Differentiation between Partial Agonists and Neutral 5-HT$_{1B}$ Antagonists by Chemical Modulation of 3-[3-(*N*,*N*-Dimethylamino)propyl]-4-hydroxy-*N*-[4-(pyridin-4-yl)phenyl]benzamide (GR-55562)

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The synthesis and binding affinity at cloned h5-HT$_{1B}$, h5-HT$_{1D}$, and h5-HT$_{1A}$ receptors of 3-[3-(*N*,*N*-dimethylamino)propyl]-4-hydroxy-*N*-[4-(pyridin-4-yl)phenyl]benzamide (**2**, GR-55562) and four O-methylated analogs are described. The functional activity of these compounds was determined at the h5-HT$_{1B}$ receptor using a [^{35}S]GTPγS binding assay. The four analogs have been prepared in order to evaluate the influence of the alkylamino side chain conformation on binding and intrinsic activity. Whereas **2** and its derivatives display a similar binding affinity profile, major differences arise from analysis of the intrinsic activity data at h5-HT$_{1B}$ receptors. The O-methylated analog of **2**, 3-[3-(*N*,*N*-dimethylamino)propyl]-4-methoxy-*N*-[4-(pyridin-4-yl)phenyl]benzamide (**3a**), and the (1*Z*)-3-(*N*,*N*-dimethylamino)prop-1-enyl derivative (**3c**) act as neutral and potent antagonists (in a similar way to **2**), while the 3-(*N*,*N*-dimethylamino)-prop-1-ynyl (**3b**) and (1*E*)-3-(*N*,*N*-dimethylamino)prop-1-enyl (**3d**) analogs display nonnegligible agonist activity. Molecular modeling studies show that, when the common triaryl portions of the molecules are overlapped, the partial agonists and the neutral antagonists differ by a near-orthogonal orientation of the NH$^+$ projection to the hydrogen-bond receptor site.

Introduction

5-HT (serotonin), a neurotransmitter, is involved in numerous physiological (e.g., thermoregulation, hemodynamics, feeding, sleeping) and pathophysiological (e.g., depression, hypertension, migraine, anxiety) processes and interacts with various distinct membrane receptors.[1] These receptors have been divided in seven classes: 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, 5-HT$_4$, 5-HT$_5$, 5-HT$_6$, and 5-HT$_7$.[2] Among them, the 5-HT$_{1B/1D}$ receptors are the most abundant 5-HT$_1$ receptor subtypes in the mammalian central nervous system (CNS).

The 5-HT$_{1B}$ binding site was first characterized in rat brain membranes and later on the 5-HT$_{1D}$ binding site in bovine caudate nucleus.[3] Molecular biological studies demonstrated that the human 5-HT$_{1D}$ receptor site is encoded by a family of two distinct genes termed initially 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$.[4] Binding studies have previously shown that the human 5-HT$_{1D\beta}$ and rat 5-HT$_{1B}$ receptors are "pharmacologically different" as demonstrated, for example, by studies with propanolol which binds to the rodent 5-HT$_{1B}$ receptor with nanomolar affinities but recognizes the 5-HT$_{1D\beta}$ binding site only very poorly (IC$_{50}$ > 1000 nM).[5] It has now been demonstrated that this difference in the pharmacological profile is the result of a single amino-acid modification.[6,7] Since the amino-acid sequence, the function, and the regional distribution of the rodent 5-HT$_{1B}$ receptor and of the human 5-HT$_{1D\beta}$ receptor are nearly identical, these two receptors are considered as species homologs. The Serotonin Club Nomenclature Committee has recently proposed[8] a revised nomenclature for 5-HT$_{1B}$, 5-HT$_{1D\alpha}$, and 5-HT$_{1D\beta}$ receptor subtypes. 5-HT$_{1D\alpha}$ receptors are now termed 5-HT$_{1D}$, while 5-HT$_{1D\beta}$ receptors are now termed 5-HT$_{1B}$ with distinction between human (h5-HT$_{1B}$) and rat (r5-HT$_{1B}$) receptors, to account for their pharmacological differences.

5-HT$_{1B/1D}$ receptors belong to the family of 7-TM G-protein-coupled receptors (both coupled to adenylate cyclase) and are implicated in important functional activities:[9] for example, in the vascular periphery, activation of 5-HT$_{1B/1D}$ receptors on blood vessels leads to vasoconstriction,[10] and 5-HT$_{1B/1D}$ receptors are also involved in the inhibition of protein extravasation,[11] two mechanisms relevant to the antimigraine activity of 5-HT$_{1B/1D}$ agonists such as sumatriptan, naratriptan, zolmitriptan, and rizatriptan. In the CNS, presynaptic 5-HT$_{1B/1D}$ receptors on serotoninergic axon terminals control the release of 5-HT.[9] This function is likely to be connected with the pathophysiology of depression, since several lines of preclinical and clinical evidence indicate that an enhancement of 5-HT-mediated neurotransmission might underlie the therapeutic effect of most antidepressant drugs. As a consequence, the blockade of terminal 5-HT$_{1B/1D}$ receptors (and probably more precisely 5-HT$_{1B}$ autoreceptors) by selective antagonists has been proposed[12,13] as a new approach toward the design of potentially more efficient and fast-acting antidepressant drugs since 5-HT$_{1B/1D}$ blockade would in theory immediately elevate terminal 5-HT release.

2′-Methyl-4′-(5-methyl[1,2,4]oxadiazol-3-yl)biphenyl-4-carboxylic acid [4-methoxy-3-(4-methylpiperazin-1-yl)-phenyl]amide (**1**, GR-127935; Chart 1) has proved most valuable as a potent and selective 5-HT$_{1B/1D}$ receptor antagonist, but it fails to discriminate between these two receptor subtypes.[14] Moreover, recent investigations at the level of cloned h5-HT$_{1B/1D}$ receptors have shown that **1** acts as a weak but full agonist at 5-HT$_{1D}$ sites and can be classified as a partial, "nonsilent" antagonist at 5-HT$_{1B}$ sites.[15,16] It has previously been

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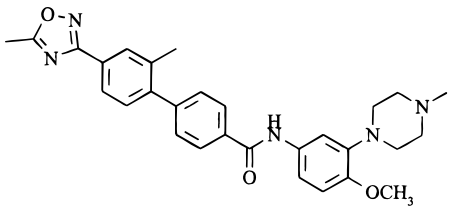
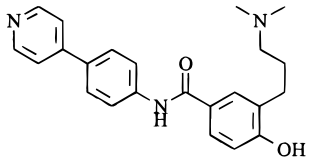
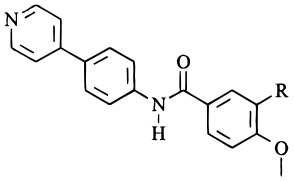
Chart 1



1



2



3a : R = $CH_2CH_2CH_2NMe_2$

3b : R = C ≡ C - CH_2NMe_2

3c : R = (Z) CH=$CHCH_2NMe_2$

3d : R = (E) CH=$CHCH_2NMe_2$

proposed that the failure of **1** to increase brain 5-HT release *in vivo* may be related to the intrinsic activity of this particular compound at 5-$HT_{1B/1D}$ sites.[14] The discovery of new, potent, neutral, and selective 5-HT_{1B} antagonists still represents a fascinating challenge to permit the evaluation of the concept of 5-HT_{1B} antagonism as a tool to improve 5-HT neurotransmission and, hopefully, to identify new antidepressant drugs.

