5-HT$_{1D}$ Receptor Agonist Properties of Novel 2-[5-[[(Trifluoromethyl)sulfonyl]oxy]indolyl]ethylamines and Their Use as Synthetic Intermediates

Tjeerd A. Barf,* Peter de Boer, and Håkan Wikström

Department of Medicinal Chemistry, University of Groningen, Antonius Deusinglaan 1, NL-9713 AV Groningen, The Netherlands

Stephen J. Peroutka

Spectra Biomedical Inc., 4040 Campbell Avenue, Menlo Park, California 94025

Kjell Svensson, Michael D. Ennis, Nabil B. Ghazal, James C. McGuire, and Martin W. Smith

CNS Diseases Research, Structural Analytical, and Medicinal Chemistry Cell Biology and Inflammation Research, Chemical and Biological Screening, Pharmacia & Upjohn Inc., Kalamazoo, Michigan 49001

Received July 5, 1996[⊗]

2-[5-[[(Trifluoromethyl)sulfonyl]oxy]-1*H*-indol-3-yl]ethylamine (**18**), its *N*,*N*-di-*n*-propyl (**12**), *N*,*N*-diethyl (**13**), and *N*,*N*-dimethyl (**14**) derivatives, and 4-[3-[2-(*N*,*N*-dimethylamino)ethyl]-1*H*-indol-3-yl]-*N*-(*p*-methoxybenzyl)acrylamide (GR46611, **19**) were synthesized and tested for binding affinities to cloned 5-HT$_{1A}$, 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$, and D$_2$ receptors. In addition, the intrinsic efficacy was measured as the reduction of forskolin-stimulated cAMP in cells transfected with 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors *in vitro*. The 5-substituted indolylethylamines investigated displayed agonist activity at the 5-HT$_{1D}$ receptors with varying degrees of preference for the 5-HT$_{1D\alpha}$ vs the 5-HT$_{1D\beta}$ receptors. The primary amine and *N*,*N*-dimethyl substitution seemed to be optimal for 5-HT$_{1D\alpha}$ affinity. Furthermore, the *N*,*N*-diethyl (**13**) and *N*,*N*-dimethyl (**14**) derivatives showed a 10−25 times preference for the 5-HT$_{1D\alpha}$ vs the 5-HT$_{1D\beta}$ receptor. In addition, all of the novel compounds showed affinity for the 5-HT$_{1A}$ receptor *in vitro* (K_i values ranging from 18 to 40 nM). The most promising derivative **14** was virtually devoid of central 5-HT$_{1A}$ agonist activity in rats, as determined by *in vivo* biochemical assays. Paradoxically, **14**, like **19**, induced a hypothermic response and a decrease in 5-HIAA levels in the prefrontal cortex and hypothalamus in guinea pigs after systemic administration. Sumatriptan failed to produce either of these effects due to a poor brain penetration.

Introduction

5-HT$_{1D}$ receptors were first defined in bovine caudate and subsequently in the brains of other species, including human.[1,2] The 5-HT$_{1D}$ receptor is the most abundant 5-HT$_1$ receptor subtype in the mammalian central nervous system (CNS), existing as a presynaptic heteroreceptor or a terminal autoreceptor, activation of which inhibits serotonin release.[3,4] Human genes encoding for the 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptor have recently been cloned,[5,6] raising questions about which of the two receptors is relevant to reported pharmacological effects. The mRNAs for the 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors appear to codistribute in the brain of nonrodent species; however, the density of the 5-HT$_{1D\alpha}$ receptor mRNA is much lower.[7] It seems that the 5-HT$_{1D\beta}$ receptors constitute the human counterpart of rodent 5-HT$_{1B}$ receptors, and have also been identified in vascular smooth muscle mediating contraction.[8,9] Stimulation of the former receptors by selective 5-HT$_{1D\beta}$ receptor agonists such as sumatriptan (**1**, Figure 1)[10] and newer 5-C-substituted tryptamine derivatives such as **2** (MK-462)[11] and **3** (311C90)[12] may account for the clinical effectiveness of these agents in the treatment of migraine. In addition, the antimigraine action of these agents has been attributed to other, both peripheral and central, mechanisms mediated by 5-HT$_{1D}$ receptors.[13] Moreover, the functional distinction between the 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptor subtypes is unclear. Obviously, selective agonists and antagonists are needed for unraveling the role of 5-HT$_{1D}$ receptors in the CNS and in the periphery. 5-HT$_{1D}$ receptor antagonists, such as (*o*-methoxyphenyl)piperazine derivative **4** (GR127935)[14] and (*o*-hydroxyphenyl)propylamine derivative **5** (GR55562)[15] are cautiously suggested to serve as centrally acting antidepressants, alone or in combination with SSRIs (selective serotonin reuptake inhibitors).[16]

In almost all 5-HT$_{1D}$ receptor agonists reported to date, a 5-substituted tryptamine serves as a structural template. It seems that selectivity for either one of the 5-HT$_1$ receptor subtypes can be achieved by modifying substituents at the 5-position of the indole portion and at the basic nitrogen atom of the ethylamino side chain.[17] Certain, mostly hydrogen bond accepting (aromatic), heterocycles have proven to be viable replacements for the C-5 hydroxy substituent of serotonin itself.[18] Other groups embarked on the synthesis of conformationally restricted tryptamine analogues.[19] However, few data have been published on the affinity

* Address correspondence to T. A. Barf, Department of Medicinal Chemistry, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands.

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1996.

S0022-2623(96)00489-X CCC: $12.00

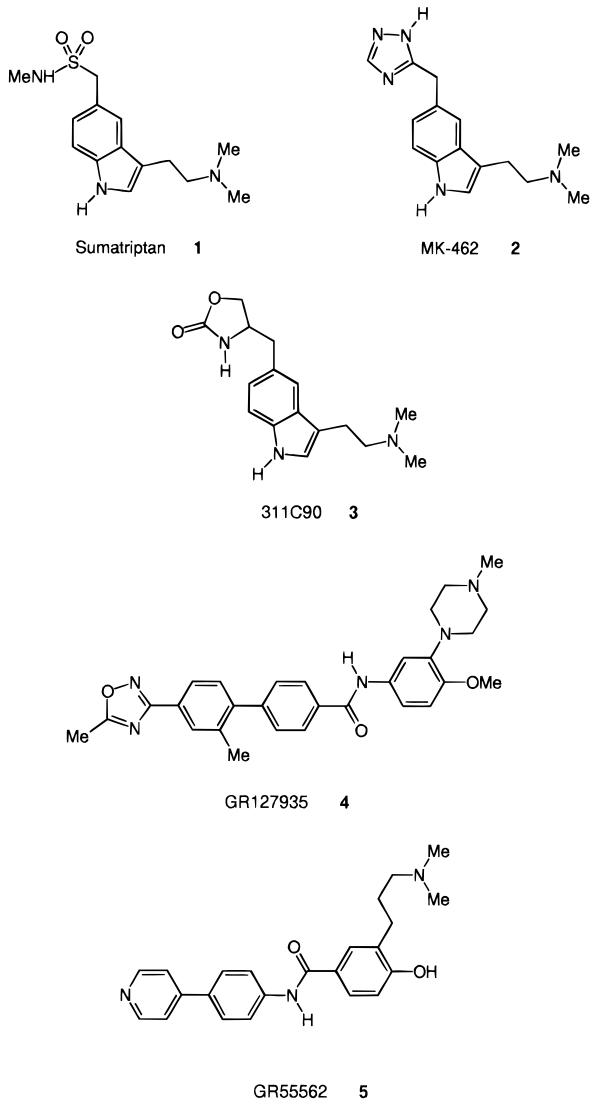
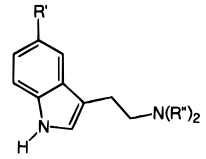


Figure 1.

of ligands for 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors individually. Sumatriptan, and other reported 5-HT_{1D} receptor ligands possess little or no selectivity for 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ receptor binding sites.

Recently, the [(trifluoromethyl)sulfonyl]oxy (triflate) group has been successfully applied as a bioisostere of a hydroxy or a methoxy functionality in a number of 2-aminotetralins.[20] These dopaminergic and serotonergic ligands generally displayed improved pharmacokinetic properties compared to their hydroxy/methoxy analogues while exhibiting the same pharmacological profile. The enhanced 5-HT_{1D} receptor affinities of the 8-triflate-substituted 2-aminotetralins relative to the hydroxy derivatives[20] prompted us to replace the [(*N*-methylamino)sulfonyl]methylene group of sumatriptan by a triflate group, enabling us to investigate the affinity of these new tryptamine analogues to 5-HT_{1A}, 5-$HT_{1D\alpha}$, and 5-$HT_{1D\beta}$ receptor subtypes. *N*,*N*-Dialkyl substituents were introduced in order to gain more insight into the structure−affinity relationships (SAFIR) and structure−activity relationships (SAR) of tryptamine derivatives. Furthermore, aromatic triflates may serve as key intermediates in the synthesis of phenyl ring substituted compounds, as was shown in a number of triflated 2-aminotetralins[21] and phenylpiperidines.[22] Correspondingly, this chemistry is applicable on triflated tryptamines, as is exemplified by the one-step synthesis of the potent 5-$HT_{1A/1D}$ receptor agonist **19** from compound **14**. The 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor-mediated inhibition of forskolin-stimulated cAMP formation was measured for a series of prepared compounds. In case of compounds **14** and **19**, these data are substantiated by neurochemical data and hypothermic effects.

