Brief Articles

New Selective and Potent 5-HT$_{1B/1D}$ Antagonists: Chemistry and Pharmacological Evaluation of *N*-Piperazinylphenyl Biphenylcarboxamides and Biphenylsulfonamides

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A series of new analogues of *N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl] 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxamide (**1**; GR127935) as potent and selective 5-HT$_{1B/1D}$ antagonists were synthesized and evaluated pharmacologically. Their receptor binding profiles were comparable to that of **1**. The 1,3,4-oxadiazole isomer **2** and the 4′-aminocarbonyl and 4′-amidinyl analogues (**9** and **10**) of **1** had higher affinities at the rat 5-HT$_{1B}$ receptor (IC$_{50}$ = 0.93, 1.3, and 0.5 nM, respectively) and calf 5-HT$_{1D}$ receptor (IC$_{50}$ = 37, 10, and 3 nM, respectively) than did **1** (1.6 and 52 nM for rat 5-HT$_{1B}$ and calf 5-HT$_{1D}$ receptors, respectively). In the functional in vitro testing of 5-HT$_{1B/1D}$ antagonistic properties, **2**, **9**, **10**, **11b** (*O*-demethylated derivative of **2**), **13a** (*O*-methylsulfonyl analogue of **2**), and **16** (which differs from **2** with a sulfonamide linker) showed more pronounced effects in the K$^+$-induced 5-HT release in the cortex of guinea pig than did **1** and **3** (SB224289). Compounds **2**, **9,** and **10** were equally potent as **1** in rabbit saphenous vein model (pA_2 > 9). A biochemical study of **2** with in vivo microdialysis in the rat brain showed that it is capable of augmenting citalopram (a selective serotonin reuptake inhibitor, SSRI) induced 5-HT release in rat ventral hippocampus, while preventing the decrease in acetylcholine release elicited by citalopram administration. The molecular structure of **2** was determined by single-crystal X-ray analysis. The log *P* and log *D* values of these compounds were calculated. This study contributes to the SAR study of *N*-piperazinylphenyl biphenylcarboxamides as selective and potent 5-HT$_{1B/1D}$ antagonists.

Introduction

The serotonin 5-HT$_{1B}$ receptor was first found in rat brain and the 5-HT$_{1D}$ receptor in bovine caudate nucleus.[1] Molecular biological studies demonstrated that the human and rabbit 5-HT$_{1D}$ receptors are encoded by a family of two distinct genes, termed initially 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ but now 5-HT$_{1D}$ and 5-HT$_{1B}$, respectively.[2] The rat 5-HT$_{1B}$ (*r*5-HT$_{1B}$) receptor and human 5-HT$_{1B}$ (*h*5-HT$_{1B}$) receptor were found to be species homologues with a single amino acid modification and pharmacologically still different as demonstrated.[3] The 5-HT$_{1D}$ receptor has 63% overall structural homology with the 5-HT$_{1B}$ receptor.[4] Unlike the 5-HT$_{1B}$ receptor subtype, the pharmacology of the cloned 5-HT$_{1D}$ receptor subtype seems conserved among various mammal species such as the human, guinea pig, and rat.[5] However, the bovine 5-HT$_{1D}$ receptor is not yet cloned, and the 5-HT$_{1D}$ receptor in calf caudate membranes was reported to be a mixture of bovine 5-HT$_{1D}$ receptor and the homologous bovine 5-HT$_{1B}$ receptor with the latter as the major constituent.[6,7] The 5-HT$_{1B}$ receptor is concentrated in the basal ganglia, striatum, and frontal cortex. It was reported that the terminal autoreceptor controlling 5-HT release in the rat, guinea pig, and human is of the 5-HT$_{1B}$ type.[4] Low levels of the 5-HT$_{1D}$ receptor mRNA are found in several areas of the rat brain, in particular in the dorsal raphe, which suggests that it may function as an 5-HT autoreceptor.[4] Nevertheless, the 5-HT$_{1B/1D}$ receptors belong to the family of seven transmembrane G-protein-coupled receptors (both negatively coupled to adenylate cyclase) and mediate serotonergic neurotransmission although their precise function remains to be determined.[5,8,9]