Interestingly enough, unlike **1**, 3-[3-(*N*,*N*-dimethylamino)propyl]-4-hydroxy-*N*-[4-(pyridin-4-yl)phenyl]-benzamide (**2**, GR-55562) has recently been characterized as a neutral, potent, and more selective h5-HT_{1B} receptor antagonist.[17] These conclusions were based on functional studies (inhibition of forskolin-stimulated adenosine 3′,5′-cyclic monophosphate production) in HeLa cells stably transfected with 5-HT_{1B} human receptor subtypes.

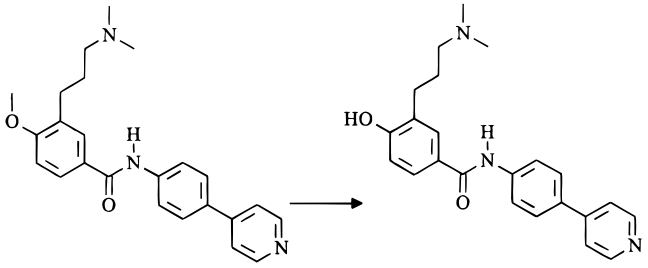
More recent investigations from our laboratory[18] have shown that [^{35}S]GTPγS binding studies in C6-glial cells stably transfected with 5-HT_{1B} or 5-HT_{1D} receptors represent a highly sensitive and quantitative way to characterize the intrinsic activity of 5-$HT_{1B/1D}$ ligands. As a consequence this assay allows the differentiation between full agonists, partial agonists, and neutral antagonists. Importantly, the detection of signs of residual agonist activity is particularly relevant to identify neutral antagonists at 5-$HT_{1B/1D}$ receptor subtypes. In this paper, we report on the synthesis and binding at cloned h5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1A} receptors of **2** and four O-methylated analogs (**3a**−**d**). Moreover the functional activity of these compounds was determined at the h5-HT_{1B} receptor using a [^{35}S]GTPγS binding assay. These particular compounds have been prepared in order to evaluate the influence of the basic amino-side chain conformation on binding and intrinsic activity at h5-HT_{1B} receptors.

Results

Chemistry. **2**[19] was prepared from its methoxy analog **3a** in 62% yield by treatment with BBr_3 in dichloromethane (Scheme 1). The preparation of the propyl analog **3a** has been previously reported.[20] It involves the palladium-catalyzed coupling of 3-iodo-4-methoxybenzoic acid and 1-(*N*,*N*-dimethylamino)-2-propyne to give **5b** followed by a complete reduction of the alkyne function and the condensation of the resulting carboxylic acid **5a** with 4-(4-pyridinyl)benzenamine (Scheme 2). All other analogs (**3b**−**d**) were obtained by coupling of the appropriate carboxylic acid intermediate **5b**−**d** with 4-(4-pyridinyl)benzenamine **9** using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI) in poor to moderate yields (Scheme 3).

The carboxylic acid intermediates **5b**−**d** were prepared from commercially available methyl 3-iodo-4-methoxybenzoate (Scheme 4). A minor modification of Sonogashira's original methodology[21] allowed the introduction of the alkynyl chain of **4b** in quantitative yield. Compound **4b** was then hydrolyzed under basic conditions to give the first carboxylic acid intermediate **5b** in 94% yield. The *Z* intermediate **5c** was obtained in quantitative yield from **5b** by catalytic hydrogenation with 2% palladium(0) over charcoal. With such a small quantity of catalyst the reaction was sufficiently slow to allow the isolation of the (*Z*)-alkene. In order to access the *E* intermediate, the hydrogenation was performed under acidic conditions on methyl ester **4b**. However a mixture of the *E* and *Z* isomers as well as traces of the fully hydrogenated propyl analog **4a** was obtained. Fortunately the *E* isomer **4d** could be separated from the mixture and was obtained in 61% yield. A mild basic hydrolysis of **4d** gave the carboxylic acid intermediate **5d** in 93% yield.

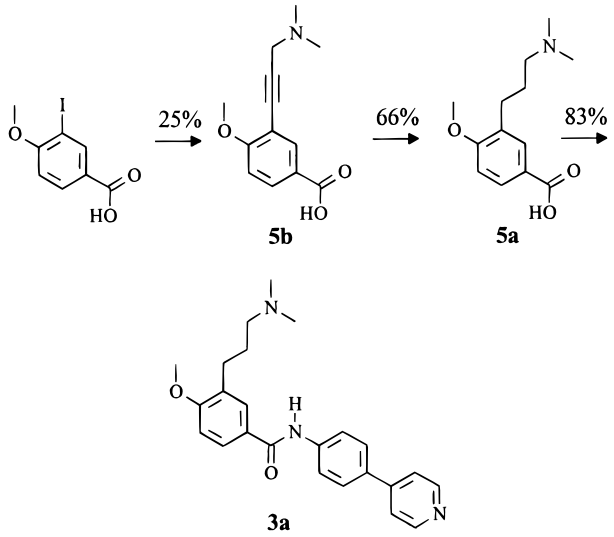
Scheme 1[a]



3a **2**

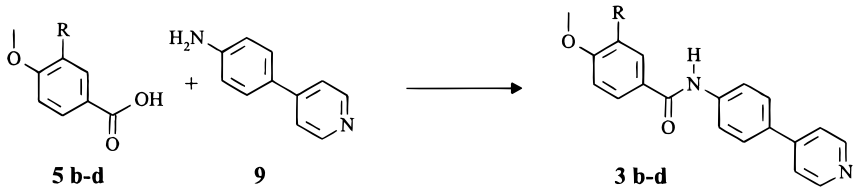
[a] Reagents: BBr_3, CH_2Cl_2, 62%.

Scheme 2



5b **5a** **3a**

Scheme 3[a]

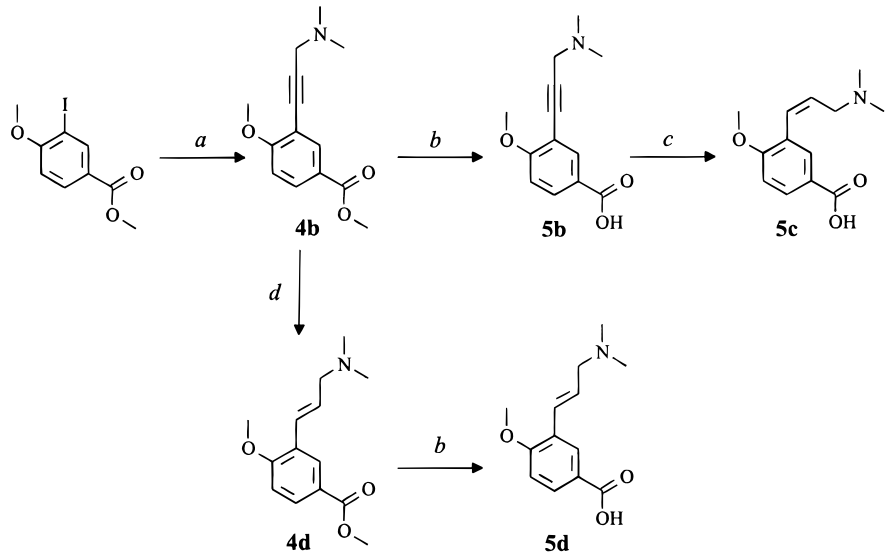


5 b-d **9** **3 b-d**

b : R = C ≡ C - CH_2NMe_2
c : R = (Z) CH=$CHCH_2NMe_2$
d : R = (E) CH=$CHCH_2NMe_2$

[a] Reagents: EDCI, Et_3N, CH_2Cl_2, 4-DMAP, 45% for **3b**, 25% for **3c**, 20% for **3d**.