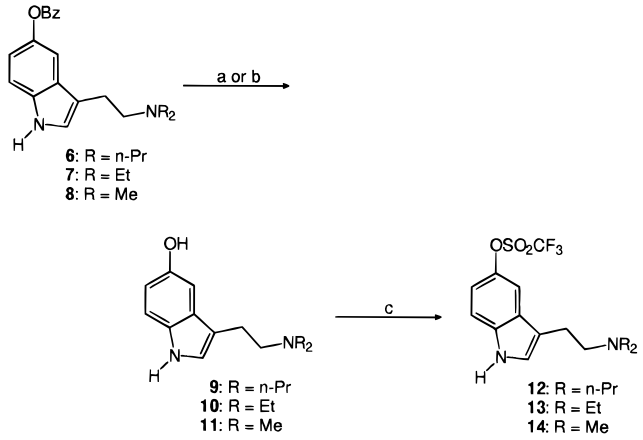
Table 1. Physical Data of Compounds **6**−**14**, **18**, and **19**



compd	R′	R″	salt	mp (°C)	formula[a]
6	OBz	*n*-propyl	oxalate	128−130	$C_{23}H_{30}N_2O\cdot C_2H_2O_4$
7	OBz	ethyl	oxalate	154−156	$C_{21}H_{26}N_2O\cdot C_2H_2O_4$
8	OBz	methyl	oxalate	167−170	$C_{19}H_{22}N_2O\cdot C_2H_2O_4$
9	OH	*n*-propyl		135−136	$C_{16}H_{24}N_2O$
10	OH	ethyl	oxalate	226−228	$C_{14}H_{20}N_2O\cdot 0.6C_2H_2O_4$
11	OH	methyl	oxalate	90−93	$C_{12}H_{16}N_2O\cdot C_2H_2O_4\cdot H_2O$
12	OTf	*n*-propyl	oxalate	148−150	$C_{17}H_{23}N_2O_3SF_3\cdot C_2H_2O_4$
13	OTf	ethyl	oxalate	142−145	$C_{15}H_{19}N_2O_3SF_3\cdot C_2H_2O_4$
14	OTf	methyl	oxalate	168−171	$C_{13}H_{15}N_2O_3SF_3\cdot C_2H_2O_4$
18	OTf	H	oxalate	166−167	$C_{11}H_{11}N_2O_3SF_3\cdot C_2H_2O_4\cdot H_2O$
19	*p*-MBAA[b]	methyl			$C_{23}H_{27}N_3O_2\cdot 0.5H_2O$

[a] Empirical formula; errors for elemental analysis of all compounds analyzed were within 0.4% of theory for C, H, N. [b] *p*-MBAA means (*p*-methoxybenzyl)acrylamide.

Scheme 1.[a] Synthetic Outline of Compounds **6**−**14**

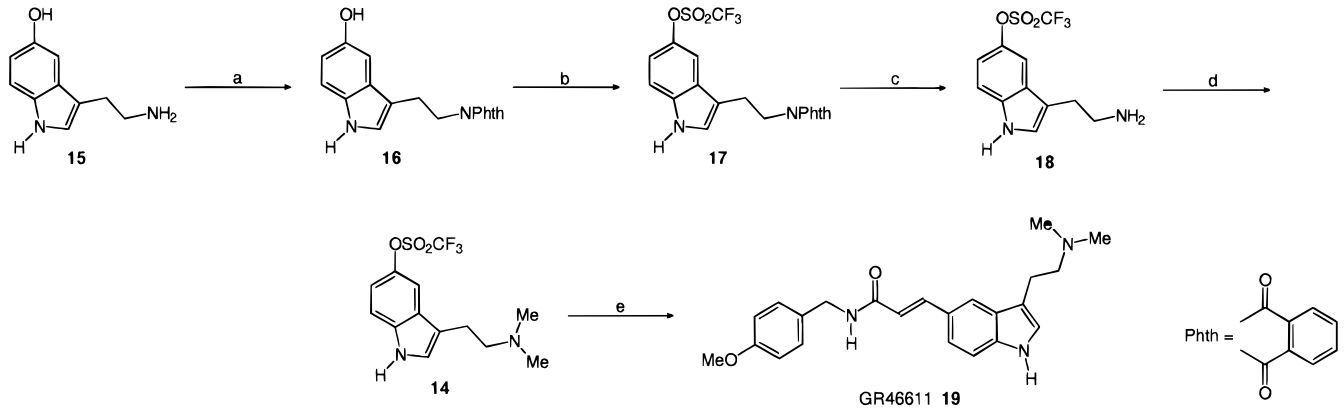


[a] Reagents: (a) Ammonium formate, 10% Pd/C, 96% EtOH; (b) H_2 (4 atm), Pd/C, MeOH; (c) $PhN(SO_2CF_3)_2$, Et_3N, CH_2Cl_2.

Chemistry

The triflates **12**−**14** were synthesized starting from the *N*,*N*-dialkylated 2-[5-(benzyloxy)indolyl]ethylamines, which were prepared according to literature procedures.[23] Catalytic debenzylation in the presence of ammonium formate or H_2 atmosphere resulted in the corresponding phenols. The triflates were synthesized by treatment with *N*-phenyltrifluoromethanesulfonimide and a base (Table 1).[24] The steps of the process are shown in Scheme 1. Since serotonin itself could not be directly triflated, the amine functionality was protected first by a phthalimido group.[25] After triflation, the amine **18** was obtained by deprotection with hydrazine (Scheme 2).[26] The synthesis of **19** was effected *via* coupling of the triflate analogue **14** and (*p*-methoxybenzyl)acrylamide in the presence of $Pd(OAc)_2$ and bis(1,3-diphenylphoshino)propane (dppp).[21]

Scheme 2.[a] Synthetic Outline of Compounds **14**, **18**, and **19**



[a] Reagents: (a) N-EtCO$_2$-phth, 10% NaHCO$_3$ (pH 8), THF/H$_2$O; (b) PhN(SO$_2$CF$_3$)$_2$, Et$_3$N, CH$_2$Cl$_2$; (c) H$_2$NNH$_2$·H$_2$O, EtOH; (d) 37% formaldehyde, NaCNBH$_3$, pH 5, CH$_2$Cl$_2$; (e) (*p*-methoxybenzyl)acrylamide, Pd(OAc)$_2$, dppp, DMF, 85 °C.

Table 2. Affinities at Cloned 5-HT$_{1A}$, 5-HT$_{1D\alpha/\beta}$, D$_2$, 5-HT$_{1D\alpha}$, and 5-HT$_{1D\beta}$ Receptors

A. Cloned 5-HT$_{1A}$, 5-HT$_{1D\alpha/\beta}$, and D$_2$ Receptors

	K_i (nM)[a]				
compd	5-HT$_{1A}$[b]	5-HT$_{1D\alpha}$[b]	5-HT$_{1D\beta}$[b]	D$_2$[c]	select. 5-HT$_{1D\alpha}$ vs 5-HT$_{1D\beta}$
12	23 (19−27)	190 (161−224)	246 (198−306)	502 (354−712)	1.3
13	27 (20−35)	12 (11−14)	171 (140−210)	637 (479−994)	14.3
14	40 (32−50)	3.2 (2.8−3.6)	32 (28−36)	538 (379−764)	10.0
18	18 (15−22)	2.8 (2.5−3.1)	14 (11−16)	658 (472−918)	5.0
19	1.3 (0.8−2.1)	0.3 (0.2−0.5)	0.2 (0.1−0.3)	>1000	0.7
1	341 (283−522)	5.7 (2.9−9.5)	22 (19−27)	>218	3.8

B. Cloned 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ Receptors

	IC_{50} (nM)[d]		
compd	5-HT$_{1D\alpha}$[e]	5-HT$_{1D\beta}$[e]	log *D* [f]
6	470 ± 100	550 ± 200	3.08
7	110 ± 7	260 ± 60	2.18
8	25 ± 4	76 ± 10	2.28
9	820 ± 100	2600 ± 500	1.24
10	77 ± 20	690 ± 100	0.28
12	170 ± 10	660 ± 50	2.21
13	21 ± 2	530 ± 30	1.31
14	8.4 ± 1	98 ± 6	1.41
18	NT[g]	NT	−0.24
19	NT	NT	1.71

[a] K_i values for displacement of the 5-HT1A receptor agonist [^{3}H]-8-OH-DPAT, the 5-HT$_{1D\alpha/\beta}$ agonist [^{3}H]-5-HT, and the dopamine D$_2$ receptor agonist [^{3}H]U86170. [b] Method A; data from cloned human receptors expressed in CHO-K1 cells. Mean values ($n = 3$). Parentheses contain 95% confidence intervals. [c] Data from cloned rat receptors expressed in CHO-K1 cells. [d] IC_{50} values for displacement of the 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptor agonist [^{3}H]-5-HT. [e] Method B; data from human 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptor clones, expressed in a human embryonic kidney (HEK 293) cell line. Mean value ± SEM ($n = 3$). [f] Calculated with Pallas 1.2 (CompuDrug Chemistry Ltd., 1994). [g] NT means not tested.

Pharmacology

***In Vitro* Binding.** The abilities of the new compounds to displace the radioligands [^{3}H]-8-OH-DPAT (5-HT$_{1A}$), [^{3}H]-5-HT (5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$), and [^{3}H]U-86170 (D$_2$) were assessed in mammalian receptor clones expressed in CHO cells (Table 2A, method A). In addition, the compounds were evaluated for their *in vitro* binding affinity at 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ human receptor clones expressed in a human embryonic kidney (HEK 293) cell line (Table 2B, method B).

cAMP Assay. The functional cAMP assay using the cloned human 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors was employed as previously described.[27,28] Compounds **12**, **13**, **14**, **18**, and **19** were tested at 10 μM, and the agonist inhibition was calculated as a percent of the 5-HT control (Table 3).

***In Vivo* Biochemistry.** The synthesis rate of 5-HT in terminal brain areas is inhibited by 5-HT$_{1A}$ receptor agonists due to stimulation of the somatodendritic 5-HT$_{1A}$ receptors in the raphe nuclei.[29] The effect of

Table 3. Intrinsic Efficacy in Cells Transfected with Human 5-HT$_{1D\alpha}$ or 5-HT$_{1D\beta}$ Receptors

	cAMP (pmol)[a]	
compound	5-HT$_{1D\alpha}$ (% 5-HT)	5-HT$_{1D\beta}$ (% 5-HT)
forskolin control	64 ± 3 (0)	166 ± 6 (0)
5-HT (1 μM)	27 ± 1 (100)	13 ± 1 (100)
1 (1 μM)	30 ± 1 (92)	24 ± 1 (93)
12 (10 μM)	36 ± 4 (75)	88 ± 11 (51)
13 (10 μM)	29 ± 3 (94)	37 ± 3 (84)
forskolin control	61 ± 4 (0)	232 ± 8 (0)
5-HT (1 μM)	20 ± 1 (100)	17 ± 1 (100)
1 (1 μM)	31 ± 4 (75)	32 ± 2 (93)
14 (10 μM)	30 ± 1 (75)	28 ± 1 (95)
18 (10 μM)	19 ± 2 (102)	18 ± 2 (100)
19 (1 μM)	(104)[b]	NT[c]

[a] Values are expressed as means ± SEM ($n = 3$). Data within parentheses denote percent of 5-HT's response. [b] Tested in a separate assay. [c] NT means not tested.