The tricyclic antidepressants and the selective serotonin reuptake inhibitors (SSRIs) are believed to increase serotonin transmission by blocking reuptake of the released 5-HT. The clinical effect of these classical drugs only becomes apparent after chronic treatment (2−3 weeks). This delayed onset of therapeutic action of SSRIs is thought to be due to the time required to desensitize somatodendritic 5-HT$_{1A}$ and terminal 5-HT$_{1B}$ autoreceptors. Therefore, the blockade of terminal 5-HT$_{1B}$ receptors by selective antagonists has been proposed[8,10] as a new approach for more efficient and/or fast-acting

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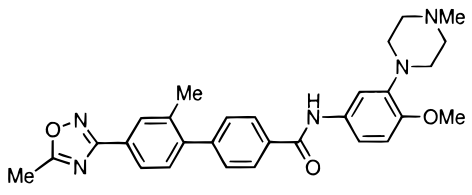
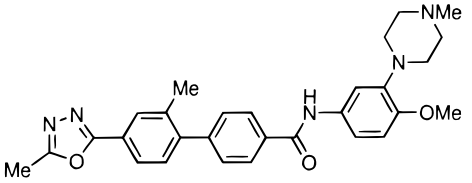
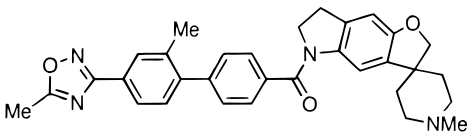
Chart 1. Structures of the Reference Compounds



1, GR127935



2



3, SB-224289

antidepressant drugs, since the acute blockade of these 5-HT autoreceptors will in theory immediately mimic their desensitization. The combination of a SSRI with a 5-HT_{1B} antagonist may thus shorten the onset of the antidepressant effect in depressed patients.[9,11] Recent studies also suggested that the supersensitive 5-$HT_{1B/1D}$ receptors may be involved in the pathophysiology of obsessive compulsive disorders (OCD) and the selective 5-$HT_{1B/1D}$ antagonists may also be potential drugs for its treatment.[12]

Until recently, GR127935 (**1**; Chart 1) was reported to be the first selective and potent 5-$HT_{1B/1D}$ antagonist,[13] which was later described as a "non-silent" antagonist (partial agonist) at human cloned 5-HT_{1B} receptors.[9,14] Following the discovery of **1**, many GR127935-like derivatives and other compounds were patented or reported subsequently as potential 5-$HT_{1B/1D}$ receptor agents.[7,9,11,15,16] The subtle structural modification of **1** generated 5-$HT_{1B/1D}$ agents, which range from partial agonists to full antagonists or inverse agonists. SB224289 (**3**; Chart 1), for instance, which has the same oxadiazole biphenylcarboxamido moiety of **1** on one side with a spiro moiety on the other side of the molecule, has been reported to be a selective *h*5-HT_{1B} inverse agonist. This compound is a potent antagonist of terminal 5-HT autoreceptor function both in vitro and in vivo.[15,17] Microdialysis studies have yielded controversial results concerning the ability of **1** to increase synaptic 5-HT levels. Local perfusion of **1** into the guinea pig frontal cortex increased 5-HT release, whereas the 5-HT release was decreased after systemic administration.[9,18−20] It is unlikely that one can explain the above microdialysis data due only to the choice of the terminal area of those measurements. A reason for this discrepancy might be that **1** is a partial agonist and not a full antagonist at the 5-HT_{1B} receptor. This is supported by the failure of the selective 5-HT_{1B} inverse agonist **3** to effect 5-HT release in the frontal cortex.[17] An additional reason for these differences in results of **1** might be the difference of its affinity to the 5-HT_{1D} in addition to the 5-HT_{1B} receptors. As the 5-HT_{1D} receptors are thought to be located in dorsal raphe nucleus, pharmacological interference with these receptors might explain the discrepancies described above. Combined systemic administration of **1** with sertraline (a SSRI), however, resulted in a pronounced, long-lasting 5-HT increase in guinea pig hypothalamus, indicative of the synergistic effect of this combined pharmacological intervention.[21]