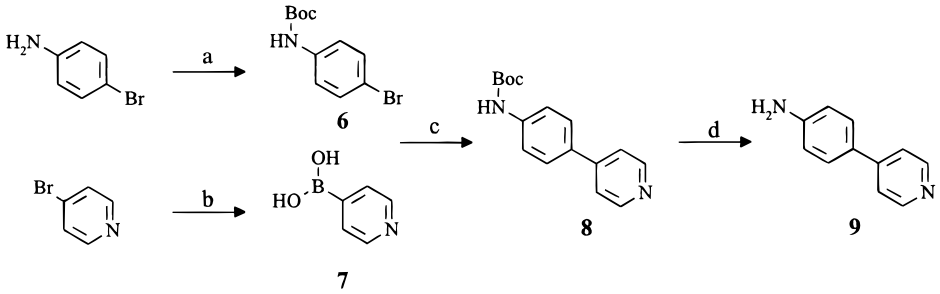
Scheme 4[a]



4b **5b** **5c** **4d** **5d**

[a] Reagents: (a) 2 equiv of pyrrolidine, 5% $Pd(PPh_3)_4$, 10% CuI, 1.7 equiv of 1-(*N*,*N*-dimethylamino)-2-propyne, THF, 100%; (b) LiOH, THF, H_2O, 94% for **5b**, 93% for **5d**; (c) 2% Pd/C, EtOH, H_2, 100%; (d) 2% Pd/C, MeOH, AcOH, H_2, 61%.

Scheme 5[a]



6 **7** **8** **9**

[a] Reagents: (a) $(Boc)_2O$, toluene, 70 °C, 100%; (b) i. *n*-BuLi, $(i\text{-PrO})_3B$, −78 °C to rt; ii. NaOH, 50%; (c) 1,2-dimethoxyethane, *t*-BuOK, 6% $Pd(PPh_3)_4$, H_2O, 84 °C, 61%; (d) HCl, MeOH, 53%.

4-(4-Pyridinyl)benzenamine (**9**) was prepared according to the general approach introduced by Terashima and colleagues[22] with some modifications that gave more reproducible results and could be easily scaled up to a few grams (Scheme 5). 4-Bromopyridine was first converted into 4-pyridinylboronic acid (**7**)[23] in 65% yield. Attempts to couple **7** with 4-bromoaniline failed to give any trace of **9**. Therefore, 4-bromoaniline was first protected as the Boc carbamate **6** in quantitative yield. Then a modified Suzuki coupling[24] between **6** and boronic acid **7** gave **8** in 61% yield. Finally the deprotection of the aniline function was carried out with hydrogen chloride in methanol to give the biaryl intermediate **9** in 53% yield after purification over a short column of alumina.

Biological Data. In a first series of experiments, the binding affinities were measured for **2** and the four derivatives **3a**−**d** at recombinant h5-HT_{1A}, h5-HT_{1B}, and h5-HT_{1D} receptors (Table 1). As previously reported, **2** showed a selective binding affinity (50−70-fold) for the h5-HT_{1B} receptor subtype versus the h5-HT_{1A} and h5-HT_{1D} receptor subtypes. The derivatives **3a**−**d** showed also a preferential binding affinity for the 5-HT_{1B} receptor subtype. h5-HT_{1B} receptor affinities were nearly identical regardless of the ligand displacing either [^{3}H]-5-CT or [^{3}H]GR-125743; slight differences (3-fold) were only apparent for **3a**,**c**. The functional activity of these compounds was determined at the h5-HT_{1B} receptor using a [^{35}S]GTPγS binding assay and a membrane preparation from C6-glial cells stably ex-

Table 1. Receptor Binding Affinities of **2** and Its Derivatives for Recombinant h5-HT_{1A}, h5-HT_{1D}, and h5-HT_{1B} Receptors

	binding affinities (K_i, nM)[a]			
compd	h5-HT_{1A}[b] [^{3}H]-8-OH-DPAT[c] HeLa[d]	h5-HT_{1D}[b] [^{3}H]-5-CT[c] Cos-7[d]	h5-HT_{1B}[b] [^{3}H]-5-CT[c] C6-glial[d]	h5-HT_{1B}[b] [^{3}H]GR-125743[c] C6-glial[d]
2	842	700	12.1 ± 0.9	14.0 ± 5.4
3a	>1000	540	70.1 ± 21.5	15.4 ± 1.9
3b	>1000	1200	16.2 ± 4.9	22.3 ± 6.2
3c	633	169	19.0 ± 3.7	6.1 ± 1.4
3d	>1000	560	13.6 ± 3.0	18.8 ± 4.9

[a] Values are presented as mean or mean ±SD of 2−3 independent experiments, each one performed in triplicate. Binding affinities at 5-HT_{1A} and 5-HT_{1D} receptors are typically within ±10−20% of the mean. [b] Receptor subtype. [c] Radioligand. [d] Transfected cell type.

pressing h5-HT_{1B} receptors.[18] This preparation is sensitive to stimulation of [^{35}S]GTPγS binding by agonists such as 5-HT and zolmitriptan. Both compounds are efficacious agonists and stimulate [^{35}S]GTPγS binding to the same magnitude; a maximal [^{35}S]GTPγS binding response of 73 ± 5% was obtained. The compounds **2** and **3a**,**c** did not affect basal [^{35}S]GTPγS binding; neither stimulation nor inhibition of [^{35}S]GTPγS binding was obtained at micromolar concentrations. However, both of them potently displaced the [^{35}S]GTPγS binding response of zolmitriptan (Figure 1A). Each compound (1 μM) competitively antagonized the zolmitriptan-mediated response. The derived K_B values varied between 19.4 and 22.7 nM in line with their respective h5-HT_{1B} binding affinities (Table 2). In contrast, compounds **3b**,**d** stimulated [^{35}S]GTPγS binding at micromolar concentrations; their intrinsic activity represented respectively 23% and 32% at 1 μM compared to a maximal response elicited by zolmitriptan (Figure 1B). Both compounds (10 μM) partially displaced the zolmitriptan response (Figure 1C), compound **3d** being more potent than compound **3b** in accordance with their intrinsic activity.

