevailing hypothesis the stimulatory effects on locomotor activity produced by (+)-1 and S-(−)-5 is thought to be mediated via enhancement of DA release due to DA autoreceptor antagonism (Table IV). This results in an increase in postsynaptic receptor stimulation by the DA released.[36] S-(−)-4 increased DA release less than (+)-1 and S-(-)-5. This may tentatively be an explanation to why it does not increase locomotor activity in non-pretreated rats. However, in nonpretreated habituated rats, where the baseline activity is low, all compounds were able to increase the locomotor activity with the efficacy rank order of (+)-2 < (+)-1 < S-(−)-4 < S-(−)-5 (Table III). Weak stereotyped sniffing and rearing was also observed.

Thus, in the behavioral assays no signs of hypolocomotion or catalepsy was noted for S-(−)-4 and S-(−)-5. This is in bright contrast to classical DA receptor antagonists such as haloperidol, which produces strong hypolocomotion and catalepsy.

The present data, indicate that S-(−)-4 and S-(−)-5 display pharmacological profiles similar to (+)-1 and (+)-2. Furthermore, S-(−)-5 appears to be more efficacious at the DA release controlling autoreceptors, as compared to the postsynaptic DA receptors, rendering this compound more stimulatory properties, as compared to S-(−)-4,(+)-1, and (+)-2.

As discussed above, the relatively low affinity in the spiperone binding assay seems to be of importance for the stimulatory properties of these compounds. Thus, it seems as though the lower affinity for the spiperone binding site the greater the increase in locomotor activity observed. These results are in good agreement with the findings by Hutchison et al.;[25] a decrease in affinity is followed by an increase in apparent preference for presynaptic DA receptors.

Both S-(−)-4 and S-(−)-5 lack direct postsynaptic agonist effects at central DA receptors. From a structural point of view it is interesting to note that the substituent on the aromatic ring has such a considerable influence on the pharmacological profile. We suggest that lack of hydrogen bond donor properties (e.g., OH) are important to prevent agonist activity. Both the triflate[37] and nitrile[38] substituents are powerful electron withdrawing groups with the nitrile being more efficacious. Due to the slight but significant biochemical and behavioral differences between S-(−)-4 and S-(−)-5, it can be questioned whether it is the low electron density in the aromatic ring, emanating from the powerful electron withdrawing groups, or the substituent itself (possible hydrogen bond accepting properties) that is the most important factor determing the DA receptor antagonistic properties of S-(−)-4 and S-(−)-5. However, it cannot be ruled out that the difference in steric factors for the substituents are of importance; a triflate group is considerably larger than a nitrile substituent. Interestingly, there seems to be opposite effects of the substituents on the phenylpiperidine- and the aminotetralin skeleton. That is, an electron withdrawing group (–CN) on the phenylpiperidine enhances the antagonistic effects, while in the 5-position on the *cis*-(+)-1-methylated aminotetralin it reduces the antagonist properties as compared to the electron-donating group (5-MeO, unpublished data).

Another physical property that has to be taken into account is the lipophilicity. The calculated ClogP values for S-(−)-4, S-(−)-5, (+)-1, and (+)-2 are 5.2, 3.4, 3.7, and 5.0, respectively.[39] As mentioned above S-(−)-5 and (+)-1 seem to be more stimulatory in the behavioral assays than S
(−)-4 and (+)-2. Thus, the ClogP values indicate a more hydrophilic character of the more behaviorally stimulant compounds.

The pharmacokinetic experiments show that both S-(−)-4 and S-(−)-5 clearly have higher oral availability (75% and 30%, respectively) than (+)-2, (+)-1, and also (−)-3 in the rat. Particularly the triflate substituted arylpiperidine S-(−)-4 has a very high oral availability. It is well known that phenolic and N,N-dialkylated aminotetralins have two major pathways for metabolic inactivation, enzymatic hydroxylation of the aromatic ring and subsequent conjugation (O-glucuronidation) or oxidative N-despropylation with subsequent conjugation (N-glucuronidation).[40] Even when the aromatic ring is protected with an electron withdrawing group the tetralin compounds will render a low oral bioavailability probably due to the N-dealkylation problem.[41] The phenyl piperidines seem to have quite high bioavailability when the aromatic ring is substituted with an electron withdrawing group.[42] A likely explanation is that the piperidine ring is more stable against enzymatic N-dealkylation as compared to the dialkylated 2-aminotetralins.