Table 4. Effect of **14** on Rat Brain 5-HT Synthesis (5-HTP Accumulation) *in Vivo* in Reserpinized and Nonpretreated Rats

	reserpinized (5-HTP accumulation)[a]				normal (5-HT levels)[b]	
compound	striatum	accumbens	frontal cortex	hippocampus	10 μmol/kg	50 μmol/kg
saline	0.37 ± 0.03	0.49 ± 0.07	0.47 ± 0.09	0.28 ± 0.02		
14	0.28 ± 0.01	0.40 ± 0.02	0.37 ± 0.01	0.28 ± 0.03		
%	75.7	81.6	78.7	100.0	108 ± 10.5	75 ± 4.0

[a] The animals received reserpine 24 h before decapitation and 45 and 30 min before the test drug (10 μmol/kg) and NSD 1015 (100mg/kg) were administered, respectively. Shown are the [5-HTP] in μg/g wet tissue (means ± SEM) in striatum ($n = 8$), accumbens ($n = 4$), frontal cortex ($n = 4$), and hippocampus ($n = 4$), after sc administration of saline or compound 14 (10 μmol/kg). [b] Determined by *in vivo* microdialysis. The values are percent of control 5-HT levels, means ± SEM ($n = 3$), 1 h after sc administration of compound 14.

Table 5. Effects on Guinea Pig Rectal Temperature and Brain 5-HT Turnover

			neurochemistry (% of vehicle controls)[b]			
			hypothalamus		prefrontal cortex	
compound	dose, μmol/kg, sc	hypothermia,[a] 0−60 min, Δ °C	5-HT	5-HIAA	5-HT	5-HIAA
saline		+0.18 ± 0.15	100 ± 6	100 ± 5	100 ± 5	100 ± 9
14	12.5	−1.70 ± 0.43*	97 ± 13	66 ± 16	104 ± 8	59 ± 9***
	50	−1.72 ± 0.36**	123 ± 16	54 ± 16*	129 ± 1	46 ± 6***
19	12.5	−1.62 ± 0.35**	NT[c]	NT	NT	NT
	50	−2.62 ± 0.05***	121 ± 11	49 ± 7**	94 ± 6	37 ± 7***
1	25	−0.14 ± 0.13	122 ± 5	133 ± 13	119 ± 6	129 ± 14
8-OH-DPAT	6.25	+0.22 ± 0.15	134 ± 17	37 ± 7**	126 ± 2	34 ± 8**

[a] The hypothermia is presented as the difference in °C from control. The data represent a 60 min reading and are expressed as mean ± SEM ($n = 4-5$). [b] The animals were decapitated, 60 min after test drug treatment. Shown are the 5-HT and 5-HIAA levels expressed as mean ± SEM ($n = 4-5$), after sc administration of the test drugs. [c] NT means not tested. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs vehicle-treated controls.

compound **14** on 5-HT synthesis was measured in four brain areas in reserpinized rats. 5-Hydroxytryptophan (5-HTP) accumulation, following decarboxylase inhibition by (3-hydroxybenzyl)hydrazine (NSD 1015), was used as an indicator of the 5-HT synthesis rate (Table 4).[30] The extracellular 5-HT levels in the hippocampus were measured by *in vivo* microdialysis after subcutaneous (sc) administration of **14** in normosensitive rats (Table 4). In addition, the 5-HT and 5-HIAA levels after administration of **14** and **19** were determined in the prefrontal cortex and the hypothalamus of guinea pigs (Table 5).

Hypothermia. The ability of compounds **14** and **19** to induce hypothermia in guinea pigs after sc administration was tested in a 60-min reading (Table 5).

Results and Discussion

Structure−Affinity and Structure−Activity Relationships. From the data presented in Table 2, two clear trends can be observed. First, the size of the *N*,*N*-dialkyl group dramatically influences the affinity for both 5-HT_{1D} receptor subtypes, and second, the nature of the 5-*O*-substituent is of major importance for both the affinity and the selectivity for these subtypes. *N*,*N*-Dimethyl substitution (IC_{50} values 8.4−25 nM) improves the affinity for 5-$HT_{1D\alpha}$ sites by approximately 20-fold as compared to the *N*,*N*-di-*n*-propyl derivatives (K_i values 170−820 nM), but only 7-fold in case of 5-$HT_{1D\beta}$ receptors (Table 2B). Notably, bulk at the protonated amine site of the tryptamine is poorly tolerated by the latter receptor, resulting in moderate binding affinities even for the *N*,*N*-dimethyl derivatives. Within the *N*,*N*-di-*n*-propyl derivative series the triflate group enhances the affinity for 5-$HT_{1D\alpha}$ sites by 3- and 5-fold relative to the benzyloxy and hydroxy substituents, respectively. Similar trends are found in the *N*,*N*-diethyl- and *N*,*N*-dimethyl-substituted tryptamines. The comparatively low affinities of the 5-benzyloxy and 5-hydroxy derivatives for the 5-HT_{1D} receptor subtypes suggest that the 5-oxygen is not of crucial importance for the 5-HT_{1D} receptor interaction. Obviously, the triflate derivatives benefit from their two additional sulfonyl oxygens which are capable of accepting a hydrogen bond. In addition, the electron-withdrawing effect of the triflate substituent may contribute to a putative interaction of the indole portion with the active-site surrounding amino acid residues.

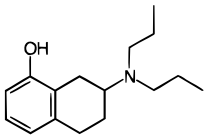
Since the novel triflate derivatives **12**−**14** and **18** displayed the most interesting binding profiles, these compounds were subjected to the *in vitro* 5-HT_{1D} receptor agonist assays. In the *in vitro* binding assays, the *N*,*N*-di-*n*-propyl-substituted analog **12** showed a comparatively weak affinity (K_i values around 200 nM) for both 5-HT_{1D} receptor subtypes, while the affinity for the 5-HT_{1A} site was higher (23 nM, Table 2A). The IC_{50} values (Table 2B) were more distinct showing a 4-fold preference for the 5-$HT_{1D\alpha}$ vs 5-$HT_{1D\beta}$ binding site (170 and 660 nM, respectively). Despite these moderate affinities, compound **12** reduced the formation of cAMP, in both the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor assays; however, the intrinsic activity appeared to be higher in the former (Table 3).

As already indicated, the 5-$HT_{1D\alpha}$ receptor affinity was greatly improved in the *N*,*N*-diethyl-substituted analogue **13**. This compound showed approximately a 15−25-fold preference for the 5-$HT_{1D\alpha}$ vs 5-$HT_{1D\beta}$ receptor, but only a 2-fold preference vs the 5-HT_{1A} receptor subtype. In the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor agonist assay, a similar and slightly lower intrinsic activity was observed, respectively, relative to sumatriptan when tested at 10 μM. Increased affinity at the expense of selectivity was observed in moving from *N*,*N*-diethyl to *N*,*N*-dimethyl substituents. Compound **14** exhibited a 10−12-fold preference for the 5-$HT_{1D\alpha}$ vs 5-$HT_{1D\beta}$ receptor subypes (K_i values (IC_{50} values) of 3.2 nM (8.4 nM) and 32 nM (98 nM), respectively). Interestingly, **14** showed both higher affinity for the 5-$HT_{1D\alpha}$ receptor subtype and a higher 5-$HT_{1D\alpha}$ vs 5-$HT_{1D\beta}$ preference

than sumatriptan (**1**). As expected, both **1** and **14** were found to be agonists with similar intrinsic efficacy in both cAMP assays. In addition, the primary amine **18** showed higher affinity for the 5-HT$_{1D\alpha}$ site (K_i = 2.8 nM). However, the affinities for the 5-HT$_{1A}$ and 5-HT$_{1D\beta}$ sites were also increased (K_i values of 18 and 14 nM, respectively). Compound **18** displayed a maximal intrinsic efficacy, similar to that of 5-HT in the cAMP assay. It is noteworthy that even compounds with moderate affinity for the 5-HT$_{1D}$ receptor subtypes can fully inhibit the forskolin-stimulated cAMP formation.

Introduction of a hydrophobic tail on the 5-position of the indole nucleus seems to favor 5-HT$_{1D\beta}$ affinity as exemplified by 5-(nonyloxy)tryptamine.[31] This 5-HT$_{1D}$ receptor ligand binds with higher affinity at human 5-HT$_{1D\beta}$ receptors than 5-HT$_{1D\alpha}$ receptors (K_i = 1.2 vs 16 nM, respectively). Similarly, the (*p*-methoxybenzyl)-acrylamido group of **19**, allowing for hydrogen bond formation, is well-tolerated by the 5-HT$_{1A/1D}$ receptor subtypes. Table 2A shows that **19** potently displaced the radioligands at these receptor subtypes, exhibiting K_i values of 1.3 (5-HT$_{1A}$), 0.3 (5-HT$_{1D\alpha}$), and 0.2 nM (5-HT$_{1D\beta}$), and was found to behave as a full 5-HT$_{1D\alpha}$ receptor agonist in the cAMP assay. Obviously, both 5-HT$_{1D}$ receptor binding sites contain a (lipophilic) pocket which can accommodate bulky, hydrogen-bond-accepting groups. None of the compounds tested showed appreciable affinity for the dopamine D$_2$ receptor (Table 2A).

Pharmacology. The 5-HT$_{1D}$ receptor agonist **14** of this series was additionally screened for its (unwanted) central 5-HT$_{1A}$ receptor activity by means of *in vivo* biochemical models. In rats, **14** failed to significantly inhibit the 5-HTP accumulation at a dose of 10 μmol/kg in the investigated brain areas, although slight decreases were observed in striatum, accumbens, and frontal cortex. The inactivity at the 5-HT$_{1A}$ receptor is substantiated by the microdialysis data, which showed no differences compared to the control 5-HT levels in the hippocampus after sc administration of **14** at a dose of 10 and 50 μmol/kg (Table 4). However, like **19**, compound **14** has a pronounced effect on the 5-HT turnover in the prefrontal cortex and the hypothalamus of the guinea pig brain using similar doses. A lower dose of **14** tested (3.1 μmol/kg, sc) did not produce a statistical significant effect. As previously reported, sumatriptan failed to affect guinea pig brain 5-HT turnover after systemic administration. This is likely explained by the fact that sumatriptan has a poor penetration across the blood−brain barrier, as indicated by its low calculated log *D* value (−0.53). This hypothesis is further strengthened by the fact that sumatriptan failed to produce hypothermia, while **19** and **14** significantly produced a hypothermic response at the 12.5 and 50 μmol/kg dose, sc.[32] The ability of **19** and **14** to lower the central 5-HT turnover is likely the result from activation of inhibitory presynaptic 5-HT$_{1D}$ or somatodendritic 5-HT$_{1A}$ and/or 5-HT$_{1D}$ receptors. Interestingly, in contrast to rats and mice, the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (**20**, Figure 2) failed to induce hypothermia in the guinea pig, indicating that 5-HT$_{1D}$ receptors are of higher importance for the temperature regulation in this species. The levels of 5-HIAA were reduced probably as a result of a stimulation of 5-HT$_{1A}$ cell body autoreceptors.



8-OH-DPAT **20**

Figure 2.