Conformational Analysis. The goal of these computations was to identify the common conformations of **3a**−**d** and to rationalize the difference in intrinsic activity observed with **3b**,**d** at the 5-HT_{1B} receptor site. To begin with, the preferred conformations of the common methoxybenzamide were determined through computation of the torsion angles τ_1, τ_2, τ_3, and τ_4 (Chart 2). A single conformation for the molecule was obtained ($\tau_1 = 56°$, $\tau_2 = -41°$, $\tau_3 = 151°$, $\tau_4 = 0°$) due to the rigidity of the benzamide function and the steric hindrance between the methoxy group and its ortho substituent. Then the different alkylamino chains were introduced (Table 3), and conformations that emerged from the analysis were checked for further fitting studies with the Disco module using the most constrained (**3b**) analog as template restricted to its conformational families to prevent redundant answers. The computation identified 14 matching points: five key atoms (three H-bond acceptors, one H-bond donor, and 1 positive), six projections from the structure to the binding site (four H-bond donors, one H-bond acceptor, and 1 negative), and three ring centroids (Chart 3A). Finally, knowing that the binding affinities of all four analogs for the $5HT_{1B}$ receptor were basically the same, an identical number of matching points were chosen for all of them. Interestingly enough, no common conformation was found that included both the H-bond donor atom (NH^+) and its corresponding H-bond acceptor site. However when

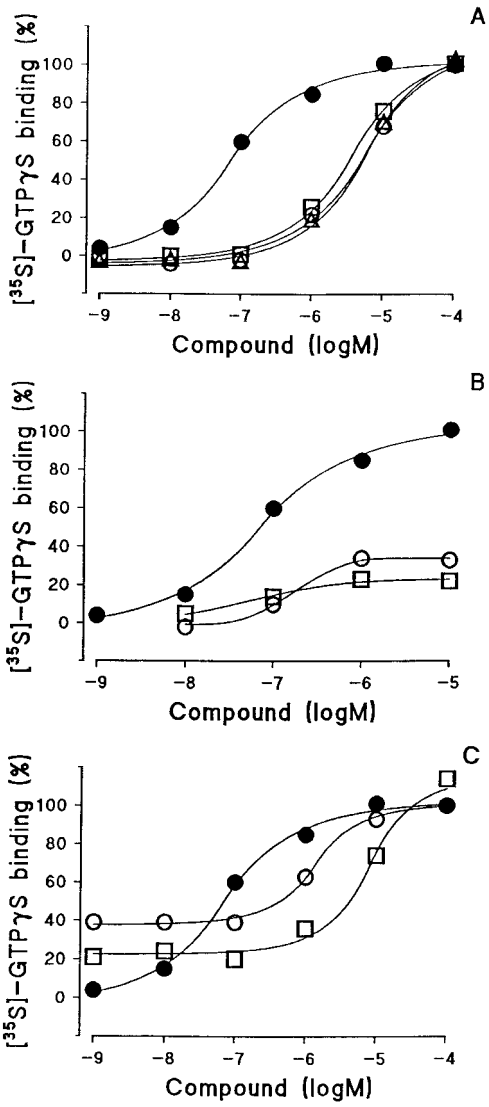


Figure 1. Intrinsic activity and antagonist effects of **2** and its derivatives on h5-HT_{1B} receptor-mediated stimulation of [^{35}S]GTPγS binding to C6-glial cellular membranes. (A) Membranes were preincubated with the indicated concentrations of zolmitriptan in either the absence (●) or presence of 1 μM **2** (○), **3a** (□), or **3c** (△) before [^{35}S]GTPγS binding was performed. (B) Membranes were incubated with the indicated concentrations of either zolmitriptan (●), **3b** (○), or **3d** (□) for [^{35}S]GTPγS binding. (C) Membranes were preincubated with the indicated concentrations of zolmitriptan in either the absence (●) or presence of 10 μM **3b** (○) or **3d** (□) before [^{35}S]-GTPγS binding was performed. Stimulation of [^{35}S]GTPγS binding is expressed as a percentage of that obtained with 10 μM 5-HT. Typical concentration binding curves are shown. Intrinsic activity, EC_{50} values, and K_B values of 2−4 independent experiments are summarized in Table 2.

the restriction for the overlapping of the NH^+ centers was dropped, several solutions were found, the best hypothesis having a tolerance of 0.7 Å. The molecules superimposed two by two (**3a** and **3c**, **3b** and **3d**), with a near orthogonality between the two projections of the H-bond donor (NH^+) to the binding site within the two groups (Chart 3B). The energy difference above the absolute minimum energy of the superimposed conformation was low for both **3b** (0.9 kcal/mol) and **3d** (1.2 kcal/mol) and high for the two neutral antagonists **3a** (3 kcal/mol) and **3c** (3.3 kcal/mol). In this hypothesis, it was clearly impossible for one molecule of one class to adopt the conformation of the other class model due

Table 2. Intrinsic Activity and Potencies of **2** and Its Derivatives for Antagonism of h5-HT_{1B} Receptor-Mediated Stimulation of [^{35}S]GTPγS Binding[a]

compd	h5-HT_{1B} receptor intrinsic activity (1 μM, %[b])	zolmitriptan EC_{50} (nM)	K_B (nM)
2	-3.7 ± 3.8	3000 ± 283	21.3 ± 2.1
3a	4.0 ± 10.5	2850 ± 495	22.7 ± 4.0
3b	32.3 ± 2.9	837 ± 612[c]	
3c	2.3 ± 6.7	3300 ± 424[c]	19.4 ± 2.6
3d	23.0 ± 0.0	4300 ± 424	

[a] Values are presented as mean $\pm$SD of 2−4 independent experiments, each one performed in triplicate. Antagonism of the zolmitriptan-mediated response (EC_{50} = 60 nM) was tested at 1 μM (**2**, **3a**,**c**) or 10 μM (**3b**,**d**). [b] Versus 10 μM 5-HT. [c] EC_{50} values are corrected for intrinsic activity.

Chart 2

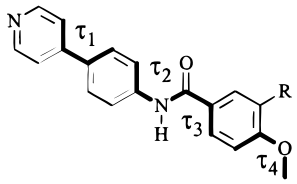


Table 3. Conformational Analysis of Compounds **3a**−**d**

compd	no. of torsion angles	no. of conformations	energy range
3a	4	68	8.4−13.4
3b	2	14	10.6−11.7
3c	3	44	12.8−16.9
3d	3	44	12.9−16.5

to the steric hindrance of the ortho methoxy group which induced different orientations for the alkylamino chain.

Discussion

Compound **2** and its derivatives display selective binding affinity for the h5-HT_{1B} receptor in contrast to the highly potent but nonselective binding affinity of **1** for h5-HT_{1B} and h5-HT_{1D} receptors.[14] The functional GTPγS data clearly show that **2** did neither stimulate nor inhibit [^{35}S]GTPγS binding in the investigated recombinant C6-glial cell line; it competitively antagonized the zolmitriptan-mediated response. Therefore, **2** can be defined as a neutral antagonist of h5-HT_{1B} receptors. Its antagonist potency correlates with its binding affinity and the antagonist potency previously determined in a cAMP-mediated assay in recombinant HeLa cells.[17] This suggests **2** differs from **1** inasmuch as this latter compound has been shown to share mixed agonist−antagonist properties.[14] The reported pharmacological properties of **2** and its *O*-methyl derivative **3a** are very similar especially when considering intrinsic activity at the h5-HT_{1B} receptor subtype (Table 2): both compounds have no intrinsic activity and thus can be considered as neutral antagonists, and both of them antagonize h5-HT_{1B} receptor-mediated stimulation of [^{35}S]GTPγS binding by zolmitriptan with equal potencies (K_B of 21.3 and 22.7 nM, respectively). Considering the comparison between the propylamino derivative **3a** with the conformationally restricted derivatives **3b**−**d**, at the binding level, it can be concluded that all four compounds bind with nearly the same affinity at the h5-HT_{1B} receptor subtype supported by the data obtained with [^{3}H]GR-125743 displacement. The low affinity for cloned h5-HT_{1D} and h5-HT_{1A} receptor subtypes is another common property for these derivatives. However, a totally different conclusion arises from analysis of intrinsic activity data at h5-HT_{1B} receptors: the results reported in Table 2 clearly show that the saturated derivative **3a** together with the (*Z*)-alkene derivative **3c** act as neutral, potent antagonists (in a very similar way to **2**) while the alkyne derivative **3b** as well as the (*E*)-alkene derivative **3d** show nonnegligible intrinsic activity which is also reflected by their poor ability to antagonize zolmitriptan.