In this study, we present the chemistry and pharmacological evaluation of a series of new structurally modified analogues of GR127935 (**1**) as selective and potent 5-$HT_{1B/1D}$ or 5-HT_{1B} antagonists.

Chemistry

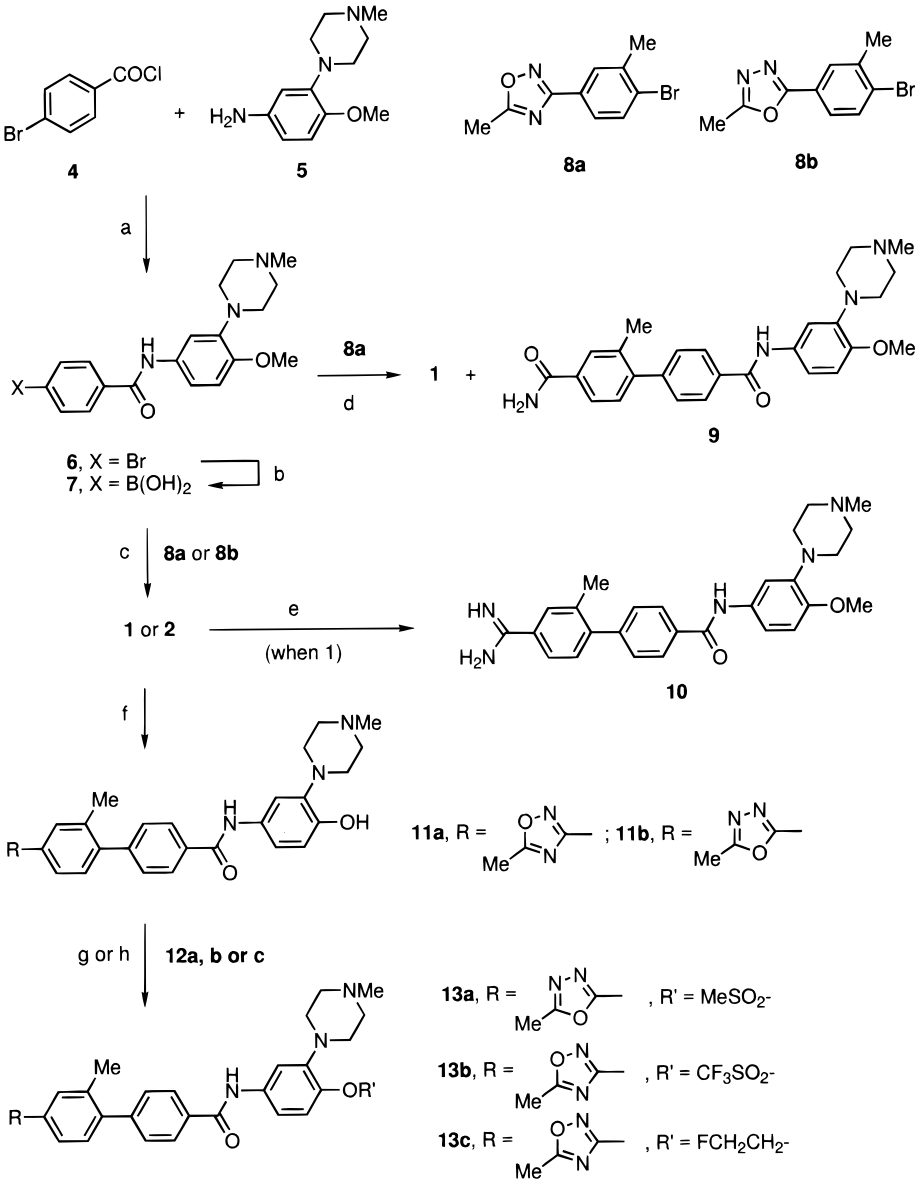
The syntheses of the tested compounds are outlined in Schemes 1 and 2. The precursors **8a**,**b**,[22] compound **1,** and its 1,3,4-oxadiazole isomer **2** were synthesized according to the method reported by Glaxo researchers[13] (i.e. steps a−c in Scheme 1). Incidentally, the application of Suzuki reaction of **8a** and **7** in a refluxing solution of *N*,*N*-dimethylformamide (DMF) instead of 1,2-dimethoxyethane (DME) in the presence of aqueous sodium carbonate (step d in Scheme 1) gave a mixture of **1** and **9**, which was isolated and characterized to be the partially hydrolyzed byproduct of the 1,2,4-oxadiazole moiety of **1**. It was found that the extensive refluxing treatment under these reaction conditions could bring out almost exclusively **9**. No partial hydrolysis of the 1,3,4-oxadiazole moiety of **2** was observed correspondingly.