R-(+)-enantiomers. The receptor binding studies (Table V) indicate that the introduction of electron withdrawing substituents in the phenyl ring is detrimental for affinity for dopamine receptors *in vitro*. The affinity for the σ-DTG binding site increased drastically for the triflate substituted analog R-(+)-4. Substitution with a cyano groups (R-(+)-5) did not increase the affinity as drastically at this site.

In nonpretreated animals, R-(+)-4 and R-(+)-5 slightly decreased the accumulation of DOPA indicating only weak effects on DA receptors (Table II). There were no effects on behavior in this model. As a comparison, the -OH substituted full DA agonist, (+)-3, decreased DOPA accumulation to about 50% in the striatum and in the limbic areas. Also, (+)-3 showed the biphasic dose-response relationship on locomotor activity, typical for DA agonists with full intrinsic activity.[22] In the reserpine model both R-(+)-4 and R-(+)-5 slightly decreased DOPA accumulation in the striatum, which suggest weak intrinsic activity at these receptors (Table II). No effects on locomotor activity were detected with these two compounds in this assay.

In habituated rats R-(+)-4 decreased the locomotor activity, while no change in DOPAC levels was detected. Interestingly, R-(+)-5 produced a strong dose-dependent locomotor stimulation up to 560% of controls (data not shown, Table III). This increase in LMA was not followed by an increase in DOPAC as for S-(−)-4 and S-(−)-5. However, a significant increase in HVA was observed, suggesting an increase in DA release (data not shown). These findings are currently under investigation.

Conclusion

The (−)-enantiomers of 4 and 5 are able to increase the DA synthesis, release and metabolism in a wide dose range. In this respect S-(−)-4 and S-(−)-5 do not differ qualitatively from classical neuroleptics. However, in sharp contrast to the latter group of dopamine receptor ligands, these compounds produce a slight but stable behavioral acti-

vation. The behavioral and biochemical data for S-(−)-4 and S-(−)-5 indicate that these two compounds display a similar pharmacological profile as the prototype DA autoreceptor antagonists (+)-1 and (+)-2. Also, in contrast to the classical dopamine antagonists, S-(−)-4 and S-(−)-5 lack high affinity for DA D_2 receptor antagonist site. However, like (+)-1 and (+)-2 these compounds have a certain preference for the DA D_3 receptor antagonist site.

From a SAR point of view it is obvious that the aromatic substituent has a crucial influence on the pharmacological profile. The physical properties of the substituent, i.e., change in lipophilicity, inductive effect as well as the possibility to form H-bonds by donating/accepting hydrogen atoms to the DA receptor seems to be important for the agonist/antagonist profile within the arylpiperidine class of compounds. To achieve DA autoreceptor antagonist properties within the phenylpiperidine class, it seems to be necessary to have an electron withdrawing group that is relatively hydrophilic and without H-bond donating properties.

The physical properties of the substituent also seems to have a considerable influence on the oral bioavailability of the phenylpiperidines. Substituting the aromatic ring with electron withdrawing groups (e.g., -CN, $-OSO_2CF_3$) results in arylpiperidines with good oral bioavailability (30% and 70%, respectively) in the rat.

Based on the available data the (−)-enantiomers of 4 and 5 are characterized as orally available, centrally acting DA autoreceptor antagonists.

Experimental Section

General Methods. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 and 75.4 MHz, respectively, with a Varian XL 300 spectrometer. Chemical shifts are reported as δ values (ppm) relative to internal tetramethylsilane. Low resolution mass spectra were obtained on HP 5970A instrument operating at an ionisation potential of 70 eV and interfaced with a HP5700 gas chromatograph. A fused silica column (11 m, 0.22 mm i.d.) coated with cross linked SE-54 (film thickness 0.3 mm, He gas, flow 40 cm/s) was used. Elemental analyses were obtained from Mikro Kemi AB, Uppsala, Sweden. Melting points were determined with a melting point microscope (Reichert Thermovar) and are uncorrected. For flash chromatography silica gel 60 (0.040–0.063 mm, E. Merck, no 9385) was used.