In conclusion, the triflate substituted tryptamines investigated all display agonist activity at the 5-HT$_{1D}$ receptors with varying degrees of preference for the 5-HT$_{1D\alpha}$ vs the 5-HT$_{1D\beta}$ receptors *in vitro*. The primary amine and *N*,*N*-dimethyl substitution seemed to be optimal for 5-HT$_{1D\alpha}$ affinity. Furthermore, the *N*,*N*-dimethyl and *N*,*N*-diethyl analogs showed a 10−25-fold selectivity for the 5-HT$_{1D\alpha}$ vs 5-HT$_{1D\beta}$ receptor. In addition, all of the compounds showed substantial affinity for the 5-HT$_{1A}$ receptor *in vitro* (K_i values ranging from 18 to 40 nM). Compound **14** seems to have a weak inhibitory effect on the 5-HT turnover at doses up to 50 μmol/kg (sc) in rats, but induces pronounced decreases of 5-HT turnover in guinea pigs. This contrasting observation may be explained by the fact that in these experiments the 5-HT turnover in rats is predominantly mediated by 5-HT$_{1A}$ cell body autoreceptors, whereas the inhibitory presynaptic 5-HT$_{1D}$ receptors have a major contribution in the observed effect in guinea pigs. The potential antimigraine action of compound **14** has been evaluated by means of a porcine carotid blood flow model and appears to be equipotent with sumatriptan.[33] The question is whether brain penetration is a desireable property of antimigraine agents or not. Animal studies have provided evidence that if putative antimigraine drugs, such as **3**, get access to the CNS, they display central neuronal actions.[13] Whether this provides increased clinical efficacy in the treatment of migraine is still in debate.

Experimental Section

General. ^{1}H 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 stated. Chemical shifts are given in δ units (ppm) and relative to TMS or deuterated solvent. Coupling constants are given in hertz. IR spectra were obtained on a ATI-Mattson spectrometer. Elemental analyses were performed in the Microanalytical department of the University of Groningen or at Parke-Davis, Warner-Lambert Co. (Ann Arbor, MI) and were within 0.4%. The chemical ionization (CI) mass spectra were obtained on a Finnegan 3300 system. Melting points were determined on a Electrothermal digital melting point apparatus and are uncorrected. log *D* values were calculated with Pallas version 1.2.[34]

Materials. All 2-[5-(benzyloxy)indolyl]ethylamines were prepared according literature procedures starting from 5-(benzyloxy)indole.[23] Chemicals used were commercially available (Aldrich) and were used without further purification.

***N*,*N*-Di-*n*-propyl-2-[5-(benzyloxy)-1*H*-indol-3-yl]-ethylamine Oxalate (6).** *N*,*N*-Di-*n*-propyl-2-[5-(benzyloxy)-1*H*-indol-3-yl]glyoxalylamide (2.20 g, 5.82 mmol) was dissolved in dry Et_2O (80 mL) and dry THF (20 mL) at room temperature. $LiAlH_4$ (2.40 g, 11 equiv) was added portionwise, and the reaction mixture was refluxed for 6 h under N_2(g). After the reaction mixture was cooled to room temperature, the reaction was quenched with the addition of water (2.4 mL), 10% NaOH (2.4 mL), and water (7.2 mL) under N_2(g). This mixture was stirred until the Li salts had turned white. These salts were filtered and washed with Et_2O. The solvent of the filtrate was evaporated under reduced pressure resulting in a

yellow oil (1.83 g, 90%). Conversion into the oxalate and recrystallization from acetonitrile gave pale brown crystals (1.68 g, 66%): mp 128−130 °C; IR (KBr, cm^{-1}) 1196 (C−O); ^{1}H NMR (CD_3OD) δ 0.94 (t, J = 7.27, 6H), 1.57−1.76 (m, 4H), 3.03−3.14 (m, 6H), 3.27−3.34 (m, 2H), 5.09 (s, 2H), 6.87 (dd, J_1 = 8.98, J_2 = 2.14, 1H), 7.12 (d, J = 2.14, 1H), 7.15 (s, 1H), 7.26−7.47 (m, 6H); ^{13}C NMR (CD_3OD) δ 10.9, 17.8, 20.8, 53.7, 55.1, 71.7, 102.5, 109.7, 113.1, 113.5, 124.8, 128.1, 128.3, 128.4, 129.1, 133.3, 154.0, 166.5; MS (CI with NH_3) *m/e* 351 (M + 1). Anal. Calcd for $C_{23}H_{30}N_2O{\cdot}C_2H_2O_4$: C, H, N.

***N,N*-Diethyl-2-[5-(benzyloxy)-1*H*-indol-3-yl]ethylamine Oxalate (7).** Reduction of *N,N*-diethyl-2-[5-(benzyloxy)-1*H*-indol-3-yl]glyoxalylamide (1.93 g, 5.51 mmol) was performed according to the procedure given for the synthesis of **6**, resulting in a brown oil after evaporation of the solvents (1.70 g, 96%). Conversion to the oxalate and recrystallization from acetone gave pale green crystals (1.75 g, 77%): mp 154−156 °C (lit.[22] mp 161−162 °C); IR (KBr, cm^{-1}) 1186 (C−O); ^{1}H NMR δ 1.14 (t, J = 7.26, 6H), 2.72 (q, J = 7.27, 4H), 2.79−2.99 (m, 4H), 5.14 (s, 2H), 6.98 (m, 2H), 7.16−7.55 (m, 7H), 8.24 (br s, NH); ^{13}C NMR δ 11.7, 22.8, 46.6, 53.3, 70.9, 103.4, 111.9, 112.8, 114.0, 122.4, 127.6, 127.7, 127.9, 128.5, 131.5, 137.6, 152.8; MS (CI with NH_3) *m/e* 323 (M + 1). Anal. Calcd for $C_{21}H_{26}N_2O{\cdot}C_2H_2O_4$: C, H, N.

***N,N*-Dimethyl-2-[5-(benzyloxy)-1*H*-indol-3-yl]ethylamine Oxalate (8).** *N,N*-Dimethyl-2-[5-(benzyloxy)-1*H*-indol-3-yl]glyoxalylamide (1.78 g, 5.53 mmol) was converted to **8** according to the procedure given for the synthesis of **6**, resulting in a colorless oil (1.53 g, 95%). This product was converted to the oxalate salt, and recrystallization from MeOH/Et_2O gave a white solid (1.79 g, 85%): mp 167−170 °C (lit.[22] mp 178−179 °C); IR (KBr, cm^{-1}) 1186 (C−O); ^{1}H NMR δ 2.38 (s, 6H), 2.66 (t, J = 7.32, 2H), 2.95 (t, J = 7.32, 2H), 5.11 (s, 2H), 6.90 (s, 1H) 6.96 (d, J = 8.79, 1H), 7.17 (d, J = 5.12, 2H), 7.41 (m, 5H), 9.03 (br s, NH); ^{13}C NMR δ 23.3, 45.0, 59.9, 70.7, 102.1, 111.7, 112.2, 113.0, 122.5, 127.3, 127.4, 128.2, 131.6, 137.5, 152.5; MS (EIPI) *m/e* 294 (M^+). Anal. Calcd for $C_{19}H_{22}N_2O{\cdot}C_2H_2O_4$: C, H, N.

***N,N*-Di-*n*-propyl-2-(5-hydroxy-1*H*-indol-3-yl)ethylamine (9).** The crystals of **6** (1.51 g, 3.43 mmol) were dissolved in 95% EtOH (50 mL), after which ammonium formate (2.16 g, 10 equiv) and Pd/C (10%, 100 mg) were added. The reaction mixture was stirred at room temperature for 2 days. The solids were filtered over Celite, and the filtrate was evaporated *in vacuo* leaving a brown oil. Next, 10% aqueous Na_2CO_3 (50 mL) was added, and the product amine was extracted with EtOAc (3 × 30 mL). The organic phases were separated, pooled, dried ($MgSO_4$), and filtered. The solvent was removed under reduced pressure, yielding a pale brown solid (0.79 g, 89%). Part of this solid (0.28 g) was recrystallized from acetonitrile to give 0.24 g of brownish crystals: mp 135−136 °C; IR (KBr, cm^{-1}) 3236 (OH); ^{1}H NMR (CD_3OD) δ 0.92 (t, J = 7.31, 6H), 1.49−1.61 (m, 4H), 2.49−2.57 (m, 4H), 2.71−2.88 (m, 4H), 6.66 (dd, J_1 = 8.55, J_2 = 2.42, 1H), 6.91 (d, J = 2.56, 1H), 6.97 (s, 1H), 7.14 (d, J = 8.45, 1H); ^{13}C NMR (CD_3OD) δ 12.0, 20.3, 22.8, 55.4, 56.8, 103.1, 112.0, 112.4, 112.9, 123.6, 129.0, 132.8, 150.7; MS (EIPI) *m/e* 260 (M^+). Anal. Calcd for $C_{16}H_{24}N_2O$: C, H, N.

***N,N*-Diethyl-2-(5-hydroxy-1*H*-indol-3-yl)ethylamine Oxalate (10).** The crystals of compound **7** (5.00 g, 12.14 mmol) were dissolved in dry MeOH (200 mL) and hydrogenated over 10% Pd/C in a Parr apparatus under a H_2 pressure of 4 atm. After 4 h the reaction mixture was filtered over Celite by suction, and after the solvent was evaporated under reduced pressure a tarry pinkish oil (3.77 g) was obtained. Recrystallization from MeOH/ether gave pale brown crystals (2.57 g; 66%): mp 226−228 °C; IR (KBr, cm^{-1}) 3300 (OH); ^{1}H NMR (CD_3OD) δ 1.09 (t, J = 7.17, 6H), 2.64 (q, J = 7.27, 4H), 2.72−2.86 (m, 4H), 6.67 (dd, J_1 = 8.65, J_2 = 2.38, 1H), 6.92 (d, J = 2.23, 1H), 6.96 (s, 1H), 7.15 (d, J = 8.68, 1H); ^{13}C NMR (CD_3OD) δ 11.0, 22.6, 47.4, 54.1, 103.1, 112.0, 112.4, 112.8, 123.6, 129.0, 132.8, 150.8; MS (CI with NH_3) *m/e* 233 (M + 1). Anal. Calcd for $C_{14}H_{20}N_2O{\cdot}0.6C_2H_2O_4$: C, H, N.