By employing molecular modeling techniques the difference in intrinsic activity between the two pairs of *O*-methyl analogs could be visualized by conformational analysis and superimposition. When the common triaryl portions of the molecules are overlapped, the partial agonists and the neutral antagonists differ by a near-orthogonal orientation of the NH^+ projection to the hydrogen-bond acceptor site. In the saturated analog **3a** and the *Z*-unsaturated analog **3c**, the NH^+ is positioned above the benzene plane due to the steric hindrance between the alkylamino chain and the ortho OMe substituent. However, for the (*E*)-olefin **3d**, this steric hindrance is lowered and a favorable electronic conjugation of the double bond with the aromatic ring will orient the NH^+ in the plane of the benzene. The very rigid acetylenic analog **3a** must keep its NH^+ close to the benzene plane.

The observation that compounds **3a**−**d** have almost the same affinity for the cloned h5-HT_{1B} receptor subtype suggests that all four derivatives bind in a very similar way to the receptor. However, the results obtained at the functional level by using the GTPγS binding assay suggest that both compounds **3a**,**c** (which appear as neutral antagonists) selectively stabilize an inactive conformation of the receptor (G-protein independent binding mode), according to the "allosteric ternary complex model".[25] On the other hand, it can be assumed that compounds **3b**,**d** (characterized as partial agonists) simultaneously stabilize both the uncoupled (inactive) form of the receptor and the active conformation which allows coupling to the G-protein. This hypothesis would explain the partial agonist properties of these two compounds.

Altogether, the results discussed above suggest that the intrinsic activity, as measured by GTPγS binding, of 5-HT_{1B} ligands such as **3a**−**d** is likely related to the conformation of the basic amino-side chain. Interestingly, replacing the piperazine ring of **1** with a more flexible amino side chain has recently been reported to modulate intrinsic activity at 5-HT_{1B} receptors.[26] For example, the [(dimethylamino)ethoxy]phenyl derivative **10** (SB-216641; Chart 4) has been reported as a partial agonist,[27] while the [(dimethylamino)propyl]phenyl derivative **11** (Chart 4) possesses negative efficacy at the 5-HT_{1B} receptor and could be therefore characterized as an inverse agonist.[26] These results together with the studies reported in this paper demonstrate the importance of the spatial orientation of this basic amino residue when designing neutral 5-HT_{1B} antagonists.

Experimental Section

Chemical Synthesis. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl, and dichloromethane was distilled from calcium hydride, both under nitrogen. All reactions were performed under positive nitrogen atmosphere, in anhydrous solvents (unless otherwise stated). Pyrrolidine

Chart 3

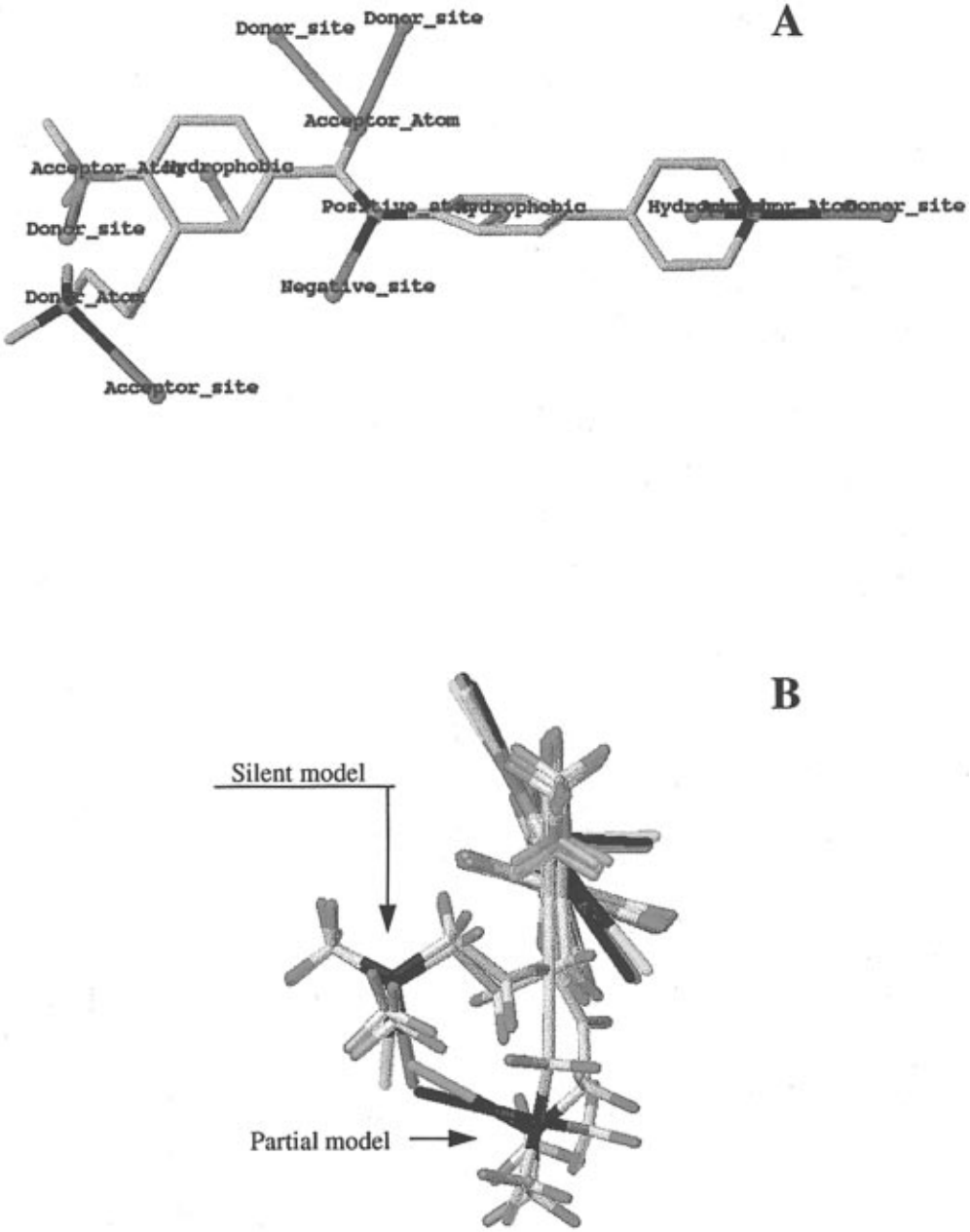
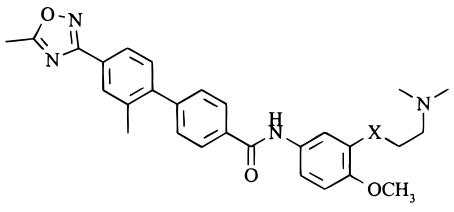