Materials. All chemicals used are commercially available and were used without further purification. 3-(3-(Cyanophenyl)-*N*-*n*-propyl)piperidine was prepared as described in the literature.[7] (+)- and (−)-3-PPP were obtained from RBI (Research Biochemicals Incorporated).

***S*-(−)-3-[(3-(((Trifluoromethyl)sulfonyl)oxy)phenyl)-*N*-*n*-propyl]piperidine (*S*-(−)-4) (Scheme I).** A solution of *S*-(−)-3-(3-(hydroxyphenyl)-*N*-*n*-propyl)piperidine (*S*-(−)-3)[43] (3.3 g, 15.07 mmol) and triethylamine (1.68 g, 16.58 mmol) in 300 mL of CH_2Cl_2 was cooled to −30 °C. Then triflicanhydride (4.68 g, 16.58 mmol) in 30 mL of CH_2Cl_2 was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 2 h at 25 °C. The reaction was quenched with cold water. The layers were separated, and the organic phase was washed with 2 portions of cold 5% HCl solution. Following a wash of the organic portion with brine and drying ($MgSO_4$), the solvent was removed under reduced pressure, and the residue was chromatographed on a silica column with $MeOH/CH_2Cl_2$ (1:19) as eluent. The solvents from the collected fractions containing pure *S*-4 were evaporated and ether was added to the residue (4.87 g, 92%) to dissolve the product and filter off the insoluble SiO_2. Addition of etheral HCl to the ether solution afforded *S*-4 × HCl: mp 156–158 °C (HCl); ^1H NMR (300 MHz, $CDCl_3$) δ 0.9 (t, 3H), 1.35–2.0 (m, 8H), 2.33 (m, 2H), 2.82–3.2 (m, 3H), 7.1–7.4 (m, 4H); ^{13}C NMR (75.4 MHz, $CDCl_3$) 12.0 (CH_3), 20.0 (CH_2), 25.4 (CH_2), 31.4 (CH_2), 42.5 (CH), 53.7 (CH_2), 60.7 (CH_2), 61.0 (CH_2), 118.2 (q, J = 320 Hz, CF_3), 119.0 (CH), 120.0 (CH), 127.4 (CH), 130.0 (CH), 148.0 (C), 149.6 (C); MS *m/e* 351.1 (M^+, 2.8), 323.15 (16.3), 322.15 (Bp, 100), 189.15 (48.5), 188.25 (10.8), 160.15 (6.8), 91.05 (10.4), 86.15 (9.0); $[\alpha]_D^{20}$ −6.6° (*c* = 1.0, MeOH).

***R*-(+)-3-[(3-(((Trifluoromethyl)sulfonyl)oxy)phenyl)-*N*-*n*-propyl]piperidine (*R*-(+)-4) (Scheme I).** This compound was prepared as described for *S*-(−)-4 from *R*-(+)-3-(3-(hydroxyphenyl)-*N*-*n*-propyl)piperidine (*R*-(+)-3)[43] (1.0 g, 4.57 mmol) and triflic anhydride (1.42 g, 5.03 mmol). Purification of the crude product by flash chromatography $MeOH/CH_2Cl_2$ (1:19) afforded 1.44 g (90%) of pure *R*-4 as an oil. The amine was converted to the hydrochloride salt with HCl-saturated ethanol, and recrystallized from ethanol/isopropyl ether: mp 154–158 °C (HCl); MS *m/e* 351.15 (M^+, 2.9), 323.05 (14.7), 322.05 (Bp, 100), 190.05 (11.6), 189.05 (78.6), 188.15 (13.4), 160.05 (8.0), $[\alpha]_D^{20}$ +6.6° (*c* = 1.0, MeOH).