***N,N*-Dimethyl-2-(5-hydroxy-1*H*-indol-3-yl)ethylamine Oxalate (11).** Compound **8** (1.51 g, 3.93 mmol) was converted to **11** according to the procedure given for the synthesis of **10**, resulting in a purple oil (1.01 g) which solidified upon standing. Pink crystals (70 mg) were obtained while being stirred in MeOH (5 mL) and were collected by filtration on a glass-sintered funnel. The filtrate was taken up in 10% aqueous Na_2CO_3 (50 mL), and then the product amine was extracted into EtOAc (3 × 30 mL). The organic phases were separated, pooled, dried ($MgSO_4$), and filtered. The solvent of the filtrate was evaporated under reduced pressure, leaving a brown oil (0.47 g, 65%): mp 90−93 °C (lit.[22] mp 93−94 °C); IR (KBr, cm^{-1}); ^{1}H NMR (CD_3OD) δ 2.23 (s, 6H), 2.54−2.82 (m, 4H), 6.71 (d, J = 8.64, 1H), 6.94 (m, 2H), 7.15 (d, J = 8.64, 1H); ^{13}C NMR (CD_3OD) δ 23.9, 45.0, 60.9, 103.3, 112.2, 112.5, 123.7, 129.0, 132.8, 150.8; MS (EIPI) *m/e* 204 (M^+). Anal. Calcd for $C_{12}H_{16}N_2O{\cdot}C_2H_2O_4{\cdot}H_2O$: C, H, N.

***N,N*-Di-*n*-propyl-2-[5-[[(trifluoromethyl)sulfonyl]oxy]-1*H*-indol-3-yl]ethylamine Oxalate (12).** *N,N*-Di-*n*-propyl-5-hydroxytryptamine (**9**, 268 mg, 1.03 mmol), Et_3N (290 μL, 2.52 mmol), and $PhN(SO_2CF_3)_2$ (550 mg, 1.55 mmol) were dissolved in CH_2Cl_2 (10 mL), and the mixture was stirred at room temperature. After 3 h the mixture was diluted with CH_2Cl_2 (20 mL) and washed with 10% aqueous Na_2CO_3 (2 × 25 mL). The aqueous layers were once more extracted with CH_2Cl_2 (30 mL), after which the organic layers were pooled, washed with brine, and dried over $MgSO_4$. The solvent was removed *in vacuo*, leaving a yellow oil which was chromatographed (SiO_2, eluting with CH_2Cl_2/MeOH (5:1)). Pure fractions were pooled and evaporated to dryness, yielding a pale yellow oil (483 mg). The residual oil was converted to the oxalate and recrystallized from MeOH/ether, giving white crystals (251 mg, 51%): mp 148−150 °C; IR (KBr, cm^{-1}) 1225, 1396 (O−SO_2); ^{1}H NMR (base) δ 0.92 (t, J = 7.36, 6H), 1.46−1.65 (m, 4H), 2.46−2.61 (m, 4H), 2.76−2.97 (m, 4H), 7.04 (dd, J_1 = 8.83, J_2 = 2.37, 1H), 7.09 (s, 1H), 7.34 (d, J = 8.83, 1H), 7.46 (d, J = 2.37, 1H), 8.93 (br s, NH); ^{13}C NMR (base) δ 11.8, 19.6, 22.2, 54.2, 55.9, 111.1, 112.2, 114.6, 114.9, 118.8 (q, J = 321, CF_3), 124.4, 127.7, 135.0, 143.2; MS (CI with NH_3) *m/e* 393 (M + 1). Anal. Calcd for $C_{17}H_{23}N_2O_3SF_3{\cdot}C_2H_2O_4$: C, H, N.

***N,N*-Diethyl-2-[5-[[(trifluoromethyl)sulfonyl]oxy]-1*H*-indol-3-yl]ethylamine Oxalate (13).** Triflation of the free base of **10** (200 mg, 0.86 mmol) was performed according to the procedure given for the synthesis of **12**, yielding an oil (317 mg, 100%) after chromatography (SiO_2, eluting with CH_2Cl_2/MeOH (5:1)). This oil was converted to the oxalic acid salt and recrystallized from MeOH/Et_2O, giving white crystals (247 mg, 63%): mp 142−145 °C; IR (KBr, cm^{-1}) 1221, 1415 (O−SO_2); ^{1}H NMR (base) δ 1.16 (t, J = 7.27, 6H), 2.84 (q, J = 7.26, 4H), 2.93 (s, 4H), 6.96 (dd, J_1 = 8.97, J_2 = 2.14, 1H), 7.05 (s, 1H), 7.31 (d, J = 8.97, 1H), 7.43 (d, J = 2.13, 1H), 9.80 (br s, NH); ^{13}C NMR (base) δ 10.2, 21.5, 46.8, 52.6, 110.7, 112.5, 112.7, 114.7, 118.8 (q, J = 321, CF_3), 125.0, 127.3, 135.2, 143.2; MS (CI with NH_3) *m/e* 365 (M + 1). Anal. Calcd for $C_{15}H_{19}N_2O_3SF_3{\cdot}C_2H_2O_4$: C, H, N.

***N,N*-Dimethyl-2-[5-[[(trifluoromethyl)sulfonyl]oxy]-1*H*-indol-3-yl]ethylamine Oxalate (14 from 11).** Triflation of the free base of **11** (257 mg, 1.26 mmol) was performed as above, yielding a colorless oil (394 mg, 93%) after column chromatography (SiO_2, eluting with CH_2Cl_2/MeOH (5:1)). This oil (369 mg) was converted to its oxalate salt with oxalic acid and recrystallized from MeOH/Et_2O, yielding white crystals (189 mg, 38%): mp 176−177 °C; IR (KBr, cm^{-1}) 1221, 1415 (O−SO_2); ^{1}H NMR (CD_3OD) δ 2.64 (s, 6H), 3.05 (s, 4H), 7.07 (d, J = 8.78, 1H), 7.33 (s, 1H), 7.47 (d, J = 8.79, 1H), 7.58 (s, 1H); ^{13}C NMR (CD_3OD) δ 22.4, 44.1, 59.6, 111.4, 112.3, 113.4, 115.2, 120.0 (q, J = 321, CF_3), 126.6, 128.2, 136.6, 144.3; MS (CI with NH_3) *m/e* 337 (M + 1). Anal. Calcd for $C_{13}H_{15}N_2O_3SF_3{\cdot}C_2H_2O_4$: C, H, N.

***N,N*-Phthalimido-2-(5-hydroxy-1*H*-indol-3-yl)ethylamine (16).** A stirred solution of serotonin creatine sulfate monohydrate (**15**, 5.00 g, 12.35 mmol) in H_2O (20 mL) and THF (20 mL) was basified to pH 8 with 10% $NaHCO_3$, after which *N*-carbethoxyphthalimide (2.75 g, 12.35 mmol) was added. The reaction mixture was stirred for 8 h, during which time a bright yellow precipitate had formed. The organic solvent was evaporated *in vacuo*, and the yellow solid (3.52 g, 94%) was collected on a sintered-glass funnel (P3) and rinsed

with Et_2O. Recrystallization from EtOH (absolute) gave 3.01 g (80%) of yellow crystals: mp 213−216 °C (lit.[25] mp 210 °C); IR (KBr, cm^{-1}) 1690 (C═O), 3370 (OH); ^{1}H NMR (acetone-d_6) δ 3.06 (t, J = 8.06, 2H), 3.92 (t, J = 8.06, 2H), 6.71 (d, J = 8.42, 1H), 7.18 (d, J = 8.79, 1H), 7.10 (d, J = 8.42, 2H), 7.53 (br s, 1H), 7.81 (m, 4H), 9.63 (br s, NH); ^{13}C NMR (acetone-d_6) δ 25.2, 39.3, 103.4, 103.5, 111.7, 112.6, 123.7, 124.2, 129.3, 133.3, 134.9, 151.7, 168.7, 206.0; MS (CI with NH_3) *m/e* 393 (M + 1).

***N,N*-Phthalimido-2-[5-[[(trifluoromethyl)sulfonyl]oxy]-1*H*-indol-3-yl]ethylamine (17).** Triflation of **16** (1.33 g, 4.35 mmol) was performed according to the procedure given for the synthesis of **12**, affording a white solid after chromatography (SiO_2, eluting with CH_2Cl_2). The solid was recrystallized from EtOH, yielding colorless needles (1.48 g, 78%): mp 165−166 °C; IR (KBr, cm^{-1}) 1206, 1398 (O−SO_2), 1719 (C═O); ^{1}H NMR δ 3.11 (t, J = 7.69, 2H), 3.97 (t, J = 7.69, 2H), 7.04 (d, J = 8.79, 1H), 7.13 (s, 1H), 7.30, (d, J = 8.78, 1H), 7.55 (s, 1H), 7.67−7.81 (m, 4H), 8.38 (br s, NH); ^{13}C NMR δ 21.6, 35.7, 108.7, 109.6, 110.7, 112.8, 116.3 (q, J = 321, CF_3), 120.7, 122.0, 125.2, 129.5, 131.4, 132.4, 141.0, 165.6; MS (EIPI) *m/e* 438 (M^+). Anal. Calcd for $C_{19}H_{13}N_2O_5SF_3$: C, H, N.

2-[5-[(Trifluoromethyl)sulfonyl]oxy]-1*H*-indol-3-yl]ethylamine Oxalate (18). The *N,N*-phthalimide **17** (1.07 g, 2.44 mmol) was dissolved in absolute EtOH (50 mL), after which hydrazine hydrate (2.0 mL) was added. The reaction mixture was stirred for 0.5 h at room temperature, after which time the volatiles were removed *in vacuo*. The residue was refluxed in $CHCl_3$ for 0.5 h, cooled to ambient temperature, and filtered in order to remove the solid phthalimidohydrazine. The filtrate was evaporated *in vacuo*, leaving a colorless oil which was converted to the oxalate. The oxalate salt was recrystallized from EtOH/ether, giving 0.86 g (89%) of white crystals: mp 166−167 °C; IR (KBr, cm^{-1}) 1210, 1412 (O−SO_2); ^{1}H NMR (base) δ 3.10−3.26 (m, 4H), 7.08 (dd, J_1 = 8.79, J_2 = 2.19, 1H), 7.36 (s, 1H), 7.48 (d, J = 8.79, 1H), 7.57 (d, J = 2.19, 1H); ^{13}C NMR (base) δ 24.9, 41.1, 111.6, 111.7, 113.8, 115.6, 120.3 (q, J = 320, CF_3), 127.4, 128.4, 137.1, 144.7, 166.7, 194.4; MS (EIPI) *m/e* 308 (M^+). Anal. Calcd for $C_{11}H_{11}N_2O_3SF_3 \cdot C_2H_2O_4 \cdot H_2O$: C, H, N.