Chart 4



10: X=O (SB-216641)
11: X=CH_2 (SB-219085)

was distilled over KOH prior to use. All others chemicals were used directly as received. Hydrogenations were carried out in a Parr 3911 hydrogenation apparatus. Analytical thin layer chromatography (TLC) was performed on Merck glass-backed silica gel 60 F 254 plates. All flash chromatography was done with SDS silica gel 60 ACC (35−70 μm, pH 7) using a gradient eluent system. ^{1}H NMR (200, 400 MHz) were recorded respectively on Brucker AC-200 and Brucker DPX-400 spectrometers. When peak multiplicity is reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), p (pentet), m (multiplet), br (broad), dd (doublet of doublets), and dt (doublet of triplets). Infrared spectra were recorded on a Nicolet 510P FT-IR spectrophotometer as solid dispersion in KBr or film and are reported in cm^{-1}. Elemental analyses were performed on a Fison EA 1108 CHN analyzer, and determined values are within 0.4% of theory. Mass spectra were recorded on a Nermag R10-10B spectrometer at Toulouse by the Laboratory of Mass Spectra at Paul Sabatier University using the chemical ionization method (NH_3) with the molecular ion as (MH^+). Melting points were measured on a Electrothermal 9200 instrument and are uncorrected.

Methyl 3-[3-(*N*,*N*-Dimethylamino)prop-1-ynyl]-4-methoxybenzoate (4b). Methyl 3-iodo-4-methoxybenzoate (5 g, 17.1 mmol), pyrrolidine (2.8 mL, 34.2 mmol), and tetrakis-(triphenylphosphine)palladium(0) (990 mg, 0.85 mmol) were dissolved in THF (34 mL). In a separate flask 1-(*N*,*N*-dimethylamino)-2-propyne (3.1 mL, 29 mmol) was reacted with copper iodide (320 mg, 1.7 mmol) in 34 mL of THF and then transferred dropwise into the first solution. The reaction mixture was heated to 60 °C for 1 h and brought back to 20 °C where the stirring was continued for 15 h. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (1:49:0.5 to 1:24:0.5 MeOH:CH_2Cl_2:

NH_4OH gradient) to give the title compound (4.28 g, 100%): ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.0−7.85 (m, 2H), 7.17 (d, 1H, J = 8 Hz), 3.89 (s, 3H), 3.82 (s, 3H), 3.46 (brs, 2H), 2,23 (s, 6H); Mass (MH^+ = 248); IR (film) 1723 (CO); R_f = 0.45 (95:5:0.5 CH_2Cl_2:MeOH:NH_4OH). Anal. ($C_{14}H_{17}NO_3 \cdot 0.25H_2O$) C, H, N.

3-[3-(*N*,*N*-Dimethylamino)prop-1-ynyl]-4-methoxybenzoic Acid (5b). To a solution of **4b** (1.5 g, 6.2 mmol) in THF (13 mL) under normal atmosphere was added dropwise a 1 M aqueous solution of NaOH (2 equiv, 12.4 mmol). The reaction mixture was stirred until total consumption of **4b** as measured by TLC; then carefully the reaction was quenched with 1 N HCl (2 equiv) and the mixture concentrated. Water was coevaporated with toluene. The residue obtained was triturated in ethanol and filtered. This operation was repeated with methanol to obtain the title compound as a solid in 100% yield (1.49 g): ^{1}H NMR (200 MHz, DMSO-d_6) δ 13.87 (brs, 1H, COOH), 11.25 (brs, 1H, NH^+), 8.05−7.90 (m, 2H), 7.21 (d, 1H, J = 9 Hz), 4.31 (s, 2H), 3.91 (s, 3H), 2.83 (s, 6H); IR (KBr) 1716 (CO); R_f = 0.1 (90:10:1 CH_2Cl_2:MeOH:NH_4OH). Anal. ($C_{13}H_{16}NO_3 \cdot 0.9H_2O \cdot 1.15HCl$) C, H, N.

3-[(1*Z*)-3-(*N*,*N*-Dimethylamino)prop-1-enyl]-4-methoxybenzoic Acid (5c). A suspension of **5b** (1.49 g, 6.38 mmol) and palladium(0) (5% on charcoal, 0.02 equiv) in DMF (18 mL) and ethanol (18 mL) was hydrogenated for 80 h at a starting pressure of 50 psi of H_2. The reaction mixture was filtered over a Celite bed, and the solvents were removed under reduced pressure. Crude **5c** (1.94 g, oil) was used in the remaining synthesis without further purification: ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.81−7.76 (m, 2H), 6.88 (d, 1H, J = 8.4 Hz), 6.57 (brd, 1H, J = 11.8 Hz), 5.68 (dt, 1H, J = 5.8, 11.8 Hz), 3.79 (s, 3H), 3.06 (brd, 2H, J = 6 Hz), 2,11 (s, 6H).

Methyl 3-[(1*E*)-3-(*N*,*N*-Dimethylamino)prop-1-enyl]-4-methoxybenzoate (4d). A suspension of **4b** (6.34 g, 25.6 mmol) and palladium(0) (5% on charcoal, 0.02 equiv) in methanol (86 mL) and glacial acetic acid (2.2 mL) was hydrogenated for 5 days at a starting pressure of 50 psi of H_2. The reaction mixture was filtered over a Celite bed, and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography (5:95:0.5 MeOH:CH_2Cl_2:NH_4OH) to give the title compound (3.81 g, 59%, oil): ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.03 (d, 1H, J = 2.1 Hz), 7.87 (dd, 1H, J = 2.1, 8.6 Hz), 7.13 (d, 1H, J = 8.7 Hz), 6.77 (brd, 1H, J = 16 Hz), 6.30 (dt, 1H, J = 16, 6.5 Hz), 3.90 (s, 3H), 3.83 (s, 3H), 3.09 (brd, 2H, J = 6.4 Hz), 2.21 (s, 6H), 1.92 (s, AcOH); Mass (MH^+ = 250); R_f = 0.3 (95:5:0.5 CH_2Cl_2:MeOH:NH_4OH). Anal. ($C_{14}H_{19}NO_3 \cdot CH_3COOH \cdot 0.4CH_2Cl_2$) C, H, N.

3-[(1*E*)-3-(*N*,*N*-Dimethylamino)prop-1-enyl]-4-methoxybenzoic Acid (5d). To a solution of **4d** (3.88 g, 15.6 mmol) in THF (31 mL) under normal atmosphere was added dropwise a 1 M aqueous solution of NaOH (2 equiv, 31 mmol). The reaction mixture was stirred until total consumption of **4d** as measured by TLC; then the reaction was carefully quenched with 1 N HCl (31 mL) and the mixture concentrated. Water was coevaporated with toluene. The residue obtained was triturated in ethanol and filtered. This operation was repeated with methanol to obtain the title compound as a solid in 93% yield (3.39 g): ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.27 (d, 1H, J = 1.9 Hz), 7.85 (dd, 1H, J = 2.1, 8.6 Hz), 7.08 (d, 1H, J = 8.7 Hz), 6.87 (brd, 1H, J = 16 Hz), 6.35 (dt, 1H, J = 16, 6.5 Hz), 3.88 (s, 3H), 3.35 (brd, 2H, J = 6.6 Hz), 2.39 (s, 6H); Mass (MH^+ = 236); R_f = 0.1 (90:10:1 CH_2Cl_2:MeOH:NH_4OH).