***S*-(−)-3-(3-(Cyanophenyl)-*N*-*n*-propyl)piperidine (*S*-(−)-5) (Scheme I).** Tetrakis(triphenylphosphine)palladium (2.47 g, 2.14 mmol) and tributyltin cyanide (1.87 g, 5.91 mmol) in 60 mL of dichloroethane were heated under argon at 80 °C for 2 h. To this refluxing solution was added in one portion *S*-(−)-4 (500 mg, 1.42 mmol) in 40 mL of dichloroethane. The reaction was heated at 80 °C under argon for 24 h. The mixture was cooled to room temperature, and the solid precipitate was filtered off. The mixture was concentrated *in vacuo* and the residue redissolved in 10% NaH_2PO_4 (35 mL). The aqueous solution was extracted with diethyl ether (3 x 30 mL) to remove impurities. The combined ether phases were extracted with two portions of 10% NaH_2PO_4. The combined aqueous phase was basified with 15% NaOH and extracted with diethyl ether (4 × 20 mL). The combined organic phases were washed with brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 12/1 by volume) and afforded 168 mg (52%) of pure *S*-5 as an oil The amine was converted to the hydrochloride salt with HCl-saturated ethanol and recrystallized from ethanol/isopropyl ether affording pure crystals: mp 198–199 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.9 (t, 3H), 1.3–2.1 (m, 8H), 2.3 (dd, 2H), 2.85 (tt, 1H), 3.0 (dd, 2H), 7.4–7.6 (m, 4H); ^{13}C NMR (75.4 MHz, $CDCl_3$) 12.0 (CH_3), 20.0 (CH_2), 25.4 (CH_2), 31.4 (CH_2), 42.5 (CH), 53.7 (CH_2), 60.7 (CH_2), 61.0 (CH_2), 112.4 (C), 119.0 (C), 129.1 (CH), 130.1 (CH), 130.8 (CH), 132.0 (CH), 146.1 (C); MS *m/e* 228.1 (M^+, 3.4), 200.0 (16.3), 199.0 (Bp, 100), 156.0 (7.5), 142.0 (3.9), 129.0 (6.4), 116.0 (11.1), 70.0 (7.0); $[\alpha]_D^{20}$ −10.6° (*c* = 1.0, methanol).

***R*-(+)-3-(3-(Cyanophenyl)-*N*-*n*-propyl)piperidine (*R*-(+)-5) (Scheme I).** This compound was prepared as described for *S*-(−)-5 from *R*-(+)-4 (500 mg, 1.42 mmol), tetrakis(triphenylphosphine)palladium (2.47 g, 2.14 mmol), and tributyltin cyanide (1.87 g, 5.91 mmol). Purification of the crude product by flash chromatography $MeOH/CH_2Cl_2$ (1:12) afforded 182 mg (56%) of pure R-5 as an oil. The amine was converted to the hydrochloride salt with HCl-saturated ethanol and recrystallized from ethanol/isopropyl ether: mp 196–198 °C (HCl); MS *m/e* 228.1 (M^+, 3.5), 200.0 (15.5), 199.0 (Bp, 100), 156.0 (5.6), 142.0 (4.0), 129.0 (6.8), 116.0 (8.9), 70.0 (6.7), 57.0 (4.0); $[\alpha]_D^{20}$ + 10.1° (*c* = 1.0, methanol).

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

Drugs. All substances to be tested were dissolved in physiological (0.9%) 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 of volume with 5.5% glucose. Injection volumes were 5 or 10 mL/kg, and all solutions had neutral pH values (except for the solutions of reserpine).

Biochemistry (Biochemically Monitored DA and 5-HT Receptor Agonist or Antagonist Activity). The concept of the biochemical screening method is that a DA or 5-HT receptor agonist stimulates the corresponding receptor and through regulatory negative feed-back systems induces a decrease in