***N,N*-Dimethyl-2-[5-[[(trifluoromethyl)sulfonyl]oxy]-1*H*-indol-3-yl]ethylamine Oxalate (14 from 18).** To a magnetically stirred solution of **18** (0.46 g, 1.5 mmol) and 37% formaldehyde (aqueous, 1.2 mL) in acetonitrile (6 mL) was added $NaCNBH_3$ (0.29 g, 4.5 mmol). The mixture was acidified to pH 5 with acetic acid and stirring continued for 3 h. NaOH (10%, 30 mL) was added, after which the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The organic layers were combined and dried over $MgSO_4$. Filtration and removal of the solvent *in vacuo* gave an oil, which was subjected to column chromatography (SiO_2, eluting with CH_2Cl_2/MeOH (5:1)), affording 193 mg (38%) of a colorless oil. Recrystallization of the oxalate salt from MeOH/ether afforded 164 mg (26%) of white crystals: mp 176−177 °C; all further spectroscopic data were analoguous to that of **14** prepared from **11**.

***(p*-Methoxybenzyl)acrylamide.** A 500 mL, oven-dried round bottom flask equipped with a pressure-equalizing dropping funnel was charged with a solution of acryloyl chloride (2.2 mL, 27 mmol) in CH_2Cl_2 (100 mL). The flask was cooled to 15 °C (ethylene glycol/dry ice bath), and the dropping funnel was charged with a solution of 4-methoxybenzylamine (3.27 mL, 25 mmol) and Et_3N (3.5 mL) in CH_2Cl_2 (100 mL). This solution was added over a period of 25 min to the acid chloride solution, stirred for an additional 30 min at −15 °C, and then slowly warmed to ambient temperature over 1.5 h. The reaction mixture was poured into an aqueous NaOH solution and extracted with CH_2Cl_2 (3 × 300 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated, affording a pale yellow solid (5.1 g). This solid was triturated with cyclohexane (4 × 450 mL) to give the title compound (4.5 g, 97%) as an ivory solid: mp 100−101 °C; IR (mull, cm^{-1}) 1614, 1654, 1666 (amide); ^{1}H NMR δ 3.79 (s, 3H), 4.42 (d, J = 5.7, 2H), 5.64 (dd, J_1 = 10.2, J_2 = 1.6, 1H), 6.09 (br s, 1H), 6.10 (dd, J_1 = 16.9, J_2 = 10.2, 1H), 6.29 (dd, J_1 = 16.9, J_2 = 1.5, 1H), 6.85 (d, J = 8.6, 2H), 7.20 (d, J = 8.6, 2H); ^{13}C NMR δ 42.9, 55.1, 126.4, 129.0, 129.9, 130.5, 158.8, 165.1; HRMS calcd (obsd) for $C_{11}H_{13}NO_2$ 191.0946 (191.0955). Anal. Calcd for $C_{11}H_{13}NO_2$: C, H, N.

4-[3-[2-(*N,N*-Dimethylamino)ethyl]-1*H*-indol-5-yl]-*N*-(*p*-methoxybenzyl)acrylamide (19). A 25-mL, oven-dried two-neck round bottom flask equipped with a water-cooled reflux condenser was charged with **14** (500 mg, 1.5 mmol), (*p*-methoxybenzyl)acrylamide (575 mg, 3.0 mmol), $Pd(OAc)_2$ (13.3 mg, 4 mol %), and 1,3-bis(diphenylphosphino)propane (27 mg, 5 mol %). An argon atmosphere was established, DMF (5.0 mL) and Et_3N (0.34 mL, 2.45 mmol) were added *via* a syringe, and the reaction mixture was degassed with argon. The mixture was heated to 85 °C for 18 h, cooled to ambient temperature, and partitioned between aqueous NaOH (1 N) and ethyl acetate. The aqueous layer was extracted with ethyl acetate (2 × 50 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to give a crude oil (1.1 g). This oil was purified by flash chromatography on 200 g of silica gel, eluting with CH_2Cl_2/MeOH/NH_4OH (92:7:1; R_f 0.16) to give 252 mg (44%) of **19** as a tan foam: IR (mull, cm^{-1}) 1610, 1652; ^{1}H NMR δ 2.34 (s, 6H), 2.63 (t, 2H), 2.93 (t, J = 7.3, 2H), 3.79 (s, 3H), 4.51 (d, J = 5.6, 2H), 5.97 (br t, 1H), 6.37 (d, J = 15.6, 1H), 6.87 (d, J = 8.6, 2H), 7.01 (s, 1H), 7.26 (m, 3H), 7.35 (d, J = 8.5, 1H), 7.72 (s, 1H), 7.80 (d, J = 15.5, 1H), 8.46 (br s, 1H); ^{13}C NMR δ 23.6, 43.3, 45.4, 55.3, 60.2, 111.6, 114.0, 115.0, 117.3, 119.8, 121.3, 122.5, 126.2, 127.7, 129.3, 130.5, 137.2, 142.9, 159.0, 166.4; HRMS calcd (obsd) for $C_{23}H_{27}N_3O_2$ 377.2103 (377.2104). Anal. Calcd for $C_{23}H_{27}N_3O_2 \cdot 0.5H_2O$: C, H, N.

Pharmacology. Animals. Adult male albino rats of a Wistar-derived strain (Harlan, Zeist, The Netherlands) weighing 275−325 g were used. Until experiments, the rats were housed in groups of six animals in plastic cages under conditions of constant temperature (20 °C) and humidity with lights on 06:30 and lights off 17:00. Food and water were available ad libitum. Animal procedures were conducted in accordance with guidelines published in the *NIH Guide for the Care and Use of Laboratory Animals*, and all protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

Dunkin-Hartley guinea pigs were ordered in from Kuipers Rabbit Ranch (Gary, IN) with a weight range of 225−275 g approximately 1 week before testing. On arrival the animals were placed in standard guinea pig cages, six to seven animals per cage. The ambient temperature of the housing room and the testing room was 22.2 ± 2.0 °C. The humidity was kept at 45−55%, and a 12 h light−dark regimen was employed (lights on between 06:00 and 18:00).

Materials. Sumatriptan was obtained from Glaxo (U.K.) and serotonin, forskolin, and reserpine were purchased from RBI (Natick, MA). The RIA kit for the cAMP assay was purchased from Biomedical Technologies (Stroughton, MA). The radioligand and other compounds were obtained from the following sources: [^{3}H]-5-HT (28.2 Ci/mmol) from New England Nuclear (Boston, MA); 5-HT, pargyline, and Tris-HCl from Sigma Chemical Co. (St. Louis, MO); STV, DME H-21, and gentamicin from UCSF (San Francisco, CA); geneticin (G-418 sulfate), fetal bovine serum, penicillin, streptomycin, and HAMS #l2 DMEM (50:50) from GIBCO Laboratories (Grand Island, NY); ascorbic acid from Mallinckrodt Inc. (Paris, KY). All substances to be tested in rats were dissolved in saline (0.9% NaCl in distilled water) and administered sc in a volume of 1.0 mL/kg.

In guinea pig experiments, the compounds were made up in a 0.25% methyl cellulose in water solution with the addition of equimolar amounts of citric acid in case the test compound was a free base. The volume of injection is 5 mL/kg for all injections. The guinea pigs that were dosed subcutaneous were injected with a Becton Dickinson 3 mL syringe with a 25 gage five-eighths in. Precision Glide needle both being disposable.

***In Vitro* Binding Assay. Method A.** Competition radioligand binding experiments employed 11 drug concentrations run in duplicate. Radioligands used were [^{3}H]-8-OH-DPAT (5-HT$_{1A}$, 85 Ci/mmol, 1.2 nM), [^{3}H]-5-HT (5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$, 85 Ci/mmol, 2.6 nM), and [^{3}H]U-86170 (D_2-dopamine, 62 Ci/mmol, 2 nM). Nonspecific binding (75−95% of total) was

defined with the following cold compounds added in excess: lisuride (5-HT_{1A}), serotonin (5-HT_{1D}), and haloperidol (D_2). Total binding was determined with buffer. Buffers (pH 7.4) used were 50 mM Tris, 5 mM $MgCl_2$ (5-HT_{1A}), the same with 0.1% ascorbic acid (5-HT_{1D}) and 20 mM HEPES, 10 mM $MgSO_4$ (D_2). Cloned human receptors permanently expressed in CHO cells were the source of the 5-HT binding sites, except for the dopamine D_2 receptor which was cloned from the rat.[27,35,36] Binding mixtures were made in 96-deep-well titer dishes by the addition of 50 μL of drug, 50 μL of radioligand, and 800 μL of membranes (20−60 μg of protein) in binding buffer. After room temperature incubation for 1 h (5-HT_{1D} reactions were protected from light), reactions were stopped by vacuum filtration with a TomTec harvester. Counting was with a 1205 Betaplate using Meltilex as scintillant. IC_{50} values were estimated by fitting the data to a one-site competition model:

$$Y = T/(1 + 10^{\log X - \log IC50})$$

where Y is the specific CPM bound at the concentration X, and T is the specific bound CPM in the absence of the competitor. Inhibition constants (K_i) were calculated with the Cheng−Prushoff equation.[37]

Method B. Human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor clones were expressed in a human embryonic kidney 293 (HEK 293) cell line.[38,39] The HEK 293 cells were grown as a monolayer in 10 mL of HAMS #12 Dulbecco's Modified Eagle Medium (50:50) supplemented with 10% fetal bovine serum, penicillin G (100 units/mL) and streptomycin (10 mg/mL). Confluent monolayers of the cell lines were harvested (PBS containing 5 mM EDTA) and centrifuged at 480*g* for 10 min at 40 °C. The cells were lysed in ice-cold buffer (50 mM Tris-HCl, pH 7.4 containing 5 mM EDTA), homogenized, and sonicated for 10 s. Nuclei and intact cells were removed by centrifugation at 1000*g* for 10 min. The supernatant was spun at 35000*g* for 30 min, and the pellet, containing the microsomal membrane fraction, was resuspended binding buffer containing 50 mM Tris-HCl, 4 mM $CaCl_2$, 0.1% ascorbic acid, 10 mM pargyline, and 1 mM leupeptine. The microsomal membrane suspension was stored at −70 °C.

Radioligand binding assays consisted of 0.1 mL of radioligand (final concentrations: [^{3}H]-5-HT, 0.01−150 nM, 0.8 mL of tissue suspension (50 mg protein), and 0.1 mL of assay buffer or displacing drug. All drugs were diluted in assay buffer. After an incubation of 30 min at 25 °C, assay mixtures were rapidly filtered through 132 glass fiber filters (Schleicher and Schuell; Keene, NH) and washed two times with 5 mL of 50 mM Tris-HCl buffer (pH 7.8). The filters were transferred to plastic counting vials, and radioactivity was measured by liquid scintillation spectroscopy in 2.5 mL of Bio-Safe II Scintillation Cocktail (Research Products International Corp.; Mount Prospect, IL). Specific binding was defined as the excess over blanks taken in the presence of 10^{-5} M 5-HT. Radioligand binding data were analyzed by the EBDA[40] and LIGAND[41] programs that utilize the nonlinear least-squares curve-fitting technique with the Marquardt−Levenberg modification of the Gauss−Newton method.