***N*-(*tert*-Butoxycarbonyl)-4-bromoaniline (6).** 4-Bromoaniline (13.0 g, 76 mmol) and di-*tert*-butyl dicarbonate (19.8 g, 91 mmol) were dissolved in toluene (380 mL) and heated at 70 °C for 15 h. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate and washed successively with HCl (0.1 N) and brine. The organic phase was dried over magnesium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography (5:95 to 10:90 EtOAc:petroleum ether (PE) gradient) to give the title compound (20.5 g, 100%): ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.49 (s, 1H), 7.42 (s, 4H), 1.47 (s, 9H); IR (KBr) 1709 (CO); R_f = 0.3 (95:5 PE:EtOAc). Anal. ($C_{11}H_{14}BrNO_2$) C, H, N.

(Pyridin-4-yl)boronic Acid (7). A stock solution of 4-bromopyridine in ether was obtained upon washing of its hydrochloride salt with cold NaOH (1 M in ice−water) and extraction with cold ether (0 °C). This solution can be stored for a few days over magnesium sulfate under nitrogen in the freezer (−10 °C). Its titer can be measured by gravimetric titration following the procedure described by Murray and Langham.[28]

A solution of 4-bromopyridine (0.63 M in ether, 110 mL, 70 mmol) was cooled to −78 °C and added to a cold solution (−78 °C) of *n*-BuLi (1.6 M in hexane, 52.5 mL, 84 mmol) in ether (225 mL). The reaction mixture was stirred a further 20 min at −78 °C prior to the addition of triisopropyl borate (21 mL, 91 mmol) and warmed to 20 °C over 1 h. After 15 h at room temperature, the reaction was quenched with 50 mL of water, and the organic layer was extracted with 100 mL of NaOH (0.5 M). The combined aqueous phases were washed with ether and acidified carefully to pH 6 (using a pH meter) with HCl (2 N). The title compound precipitated and was collected by a simple filtration (5.69 g, 65% yield): ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.52 (brs, 2H), 7.63 (brs, 2H). Anal. ($C_5H_6BNO_2$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-4-(pyridin-4-yl)aniline (8).** The reaction was carried out under argon. To a solution of **6** (17 g, 62 mmol) and **7** (7.67 g, 62 mmol) in 1,2-dimethoxyethane (700 mL) was added a solution of potassium *tert*-butoxide (61.9 g, 552 mmol) and tetrakis(triphenylphosphine)palladium(0) (4.33 g, 3.7 mmol) in water (276 mL). The reaction mixture was stirred at 84 °C for 8 h, then cooled, and evaporated. The residue was dissolved in dichloromethane, washed with NaOH (0.1 N), dried over magnesium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography (1:99:0.4 to 1:49:0.4 MeOH:CH_2Cl_2:NH_4OH gradient) to give the title compound (10.2 g, 61%): ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.56 (brd, 2H, J = 5.9 Hz), 7.73 (d, 2H, J = 8.7 Hz), 7.65 (d, 2H, J = 6.2 Hz), 7.58 (d, 2H, J = 8.6 Hz), 1.47 (s, 9H); IR (KBr) 1719 (CO); R_f = 0.3 (95:5:0.5 CH_2Cl_2:MeOH:NH_4OH). Anal. ($C_{16}H_{18}N_2O_2$) C, H, N.

4-(Pyridin-4-yl)aniline (9). *N*-(*tert*-Butoxycarbonyl)-4-(pyridin-4-yl)aniline (**8**; 4.1 g, 15 mmol) was treated with a solution of 1 M HCl in methanol at 20 °C for 2 days. The reaction mixture was concentrated, and the residue was charged on a short alumina column. Elution with 10% methanol in dichloromethane allowed the removal of the impurity. Then the column was washed with a more polar eluent (9:90:1 MeOH:CH_2Cl_2:NH_4OH) to give the title compound as the free base (1.71 g, 57%): ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.46 (dd, 2H, J = 1.6, 4.7 Hz), 7.63−7.49 (m, 4H), 6.61 (brd, 2H, J = 8.6 Hz), 5.51 (s, 2H, NH_2); Mass (MH^+ = 171). R_f = 0.3 (95:5:0.5 CH_2Cl_2:MeOH:NH_4OH).

3-[3-(*N*,*N*-Dimethylamino)prop-1-ynyl]-4-methoxy-*N*-[4-(pyridin-4-yl)phenyl]benzamide (3b). Carboxylic acid **5b** (0.5 g, 1.85 mmol), aniline **9** (682 mg, 2.7 mmol), EDCI (389 mg, 2.1 mmol), triethylamine (785 μL, 5.6 mmol), and 4-(dimethylamino)pyridine (0.1 equiv) were dissolved in THF (10 mL) and dichloromethane (20 mL) and stirred at 20 °C for 72 h. The solvents were removed under reduced pressure, and the residue was dissolved in dichloromethane and washed with 1 M NaOH. The organic phase was dried over magnesium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography (4:96:0.4 to 10:90:0.4 MeOH:CH_2Cl_2:NH_4OH gradient) to give the title compound (317 mg, 45%). The fumarate salt was prepared by addition of 1 equiv of fumaric acid in hot methanol and precipitation of the salt at 0 °C: ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.61 (d, 2H, J = 5.9 Hz), 8.06 (d, 1H, J = 2.2 Hz), 8.02 (dd, 1H, J = 2.3, 8.7 Hz), 7.95 (d, 2H, J = 8.8 Hz), 7.84 (d, 2H, J = 8.8 Hz), 7.72 (brd, 2H, J = 5.5 Hz), 7.21 (d, 1H, J = 8.7 Hz), 6.61 (s, 3H, fumarate), 3.92 (s, 3H), 3.55 (s, 2H), 2.31 (s, 6H); Mass (MH^+ = 386); IR (film) 1716, 1677 (CO); R_f = 0.36 (92:8:0.2 CH_2Cl_2:MeOH:NH_4OH); mp = 193 °C. Anal. ($C_{24}H_{23}N_3O_2 \cdot 1.5C_4H_4O_4$) C, H, N.