tyrosine or tryptophan hydroxylase activity, respectively, and a subsequent reduction in the synthesis rate of DA and 5-HT. DOPA and 5-HTP formation, as determined after *in vivo* inhibition of the aromatic L-amino acid decarboxylase with NSD 1015 (3-hydroxybenzylhydrazine hydrochloride), are taken as indirect measures of DA and 5-HT synthesis rates, respectively.[20,44] The biochemical experiments and the determinations of DOPA and 5-HTP by means of HPLC with electrochemical detection were performed according to a modification of a previously described method.[45,46] Receptor antagonist effects were seen as increases in the synthesis rate of corresponding neurotransmitter. This is a result of inhibition of the negative feed-back down regulation of transmitter synthesis. The effects on DOPA accumulation are expressed as percent of controls, which are as follows: DOPA limbic system = 447 ± 23 ng/g and DOPA striatum = 1045 ± 47 ng/g, mean ± SEM, n = 4 (Table II). In the experiments with habituated rats no NSD1015 was administered and the animals were killed 1 h after drug administration. The brains were dissected and the levels of DA, DOPAC (control levels: limbic 304 ± 11 ng/g and striatum 843 ± 24 ng/g, mean ± SEM, n = 4), were assayed by means of HPLC with electrochemical detection.[47] The levels were expressed as percent of controls (DOPAC, Table II).

Motor Activity. Reserpine-Pretreated Animals. The motor activity was measured by means of photocell recordings (M/P 40 Fc Electronic Motility Meter, Motron Products, Stockholm) as previously described.[44] 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 s.c.). 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 (one rat/cage). Motor activity was then followed and recorded for the subsequent 30 min (reserpine control values 3 ± 1 counts/30 min, mean ± SEM; n = 13, Table III). Observations of gross behavior were made throughout the activity sessions through semitransparent mirrors.

Nonpretreated Animals. The motor activity was measured as described for reserpine pretreated animals. The different test compounds were administered subcutaneously in the flank or orally (in animals that had been starved over night). Immediately after drug administration, the rats were placed in the test cages (one rat/cage) and put into the motility meters. Motor activity was then followed and recorded for the subsequent 30 min (Table III). Observations of gross behavior were made throughout the activity sessions through semi-transparent mirrors. Control levels were 229 ± 24 counts/30 min (means ± SEM, n = 4).

Nonpretreated Habituated Animals. These experiments were performed as described above, but the animals were habituated in the test cages 1 h before the injection of the test compound or saline (controls). The habituation resulted in a locomotor activity of about 10% of that seen in nonpretreated animals. The locomotor activity after the test compound was then recorded for 60 min (Table III). Control levels were 44 ± 15 counts/60 min (means ± SEM, n = 4).

Microdialysis. Following the placement of a small burr hole in the appropriate location on the cranium over the corpus striatum (A 1, L 2.6, and D 6) or the nucleus accumbens (A 1.7, L 1.6, D 7.8, 4), male Sprague–Dawley rats were stereotaxically implanted with a flexible plastic dialysis probe (Waters et al. in preparation). The rats were then allowed to recover for 48 h before the experiment started. After connection to a perfusion pump, delivering a Ringers solution containing in mmol/L NaCl 140, $CaCl_2$ 1.2, KCl 3.0, $MgCl_2$ 1.0, and ascorbic acid 0.04, the rats were placed in an open cage allowed to move freely within its domains. The dialysates content of DA and its metabolites were analyzed on a HPLC-EC system allowing 5-min runs for each sample using a sample splitting technique (Waters et al., manuscript in preparation). Drugs were dissolved in physiological saline and injected s.c. in the flank. The effect of the drugs were studied during 3 h. After the experiment the rats were decapitated and the brains were taken out and frozen on a block of dry ice. The location of the probes was controlled by means of a Leitz freezing micro tome.

Receptor Binding. IC_{50} values were estimated from a nonlinear single site fit to data obtained from competition binding experiments run in single, duplicate, or triplicate. Radio receptor binding studies with [^{3}H]-8-OH-DPAT (5-HT_{1A} agonist, 143–158 Ci/mmol, New England Nuclear, Boston, MA, USA), [^{3}H]spiperone (D2 antagonist, 21–24 Ci/mmol, New England Nuclear, Boston, MA, USA), and [^{3}H]N-0437 (D2 agonist) were performed using rat striatal membrane preparations as previously described.[41,48]