Forskolin-Stimulated cAMP Inhibition. The funcional cAMP assay using the cloned human 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ receptors was employed as previously described.[26,27] Briefly, confluent cells were preincubated with α-MEM 10 mM/HEPES/1 mM IBMX for 10 min and then stimulated with 25 mM forskolin with or without test drug (either 1 μM 5-HT or 10 μM test compound) for 20 min. The reaction was quenched with TCA and an aliquot assayed for cAMP using a RIA kit. The results were expressed as pmol cAMP/well (n = 3) and agonist inhibition calculated as a percent of 5-HT response.

5-HTP Measurements.[30] Rats were reserpinized (5 mg/kg, ip) 24 h prior to administration of the test compound. The test compound was administered 15 min prior to the administration of 100 mg/kg NSD. After 30 min the rats were decapitated and the brain quickly dissected on ice. Striatum, accumbens, frontal cortex, and hippocampus were stored at −70 °C until analysis. Before analysis the samples were homogenized in TCA and centrifuged. The supernatant was analyzed by means of HPLC with electrochemical detection for 5-HTP and L-DOPA (not shown). 5-HTP accumulation was expressed as [5-HTP] in μg/g wet tissue.

Surgery and Microdialysis Experiments. The microdialysis probes that were used were of a ventrical, concentric design.[42] The exposed tip of the dialysis membrane was 4 mm. The dialysis tube (i.d., 0.22 mm; o.d., 0.31 mm) was prepared from polyacrylonitrile/sodium methyl sulfonate copolymer (AN 69, Hospal, Bologna, Italy). The microdialysis probes were implanted under chloral hydrate anesthesia (400 mg/kg, ip) at the following coordinates: AP −5.2, ML ±4.8 relative to bregma, and V −8.0 below dura (hippocampus). During surgery, lidocaine HCl salt (6% in saline, brought to pH 6.0 with 1 N NaOH) was used as an adjuvant local anesthesic. Probes were secured to the skull with two set screws and fast-securing dental cement. Microdialysis experiments were carried out 24−48 h after implantation of the probe. Samples were collected on-line every 15 min in a 20 μL sample loop of an HPLC system. In brief, the inlet of the microdialysis probe was connected to a piece of polyethylene tubing (450 × 0.28 mm), whereas the outlet of the microdialysis probe was connected to a piece of peek tubing (450 × 0.12 mm). The inlet tube was connected to the perfusion pump and the peek tube directly into the injection valve of the HPLC apparatus. The connection with the HPLC equipment introduced a lag time of about 8 min, for which the presented data are corrected. With the help of an electronic timer, the injection valve was held in the load position for 15 min, which was the time needed to record a complete chromatogram. The perfusion was carried out with an aCSF solution at a flow rate of 1.5 μL/min using Carnegie CMA (Stockholm, Sweden) perfusion pump. The composition of the aCSF solution was (mM): NaCl, 147.0; KCl, 4.0; $CaCl_2$, 1.2; and $MgCl_2$, 1.0. After finishing the experiment, the rat was terminated with an overdose pentothal and the brain was fixed with 4% paraformaldehyde via intracardiac perfusion. Coronal sections (40 μm thick) were cut, and dialysis probe placement was verified with the help of the atlas of Paxinos and Watson.[43]

Analysis of the Dialysates. 5-HT was quantified by HPLC with electrochemical detection. A Shimadzu LC10-AD pump was used in conjunction with an electrochemical detector (Antec, Leiden, The Netherlands) set at 650 mV vs an Ag/AgCl reference electrode. A reversed-phase C_{18} Supelco LC18DB column (150 × 4.6 mm; 5 μm) was used. The mobile phase consisted of an aquous solution of 2.0 g/L of citric acid, 5.0 g/L of sodium acetate, 100 mg/L of EDTA, 300 mg/L of TMA, 300 mg/L of MSA, 10% methanol (v/v), and 5% acetronitile (v/v) and was delivered at a flow rate of 1 mL/min. 5-HT eluted after 8 min.

Guinea Pig Brain Neurochemistry. Male Dunkin-Hartley guinea pigs were decapitated 60 min after test drug administration by means of guillotine. Their brains were rapidly removed and put on an ice-chilled Petri dish. The prefrontal cortex and the hypothalamus were dissected, and the tissue parts were stored at −80 °C until further analysis. The levels of 5-HT and 5-HIAA were measured by means of HPLC with electrochemical detection according to methods described in the literature with minor modifications.[30]

Hypothermia. At least 1 h before animals were to be tested they were removed from the gang cages and placed in individual plastic cages (20 × 30 × 15 cm) and then taken to the testing room. The guinea pigs were tested in groups of five animals per group. The zero rectal temperature was taken using a Digital Thermometer VWR Scientific Inc. with a range of −40 to a 300 °F, or −40 to 150 °C. The probe used in this study is a Yellow Springs Instruments 423-series probe. The probe was lubricated with a drop of silicon and then inserted 3−4 cm in the rectum and left until the reading on the Digital Thermometer is stable, usually around 10 s. The temperature is recorded to the nearest 0.1 °C. This is the control measurement for the other time intervals used which are 30, 60, and 120 min after dosing. In the present study we present data from the 60 min readings.

Expression of Results and Statistics. Differences between 5-HTP concentration of the control and drug treatment were analyzed with one-way ANOVA followed by a posthoc *t*-test. In microdialysis, the average of the last four stable

samples (less than 20% variation) before drug treatment was considered as the control value and was defined as 100%. Values given are expressed as percentages of controls. Differences between the average dialysate concentrations of the control and drug treatment were compared with Friedman's one-way ANOVA with repeated measures ($p \leq 0.05$) followed by Dunn's posthoc test.

The data from the guinea pig brain neurochemistry are expressed as mean ± SEM ($n = 4-5$) (%) of vehicle treated controls. Statistical analyis was performed by means of ANOVA followed by Fishers' PLSD. In the hypothermia test, the mean difference, the SEM, and the probablility value between the 0 min control and the 60 min value were calculated *via* the RS-1 statistical program.

References

(1) Heuring, R. E.; Peroutka, S. J. Characterization of a novel [^{3}H]-5-hydroxytryptamine binding subtype in bovine brain membranes. *J. Neurosci.* **1987**, *7*, 894−903.

(2) Waeber, C.; Schoeffter, P.; Palcios, J. M.; Hoyer, D. Molecular pharmacology of 5-HT$_{1D}$ recognition sites: Radioligand binding studies in human, pig and calf brain membranes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1988**, *337*, 595−601.

(3) Hoyer, D.; Middlemiss, D. N. Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. *Trends. Pharmacol. Sci.* **1989**, *10*, 130−132.

(4) Maura, G.; Thellung, S.; Andreoli, G. C.; Ruelle, A.; Raiteri, M. Release-regulating 5-HT$_{1D}$ serotonin autoreceptors in human cerebral cortex. *J. Neurochem.* **1993**, *60*, 1179−1182.

(5) Hartig, P. R.; Branchek, T. A.; Weinshank, R. L. A subfamily of 5-HT$_{1D}$ receptor genes. *Trends Pharmacol. Sci.* **1992**, *13*, 152−159.

(6) Weinshank, R. L.; Zgombick, J. M.; Macchi, M. J.; Branchek, T. A.; Hartig, P. R. Human sertonin 1D receptor is encoded by a subfamily of two distinct genes: 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3630−3634.

(7) Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humprey, P. P. A. VII. International Union of Pharmacology Classification of Receptors for 5-Hydroxytryptamine (Serotonin). *Pharmacol. Rev.* **1994**, *46*, 157−203.

(8) Hamel, E.; Fan, E.; Linville, D.; Ting, V.; Villemure, J. G.; Chia, L. S. Expression of mRNA for the serotonin 5-hydroxytryptamine$_{1D\beta}$ receptor subtype in human and bovine cerbral arteries. *Mol. Pharmacol.* **1993**, *44*, 242−246.

(9) Hamel, E.; Gregoire, L.; Lau, B. 5-HT$_1$ receptors mediating contraction in bovine cerebral arteries: a model for human cerebrovascular '5-HT1Dβ' receptors. *Eur. J. Pharmacol.* **1993**, *242*, 75−82.

(10) (a) Ferrari, M. D.; Saxena, P. R. Clinical and experimental effects of sumatriptan in humans. *Trends Pharmacol. Sci.* **1993**, *14*, 129−133. (b) Peroutka, S. J.; McCarthy, B. G. Sumatriptan (GR 43175) interacts selectively with 5-HT$_{1B}$ and 5-HT$_{1D}$ binding sites. *Eur. J. Pharmacol.* **1989**, *163*, 133−136.

(11) Street, L. J.; Baker, R.; Davey, W. B.; Guiblin, A. R.; Jelley, R. A.; Reeve, A. J.; Routledge, H.; Sternfeld, F.; Watt, A. P.; Beer, M. S.; Middlemiss, D. N.; Noble, A. J.; Stanton, J. A.; Scholey, K.; Hargreaves, Sohal, B.; R. J.; Graham, M. I.; Matassa, V. G. Synthesis and Serotonergic Activity of N,N-Dimethyl[5-(1,2,4-triazol-1-ylmethyl)-1*H*-indole-3-yl]ethylamine and Analogues: Potent Agonists for 5-HT$_{1D}$ Receptors. *J. Med. Chem.* **1995**, *38*, 1799−1810.

(12) Glen, R. C.; Martin, G. R.; Hill, A. P.; Hyde, R. M.; Woollard, P. M.; Salmon, J. A.; Buckingham, J.; Robertson, A. D. Computer-Aided Design and Synthesis of 5- Substituted Tryptamines and Their Pharmacology at the 5-HT$_{1D}$ Receptor: Discovery of Compounds with Potential Anti-Migraine Properties. *J. Med. Chem.* **1995**, *38*, 3566−3580.

(13) For a review, see: Ferrari, M. D.; Saxena, P. M. 5-HT$_1$ receptors in migraine pathophysiology and treatment. *Eur. J. Neurol.* **1995**, *2*, 5−21.

(14) Clitherow, J. W.; Scopes, D. I. C.; Skingle, M.; Jordan, C. C.; Feniuk, W.; Campbell, I. B.; Carter, M. C.; Collington, E. W.; Connor, H. E.; Higgins, G. A.; Beattie, D.; Kelly, H. A.; Mitchell, W. L.; Oxford, A. W.; Wadsworth, A. H.; Tyers, M. B. Evolution of a Novel Series of [(*N*,*N*-Dimethylamino)propyl]- and Piperazinylbenzanilides as the First Selective 5-HT$_{1D}$ Antagonists. *J. Med. Chem.* **1994**, *37*, 2253−2257.