3-[(1*Z*)-3-(*N*,*N*-Dimethylamino)prop-1-enyl]-4-methoxy-*N*-[4-(pyridin-4-yl)phenyl]benzamide (3c). Carboxylic acid **5c** (1.5 g, 6.3 mmol), aniline **9** (1.8 g, 9 mmol), EDCI (1.34 g, 7 mmol), triethylamine (2.7 mL, 19 mmol), and 4-(dimethylamino)pyridine (0.1 equiv) were dissolved in THF (30 mL) and dichloromethane (30 mL) and stirred at 20 °C for 72 h. The

solvents were removed under reduced pressure, and the residue was dissolved in dichloromethane and washed with 1 M NaOH. The organic phase was dried over magnesium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography (4:96:0.4 to 10:90:0.4 MeOH:CH_2Cl_2:NH_4OH gradient) to give the title compound (621 mg, 25%). The fumarate salt was prepared by addition of 1 equiv of fumaric acid in hot methanol and precipitation of the salt at 0 °C: ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.3 (s, 1H), 8.60 (d, 2H, J = 5.9 Hz), 8.05−7.75 (m, 6H), 7.70 (d, 2H, J = 6 Hz), 7.17 (d, 1H, J = 8.8 Hz), 6.70 (d, 1H, J = 11.9 Hz), 6.56 (s, 3H, fumarate), 5.86 (dt, 1H, J =11.9, 6 Hz), 3.87 (s, 3H), 3.36 (d, 2H, J = 6 Hz), 2.33 (s, 6H); Mass (MH^+ = 388); R_f = 0.25 (92:8:0.1 CH_2Cl_2:MeOH:NH_4OH); mp = 196 °C. Anal. ($C_{24}H_{25}N_3O_2 \cdot 1.5C_4H_4O_4 \cdot 1.25H_2O$) C, H, N, H_2O.

3-[(1*E*)-3-(*N*,*N*-Dimethylamino)prop-1-enyl]-4-methoxy-*N*-[4-(pyridin-4-yl)phenyl]benzamide (3d). Carboxylic acid **5d** (3.36 g, 14.3 mmol), aniline **9** (3.8 g, 18.6 mmol), EDCI (3 g, 15.7 mmol), triethylamine (6 mL, 43 mmol), and 4-(dimethylamino)pyridine (0.1 equiv) were dissolved in THF (70 mL) and dichloromethane (70 mL) and stirred at 20 °C for 12 h. The solvents were removed under reduced pressure, and the residue was dissolved in dichloromethane and washed with 1 M NaOH. The organic phase was dried over magnesium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography (4:96:0.4 to 10:90:0.4 MeOH:CH_2Cl_2:NH_4OH gradient) to give the title compound (1.1 g, 20%). The fumarate salt was prepared by addition of 1 equiv of fumaric acid in hot methanol and precipitation of the salt at 0 °C: ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.60 (brd, 2H, J = 6.2 Hz), 8.15 (d, 1H, J = 2 Hz), 8.00−7.80 (m, 5H), 7.71 (brd, 2H, J = 6.2 Hz), 7.17 (d, 1H, J = 8.7 Hz), 6.92 (d, 1H, J = 16 Hz), 6.56 (s, 3H, fumarate), 6.45 (dt, 1H, J = 16, 6.6 Hz), 3.9 (s, 3H), 3.43 (d, 2H, J = 6.7 Hz), 2.44 (s, 6H); Mass (MH^+ = 388); IR (film) 1716, 1671 (CO); R_f = 0.36 (92:8:0.2 CH_2Cl_2:MeOH:NH_4OH); mp = 220 °C. Anal. ($C_{24}H_{25}N_3O_2 \cdot 1.5C_4H_4O_4$) C, H, N, H_2O.

3-[3-(*N*,*N*-Dimethylamino)propyl]-4-hydroxy-*N*-[4-(pyridin-4-yl)phenyl]benzamide (2). 3-[3-(*N*,*N*-Dimethylamino)propyl]-4-methoxy-*N*-[4-(pyridin-4-yl)phenyl]benzamide (**3a**; 840 mg, 2.16 mmol) was dissolved in dichloromethane (14 mL) and cooled to −78 °C. Boron tribromide (1 M in CH_2Cl_2, 8.6 mL, 8.6 mmol) was added, and the reaction was allowed to warm progressively to 20 °C. A precipitate appeared. The stirring was continued for 20 h, after which methanol (3 mL) was added dropwise. The title compound was filtered and washed thoroughly with dichloromethane to give the title compound as a pale yellow solid (616 mg, 62%): ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.79 (d, 2H, J = 6.4 Hz), 8.36 (d, 2H, J = 6.5 Hz), 8.04 (s, 4H), 7.84 (d, 1H, J = 1.8 Hz), 7.77 (dd, 1H, J = 2, 8.3 Hz), 6.91 (d, 1H, J = 8.4 Hz), 3.20 (m, 2H), 2.91 (s, 6H), 2.79 (t, 2H, J = 7.6 Hz), 2.10 (p, 2H, J = 7.6 Hz); Mass (MH^+ = 375); R_f = 0.2 (95:5:0.5 CH_2Cl_2:MeOH:NH_4OH); mp > 240 °C. Anal. ($C_{23}H_{25}N_3O_2 \cdot 2HBr \cdot 0.6H_2O$) C, H, N, HBr, H_2O.

Biological Methods. Radioligand binding experiments were performed on membrane preparations of the HeLa/HA7 cell line for h5-HT_{1A} receptors, Cos-7 cells transfected with h5-HT_{1D} receptors, and the C6-glial cell line stably transfected with h5-HT_{1B} receptors as previously described.[18] [^{35}S]GTPγS binding to membrane preparations of C6-glial/h5-HT_{1B} cells was performed in glass tubes and consisted of 0.4 mL of membrane preparation (20−120 μg of protein) and 0.05 mL of compound in either the absence or presence of antagonist in 20 mM Hepes (pH 7.4) supplemented with 30 μM GDP, 100 mM NaCl, 3 mM $MgCl_2$, and 0.2 mM ascorbic acid. After an incubation period of 30 min at 25 °C, 0.05 mL of [^{35}S]GTPγS (500 pM) was added for an additional period of 30 min. The reactions were stopped as previously described.[18] Maximal stimulation of [^{35}S]GTPγS binding was defined in the presence of 10 μM 5-HT. E_{max} values are expressed as a percentage of the maximal response obtained with 10 μM 5-HT. EC_{50} values are expressed as the concentration of compound at which 50% of their own maximal stimulation was obtained. For antagonist studies, concentration ratios were calculated and used to obtain estimates of pA_2 values using the following equation: pA_2 = log(concentration − 1) − log(antagonist concentration). Radioligands were obtained from New England Nuclear (Les Ulis, France) or Amersham (Les Ulis, France).

Molecular Modeling. Molecular modeling was performed on a Silicon Graphics Indigo workstation using the Sybyl Molecular Modeling Software, version 6.3, from Tripos Associates, Inc., St. Louis, MO. Optimization by energy minimization was done with the Tripos force field including the electrostatic term calculated from Gasteiger and Marsili atomic charges. The BFGS method was used for minimization (convergence at 0.001 kcal/mol). Compound **3b** was submitted to the Gridsearch option from Sybyl with stepwise rotation of 20° around the two alkylamine torsion angles which gave 361 conformations and then classified in 14 conformational families using a cluster analysis method from family spl macro (written by M. Neuwels and E. Vande Water from UCB SA). The other analogs having three or four alkylamine torsion angles were submitted to the Randomsearch option from Sybyl with maximum cycles at 1000, energy cutoff at 70 kcal/mol, rms threshold at 0.2 Å, and convergence threshold at 0.005. This option located energy minima by randomly adjusting the chosen torsion angles, minimizing the energy of the resulting geometry, and eliminating duplicates (<5 kcal/mol above the absolute minimum energy). The three-dimensional pharmacophore mapping was generated with the Disco module from Sybyl with default values of disco file2.dat option. Searchs were generated using **3b** as the reference compound, detailed by class with a minimum of 13 feature requirements to fulfill the overall fitting (distance tolerance 0.5−1.0 Å, increment 0.1 Å).

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