Hydrogenation of **1** with Raney nickel water slurry in a mixture of methanol and acetic acid (step e in Scheme 1) generated successfully **10**, which has an amidino group instead of the 1,2,4-oxadiazole moiety of **1**. *O*-Demethylations of **1** and **2** were carried out by treatment with aluminum chloride in ethylmercaptan and CH_2Cl_2 at room temperature, affording **11a**,**b**, respectively, which were further converted to their sulfonyloxy derivatives (**13a**,**b**) or the *O*-alkylated analogue **13c** under the conditions shown in Scheme 1 (step f, g, or h).

Compound **16**, which differs from **2** with a sulfonamide linker, was synthesized in the manner outlined in Scheme 2. Compound **4b** reacted with **5** to give **14**, which was converted to its boronic acid derivative **15** and further coupled with **8b** by Suzuki reaction, affording **16** in good total yield.

Attempts of obtaining crystals of **1** and **2** suitable for X-ray studies were successful in the case of **2**, where its crystals grew by slow evaporation from ethanol. Subsequently, the single-crystal X-ray analysis revealed the molecular conformation of **2** (Figure 1), thus permitting the molecular appearance of GR127935-like benzanilide derivatives to be inferred. The basic features of the molecule **2** are as follows: (i) The molecule has an elongated conformation due to a trans configuration at the central amide bond. The torsion angles around the C_1-N_{22} and $N_{22}-C_{23}$ bonds are 179° and 176°, respectively. This makes the amide linker and the phenyl ring bearing the piperazine ring approximately coplanar. (ii) The piperazine ring is in a chair conforma-

Scheme 1[a]



[a] Reagents and conditions: (a) triethylamine, CH_2Cl_2 or THF, 0 °C to rt, overnight; (b) B(O-*i*-Pr)$_3$, *n*-BuLi, −100 to −78 °C, 3 h, then −78 °C to rt, overnight; (c) Pd(PPd$_3$)$_4$, Na_2CO_3, DME−H_2O (1:1), reflux 24 h; (d) Pd(PPh$_3$)$_4$, Na_2CO_3, DMF−H_2O (1:1), reflux 24 h; (e) H_2, Raney-Ni, H_2O, MeOH−AcOH; (f) $AlCl_3$, EtSH−CH_2Cl_2, rt, 4−6 h; (g) triethylamine, CH_2Cl_2, −78 to 0 °C, 2 h, $MeSO_2Cl$ (**12a**) or $(CF_3SO_2)_2O$ (**12b**); (h) $CsCO_3$, CH_3CN, reflux 3 h, FCH_2CH_2Br (**12c**).

tion. The torsion angle of the $C_{25}-N_{31}$ bond ($C_{24}-C_{25}-N_{31}-C_{32}$) connecting the piperazine ring to the benzene ring is 15°. (iii) The torsion angles around the linking bonds $N_{22}-C_1-C_3-C_4$, $C_5-C_6-C_9-C_{14}$, and $C_{13}-C_{12}-C_{16}-N_{20}$ of the linear arranged biphenyl rings and the 1,3,4-oxadiazole ring are −45°, −48°, and 9°, respectively. Other detailed information about the molecular geometry is given as Supporting Information.