(15) Walsh, D. H.; Beattie, D. T.; Connor, H. E. The activity of 5-HT$_{1D}$ receptor ligands at cloned human 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors. *Eur. J. Pharmacol.* **1995**, *287*, 79−84.

(16) Clitherow, J. W.; Scopes, D. I. C.; Beattie, D. T.; Skingle, M. The evolution of selective 5-HT$_{1D}$ receptor antagonists. *Exp. Opin. Invest. Drugs.* **1995**, *4*, 323−331.

(17) Glennon, R. A.; Ismaiel, A. M.; Chaurasia, C.; Titeler, M. 5-HT$_{1D}$ Serotonin Receptors: Results of a Structure-Affinity Investigation. *Drug Dev. Res.* **1991**, *22*, 25−36.

(18) (a) Castro. J. L.; Baker, R.; Guiblin, A. R.; Hobbs, S. C.; Jenkins, M. R.; Russell, M. G. N.; Beer, M. S.; Stanton, J. A.; Scholey, K.; Hargreaves, R. J.; Graham, M. I.; Matassa, V. G. Synthesis and Biological Activity of 3-[2-(Dimethylamino)ethyl-5-[(1,1-dioxo-5-methyl-1,2,5-thiadiazolidin-2-yl)-methyl]-1*H*-indole and Analogues: Agonists for the 5-HT$_{1D}$ Receptor. *J. Med. Chem.* **1994**, *37*, 3023−3032. (b) Perez, M.; Fourrier, C.; Sigogneau, I.; Pauwels, P. J.; Palmier, C.; John, G. W.; Valentin, J.-P.; Halazy, S. Synthesis and Serotonergic Activity of Arylpiperazide Derivatives of Serotonin: Potent Agonists for 5-HT$_{1D}$ Receptors. *J. Med. Chem.* **1995**, *38*, 3602−3607.

(19) (a) Macor, J. E.; Blank, D. H.; Post, R. J.; Ryan, K. The Synthesis of Conformationally Restricted Analog of the Anti-migraine Drug Sumatriptan. *Tetrahedron Lett.* **1992**, *33*, 8011−8014. (b) King, F. D.; Brown, A. M.; Gaster, L. M.; Kaumann, A. J.; Medhurst, A. D.; Parker, S. G.; Parsons, A. A.; Patch, T. L.; Raval, P. (±) 3-Amino-6-carboxamido-1,2,3,4-tetrahydrocarbazole: A Conformationally Restricted Analogue of 5-Carboxamidotryptamine with Selectivity for the Serotonin 5-HT$_{1D}$ Receptor. *J. Med. Chem.* **1993**, *36*, 1918−1919. (c) Macor, J. E.; Blank, D. H.; Fox, C. B.; Lebel, L. A.; Newman, M. E.; Post, R. J.; Ryan, K.; Schmidt, A. W.; Schulz, D. W.; Koe, B. K. 5-[(3-Nitropyrid-2-yl)amino]indoles: Novel Serotonin Agonists with Selectivity for the 5-HT$_{1D}$ Receptor. Variation of the C3 Substituent on the Indole Template Leads to Increased 5-HT$_{1D}$ Receptor Selectivity. *J. Med. Chem.* **1994**, *37*, 2509−2512.

(20) (a) Sonesson, C.; Boije, M.; Svensson, K.; Ekman, A.; Carlsson, A.; Romero, A. G.; Martin, I. J.; Duncan, J. N.; King, L. J.; Wikström, H. Orally Active Central Dopamine and Serotonin Receptor Ligands: 5-, 6-, 7- and 8-[(Trifluoromethyl)sulfonyl]-oxy-2-(di-n-propylamino)tetralins and the Formation of Active Metabolites *in Vivo*. *J. Med. Chem.* **1993**, *36*, 3409−3416. (b) Sonesson, C.; Barf, T.; Nilsson, J.; Dijkstra, D.; Carlsson, A.; Svensson, K.; Smith, M. W.; Martin, I. J.; Duncan, J. N.; King, L. J.; Wikström, H. Synthesis and Evaluation of Pharmacological and Pharmacokinetic Properties of Monopropyl Analogs of 5-, 7- and 8-[[(Trifluoromethyl)sulfonyl]oxy]-2-aminotetralins, *J. Med. Chem.* **1995**, *38*, 1319−1329.

(21) (a) Liu, Y.; Yu, H.; Svensson, B. E.; Cortizo, L.; Lewander, T.; Hacksell, U. Derivatives of 2-(Dipropylamino)tetralin: Effect of the C8-Substituent on the Interaction with 5-HT$_{1A}$ Receptors. *J. Med. Chem.* **1993**, *36*, 4221−4229. (b) Liu, Y.; Yu, H.; Mohell, N.; Nordvall, G.; Lewander, T.; Hacksell, U. Derivatives of *cis*-2-Amino-8-hydroxy-1-methyltetralin: Mixed 5-HT$_{1A}$-Receptor Agonists and Dopamine D$_2$-Receptor Antagonists. *J. Med. Chem.* **1995**, *38*, 150−160.

(22) Sonesson, C.; Lin, C.-H.; Hansson, L.; Waters, N.; Svensson, K.; Carlsson, A.; Smith, M. W.; Wikström, H. Substituted (*S*)-Phenylpiperidines and Rigid Congeners as Preferential Dopamine Autoreceptor Antagonists: Synthesis and Structure-Activity Relationships. *J. Med. Chem.* **1994**, *37*, 2735−2753.

(23) (a) Speeter, M. E.; Anthony, W. C. The action of oxalyl chloride on indoles: a new approach to tryptamines. *J. Am. Chem. Soc.* **1954**, *76*, 6208−6210. (b) Kondo, H.; Kataoka, H.; Hayashi, Y.; Dodo, T. *Chem. Abstr.* **1960**, *54*, 492.

(24) Hendrickson, J. B.; Bergeron, R. Triflamides: New acylating and triflating reagents. *Tetrahedron Lett.* **1973**, 4607−4610.

(25) De Silva, S. O.; Snieckus, V. Indole N-alkylation of tryptamines via dianion and phthalimido intermediates. New potential indolealkylamine haptens. *Can. J. Chem.* **1978**, *56*, 1621−1627.

(26) Kukolja, S.; Lammert, S. R. Azetidone antibiotics. XIV. Removal of a phthaloyl protective group from acid and base sensitive compounds. *J. Am. Chem. Soc.* **1975**, *97*, 5582−5583.

(27) Veldman, S. A.; Bienkowski, M. J. Cloning and Pharmacological Characterization of a Novel Human 5-Hydroxytryptamine-1D Receptor Subtype. *Mol. Pharmacol.* **1992**, *42*, 439−444.

(28) McCall, R. B.; Romero, A. G.; Bienkowksi, M. J.; Harris, D. W.; McGuire, J. C.; Piercey, M. F.; Shuck, M. E.; Smith, M. W.; Svensson, K. A.; Schreur, P. J. K. D.; Carlsson, A.; VonVoightlander, P. F. Characterization of U-92016A as a Selective, Orally Active, High Intrinsic Activity 5-Hydroxytryptamine$_{1A}$ Agonist. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 875−883.

(29) Dourish, C. T.; Hutson, P. H.; Curzon, G. Putative anxiolytics 8-OH-DPAT, buspirone and TVX Q 7821 are agonists at 5-HT$_{1A}$ autoreceptors in the raphe nuclei. *Trends Pharmacol. Sci.* **1986**, *7*, 212−214.

(30) Shum, A.; Sole, M. J.; van Loon, G. R. Simultaneous measurement of 5- hydroxytryptophan and L-dihydroxyphenylalanine by high performance liquid chromatography with electrochemical detection. Measurement of serotonin and catecholamine turnover in discrete brain areas. *J. Chromatogr.* **1982**, *228*, 123−130.

(31) Glennon, R. A.; Hong, S.-S.; Dukat, M.; Teitler, M.; Davis, K. 5-(Nonyloxy)tryptamine: A Novel High-Affinity 5-HT$_{1D\beta}$ Serotonin Receptor Agonist. *J. Med. Chem.* **1994**, *37*, 2828−2830.

(32) Skingle, M.; Higgins, G. A.; Feniuk, W. J. Stimulation of central 5-HT_{1D} receptors causes hypothermia in the guinea-pig. *J. Psychopharmacol.* **1994**, *8*, 14−21.
(33) Saxena, P. R.; De Vries, P.; Heiligers, J. P. C.; Maassen-Van Den Brink, A.; Barf, T.; Wikström, H. Investigation with GMC2021, a triflated analogue of sumatriptan, in experimental models predictive of antimigraine activity and coronary side-effect potential. *Eur. J. Pharmacol.* **1996**, *312*, 53−62.
(34) Pallas version 1.2 is commercially available software of CompuDrug Chemistry Ltd., 1994.
(35) Chio, C. L.; Hess, G. F.; Graham, R. S.; Huff, R. M. A second molecular form of D_2 dopamine receptor in rat and bovine caudate nucleus. *Nature* **1990**, *343*, 266−269.
(36) Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. The genomic clone G-21 which resembles a β-adrenergic receptor sequence encodes the 5-HT_{1A} receptor. *Nature* **1988**, *335*, 358−360.
(37) Cheng, Y. C.; Prushoff, W. H.; Relationship Between the Inhibition Constant and the Concentration of Inhibitor which Causes 50 Percent Inhibition of Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(38) Oksenberg, D.; Marsters, S. A.; O'Dowd, B. F.; Jin, H.; Havlik, S.; Peroutka, S. J.; Ashkenazi. A single aminoacid difference confers to major pharmacological variation between human and rodent 5-HT_{1B} receptors. *Nature* **1992**, *360*, 161−163.
(39) Jin, H.; Oksenberg, D.; Ashkenazi, A.; Peroutka, S. J.; Duncan, A. M. V.; Rozmahel, R.; Yang, Y.; Mengod, G.; Palacios, J. M.; O'Dowd, B. F. Characterization of the human 5-hydroxytryptamine$_{1B}$ receptor. *J. Biol. Chem.* **1992**, *267*, 5735−5738.
(40) McPherson, G. A. A Computer-based Approach to the Analysis of Radioligand Binding Experiments. *Comput. Prog. Biomed.* **1983**, *17*, 107−114.
(41) Munson, P. J.; Rodbard, D. Ligand: A versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **1980**, *107*, 220−239.
(42) Santiago, M.; Westerink, B. H. C. Role of adenylate cyclase in the release of dopamine: A microdialysis study in the striatum of the rat. *J. Neurochem.* **1990**, *55*, 169−174.
(43) Paxinos, G.; Watson, C. *Rat Brain in Stereotaxic Coordinates*; Academic Press: New York, 1982.

JM9604890