Radioligands used, in cloned mammalian receptors expressed in CHO-K1 cells,[26,49,50] were [^{3}H]-U-86170 (62 Ci/mmol, 2 nM)[51] and [^{3}H]spiperone (107 Ci/mmol, 0.5 nM) for D2-dopamine and [^{3}H]spiperone (107 Ci/mmol, 0.6 nM) for D3- and D4-dopamine receptors. Buffers used were 20 mM HEPES, 10 mM $MgSO_4$, and pH 7.4 for D2-dopamine receptors. The buffer used for D3- and D4-dopamine receptors were 20 mM HEPES, 10 mM $MgCl_2$, 150 mM NaCl, 1 mM EDTA, and pH 7.4. Incubation of the 0.9 mL binding mixtures was for 1 h at room temperature. Reactions were stopped by vacuum filtration. Counting was with a 1205 Betaplate (Wallac) using MeltiLex B/HS (Wallac) as scintillant. Dissociation constants (K_i) were calculated with the Cheng and Prushoff equation.[52] The data in Table V are in nM ± SEM.

Inhibition of [^{3}H]DTG binding to σ receptors in rat brain minus cerebellum is determined in vitro as modified from Weber et al.[14] Rats (150–250 g) are decapitated, and the brains (minus cerebellum) quickly removed, placed on ice, weighted, and homogenized in 100 vol ice-cold (0 °C) 50 mM Tris-buffer (pH 7.7) in ethanol-rinsed glass/Teflon homogenizer at 0 °C and kept on ice until use. Tris-buffer (0.5 mL, 50 mM, pH 7.7), 0.25 mL of displacer (DTG, test compound, or Tris-buffer), and 0.25 mL of [^{3}H]DTG are mixed into 5 mL plastic test tubes and kept at 4 °C until use. The binding reaction is initiated by mixing 0.5 mL of tissue suspension into this solution and incubating at 25 °C for 20 min.

Glass fiber filters (Whatman GF/B) are placed on the filter machine which is then closed tightly. Just before filtration vaccum is turned on, and the filters were washed with 0.1% polyethylamine solution from a spray bottle followed by one wash with Tris-buffer (pH 7.7). The binding reaction is stopped by filtration of the assay mixture at reduced pressure (560 mm Hg) followed by further 3 washes with 5 mL of ice-cold Tris-buffer (pH 7.7). The filters are placed in counting vials and 4 mL of scintillation solution added. The vials are counted in a Beckmann scintillation counter. Specific binding is estimated by subtracting nonspecific binding in the presence of 100 μM DTG. The data in Table V are in nM and reported as IC_{50} values.

Estimation of Oral Bioavailability. Blood levels of (+)-1, (+)-2, *S*-(−)-3, *S*-(-)-4, and *S*-(−)-5, were measured by means of gas chromatography (Hewlett–Packard)-mass spectrometry (VG Trio II). The blood samples (150 mL) were collected from arterial catheters in male Sprague–Dawley rats (300 g). The experiment started 24 h after operation, and blood samples were collected at various time intervals up to 12 h after drug injection. The samples were then diluted with 1 mL of water. Then to 50 μL of internal standard (*S*-(−)-3-((3-(((trifluoromethyl)sulfonyl)-oxy)phenyl)-*N*-ethyl]piperidine)[53] for *S*-4 and *S*-5 (3-[3-(hydroxyphenyl)-*N*-*n*-butyl]piperidine for compound 3 and *cis*-5-methoxy-1-methyl-2-((cyclopropylmethyl)amino)tetralin for compounds (+)-1 and (+)-2) was added 10 pmol/μL was added. The pH was adjusted to 11.0 by addition of 50 μL of saturated Na_2CO_3. 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 the compound to be tested 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 cross-linked PS 264 capillary column (15 m x 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 was then increased by 30 °C/min to the final temperature 290 °C. Each sample was run in duplicate. The absolute oral availability of the compounds was assessed by comparing the areas under the curves (AUC), for po (n = 4) and iv (n = 4) administration, in graphs where the blood concentrations of the

compound were plotted as a function of time. Rats treated orally with the drug were starved 18 h before the experiment.

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