The log *P* and log *D* values of this series of compounds were calculated for indicating their lipophilicity and solubility (Table 1). Compounds **3** and **13b**,**c** have log *P* values greater than 5 (6.29, 6.06, and 5.33, respectively), implicating high lipophilicity and thus alerting the likely absorption problems as drugs for oral administration.[23] Compound **1** has a log *P* value (4.90) approaching 5. In comparison with them, **2**, **9**−**11**, **13a,** and **16** have smaller log *P* values and thus are less lipophilic, with **13a** being optimal (log $P = 3.38$). The calculated log *D* values, which are pH-dependent, are more realistic to predict solubility and permeability of a drug. We need: log *D* (at pH = 7.4) > −0.80 for good absorption in the intestine and slow elimination and in an optimal range of 0.00−3.00 for good absorption and good penetration through the blood−brain barrier. Generally, a compound with a log *D* value greater than 4 could have solubility problems and often shows a high plasma protein binding (>98%). This leads to low brain concentration of a drug.[32] As can be seen, log *D* values for **1**, **3**, and **13b**,**c** are greater than 4 and that of **2, 11b, 13a,** and **16** are improved (<4). Compounds **11b** and **13a** have the most favorable log *D* values (3.12 and 2.96, respectively) in this respect. Interestingly, **10** with a basic amidine moiety has a log *D* value (−0.35) approaching the opposite territory due to its high pK_a value (11.98) and is expected to have very good solubility although its ability to penetrate the blood−brain barrier needs to be examined experimentally.

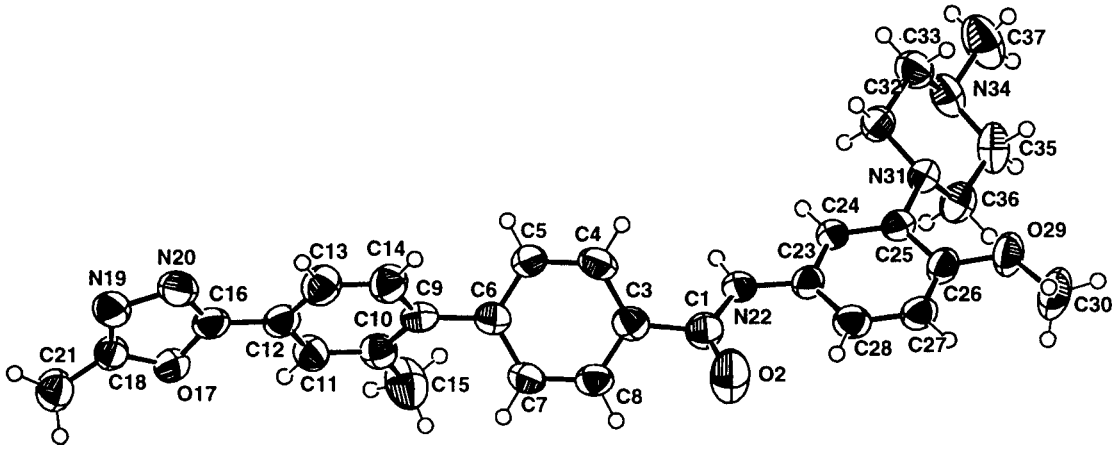
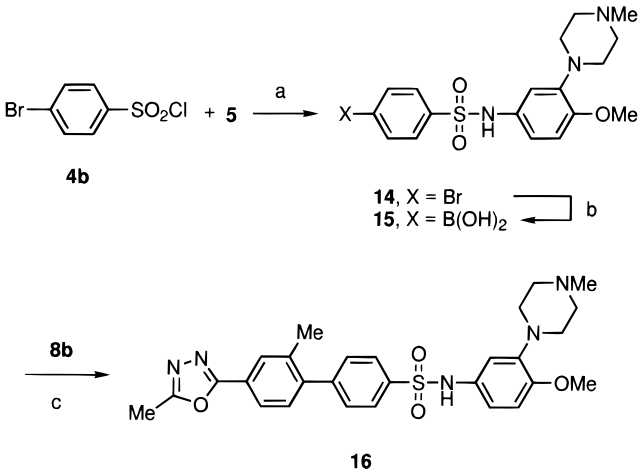


Figure 1. Molecular structure of compound **2**, showing 50% probability thermal ellipsoids for the non-H atoms and the atom-numbering scheme.

Scheme 2[a]



[a] Reagents and conditions: (a) triethylamine, CH_2Cl_2 or THF, 0 °C to rt, overnight; (b) B(O-*i*-Pr)$_3$, *n*-BuLi, −100 to −78 °C, 3 h, then −78 °C to rt, overnight; (c) $Pd(PPd_3)_4$, Na_2CO_3, DME−H_2O (1:1), reflux 24 h.

Pharmacological Results and Discussion

In Vitro Receptor Binding Assays. The compounds were tested in binding assays at the 5-HT_{1B} receptor in rat frontal cortex (*r*5-HT_{1B}), 5-HT_{1D} receptor in calf striatum membranes (*c*5-HT_{1D}), and 5-HT_{1A} receptor in rat hippocampus membranes. As shown in Table 1, **1** had affinities (IC_{50} values) of 52 and 1.6 nM at the 5-HT_{1D} and 5-HT_{1B} receptors, respectively, in our testing system, while **3** showed high affinity only at the 5-HT_{1B} receptor (IC_{50} = 2 nM). Compounds **2**, **9,** and **10** were found to be more potent than **1** at the 5-HT_{1B} and 5-HT_{1D} receptors, with **10** being the most potent ligand to date (IC_{50} = 0.5 and 3 nM for 5-HT_{1B} and 5-HT_{1D} receptors, respectively). Compounds **11a**,**b** and **13c** had decreased affinities at the 5-HT_{1D} receptor (IC_{50} > 400 nM) but comparable affinities at the 5-HT_{1B} receptor (IC_{50} = 7.3, 6.3, and 10 nM, respectively) to that of **1**. Compounds **13a**,**b** and **16** lost affinities at the 5-HT_{1D} receptor (IC_{50} > 700 nM), but **13a** and **16** still retained good affinities at the 5-HT_{1B} receptor (IC_{50} = 70 and 60 nM, respectively).

The reference compound **1** had low affinity (IC_{50} = 425 nM) at the 5-HT_{1A} receptor. Compounds **9** and **10** showed moderate to good affinities at the 5-HT_{1A} receptor (IC_{50} = 57 and 23 nM, respectively), however, these were relatively lower in comparison with their high affinities at the 5-$HT_{1B/1D}$ receptors. Other test compounds had virtually low or no affinities (IC_{50} > 700 nM) at the 5-HT_{1A} receptor.

In the literature, **1** has been reported to display only low affinities at other CNS receptors, like 5-HT_2, α_1, and dopamine DA receptors.[13,15] In testing of the selected compounds at other CNS receptors, **2**, **9**, **10,** and **16** were found to have weak and thus negligible affinities at the 5-HT_{2A}, 5-HT_{2C}, α_1, α_2, and hD_2 receptors (data not presented) as compared to their affinities at the *r*5-HT_{1B} and *c*5-HT_{1D} receptors.

In Vitro Functional Study. For determining the 5-$HT_{1B/1D}$ antagonistic activity, the inhibition of contractile responses elicited by sumatriptan in rabbit saphenous vein (RSV), challenged by test compound, was measured. Compounds **1**, **2**, **9**, **10**, **11b,** and **13a,c** all showed significant effects (pA_2 values > 8.0) as potent 5-$HT_{1B/1D}$ antagonists. Among them, **2**, **9,** and **10** are equally potent as **1** (pA_2 >9.1). Interestingly, **11a**, **16**, and the reported inverse 5-HT_{1B} agonist **3** showed insignificant effects (pA_2 < 7.4) in this model. Considering the binding profiles of these compounds, it appeared that the potent 5-$HT_{1B/1D}$ ligand had a much more pronounced effect than did the selective 5-HT_{1B} ligand in this model.

In testing of [^{3}H]5-HT release to determine the 5-$HT_{1B/1D}$ antagonistic properties, the effect on the sumatriptan-induced inhibition of K^+-evoked [^{3}H]5-HT release from guinea pig cortex slices by test compound was measured. Compound **1** only slightly increased the 5-HT release (114% of the control level), and **3** increased the 5-HT release to 134%. Correspondingly, **2**, **9**, **10**, **11b**, **13a,** and **16** showed much more pronounced effects on the 5-HT release (in a range of 147−166%) with **13a** being the most potent one. Interestingly, not only the potent 5-$HT_{1B/1D}$ ligands such as **2**, **9,** and **10** but also compounds such as **11b**, **13a,** and **16** with low affinities at the 5-HT_{1D} receptor showed such pronounced effects on the 5-HT release.

As mentioned above it could be favorable to combine the potent 5-$HT_{1B/1D}$ antagonist with a 5-HT reuptake inhibitor. To check if such double properties were present in this series of compounds, they were tested for their abilities to inhibit 5-HT reuptake. None of the

Table 1. Calculated log *P* and log *D* Values, Affinities of Compounds at the *r*5-HT$_{1A}$, *r*5-HT$_{1B}$, and *c*5-HT$_{1D}$ Receptors and for 5-HT Reuptake Inhibition, pA_2 Values for the Effects in RSV, and 5-HT Level in K^+-Induced 5-HT Release in Guinea Pig Cortex

		IC$_{50}$, nM[c]					
compd	log *P*[a] (log *D*[b])	*c*5-HT$_{1D}$	*r*5-HT$_{1B}$	*r*5-HT$_{1A}$	5-HT reuptake	pA_2	5-HT release, % of control[d]
1	4.90 (4.48)	52 ± 13.2	1.6 ± 0.7	425 ± 48	1000	9.9	114 ± 12
2	4.11 (3.69)	37 ± 7	0.93 ± 0.07	>1000	>1000	9.1	147 ± 4
3	6.29 (4.64)	>1000	2.0 ± 0.0	>10000	4700	7.4	134 ± 8
9	3.60 (3.18)	10 ± 0.0	1.3 ± 0.3	57 ± 9	>1000	10.0	162 ± 5
10	3.61 (−0.35)	3.0 ± 0.6	0.5 ± 0.06	23 ± 3	407 ± 70	9.6	155 ± 11
11a	4.33 (3.91)	467 ± 88	7.3 ± 1.3	>1000	>1000	7.3	122 ± 12
11b	3.54 (3.12)	400 ± 100	6.3 ± 0.9	>100	4700	8.0	147 ± 4
13a	3.38 (2.96)	>1000	70 ± 20	7000	3800	8.3	166 ± 9
13b	6.06 (5.64)	>1000	700 ± 58	>10000	>1000	NT[e]	NT[e]
13c	5.33 (4.91)	600 ± 252	10 ± 5	5000	900	8.3	107 ± 13
16	4.04 (3.60)	700 ± 58	60 ± 11	700	>1000	7.0	155 ± 4

[a] The log *P* values of the nonionized compounds in octanol/water system were calculated with SRC Logkow 1.50 (Syracus Research Corp.).[33] [b] The log *D* values (at pH 7.4) of the ionized compounds (*in parentheses*) were calculated according to the equation: $\log D = \log[10^{\log P} + 10^{(\log P_{ion} + pK_a - pH)}] - \log[1 + 10^{(pK_a - pH)}]$, in which the p$K_a$ values were calculated with pKa/c 1.2 (Pallas 1.2, Compu Drug Chemistry Ltd.). [c] Results are expressed as IC$_{50}$ values (nM), mean of at least 3 determinations ± SEM, otherwise indicated. [d] For the method description and data calculation, see Experimental Section and refs 24−29. [e] NT, not tested.

tested compounds showed activities for the functional 5-HT reuptake inhibition, except for **10**, which showed weak inhibitory activity (IC$_{50}$ = 407 nM).

In Vivo Microdialysis. Desensitization of the terminal 5-HT$_{1B/1D}$ autoreceptors can be mimicked by co-administration of a 5-HT$_{1B/1D}$ antagonist with a SSRI. The augmentation of the SSRI-induced increase in extracellular 5-HT in terminal area would equal the changes in serotonergic neurotransmission after chronic treatment with the SSRIs. This strategy is believed to induce instantaneous antidepressant effects of a SSRI. In rat ventral hippocampus, compound **2** alone had no effect on the 5-HT level, which is likely indicative of being a silent antagonist. Administration of citalopram (10 μmol/kg sc) increased the 5-HT level to 300%, and co-administration of **2** (10 μmol/kg) and citalopram (10 μmol/kg) sc increased the 5-HT level up to 800%. Compound **2** was indeed capable of augmenting the increase of the 5-HT level induced by citalopram as a SSRI (Figure 2). The postsynaptic effect of SSRI treatment became a focus of interest in recent years. In ventral hippocampus, the 5-HT$_{1A}$ and 5-HT$_3$ receptors are thought to increase the acetylcholine (ACh) release, whereas the 5-HT$_{1B/1D}$ receptors are postulated to inhibit the ACh release.[30,31] Co-administration of **2** and citalopram also prevented the 5-HT-mediated inhibition of ACh release elicited by citalopram administration (Figure 3). This effect is mediated either by blockade of postsynaptic 5-HT$_{1B/1D}$ receptors (inhibiting ACh release) or by augmenting extracellular 5-HT, thereby stimulating other 5-HT receptors to facilitate the ACh release.

In summary, the 1,3,4-oxadiazole analogue **2** of **1** was found to be a very potent and selective 5-HT$_{1B/1D}$ antagonist both in vitro and in vivo. In the 5-HT release model, it showed a more pronounced effect than **1** and **3**. The successful single-crystal X-ray analysis of **2** demonstrated the molecular geometry of GR127935-like benzanilides, which will allow further modeling or QSAR studies by docking its 3D structure to the computational receptor models to better understand the receptor−antagonist interactions and to direct further SAR studies.

Manipulation of the oxadiazole moiety of **1** led to the most potent 5-HT$_{1B/1D}$ receptor ligands, **9** and **10**, with very potent 5-HT$_{1B/1D}$ antagonistic properties functionally not only in the RSV model but also in the 5-HT release model. Manipulations of the methoxy moiety of **1** or **2** dramatically decreased the *c*5-HT$_{1D}$ receptor binding; therefore, **11a**,**b** and **13a,c** appeared to be selective at the *r*5-HT$_{1B}$ receptor. Compounds **11a**,**b** and **13c** displayed the selectivity of affinities at the *r*5-HT$_{1B}$ receptor versus the 5-HT$_{1D}$ receptor in calf striatum membranes to be at least 60-fold. The selective *r*5-HT$_{1B}$ ligands **11b** and **13a** showed very potent antagonistic effects in the 5-HT release model although they are not as highly potent as **1**, **2**, **9,** and **10** in the RSV model. Replacement of the amide linker of **2** by a sulfonamide linker led to **16** which also has a very pronounced effect in the 5-HT release model but not in the RSV model although its affinities at the 5-HT$_{1B/1D}$ receptors decreased comparably.

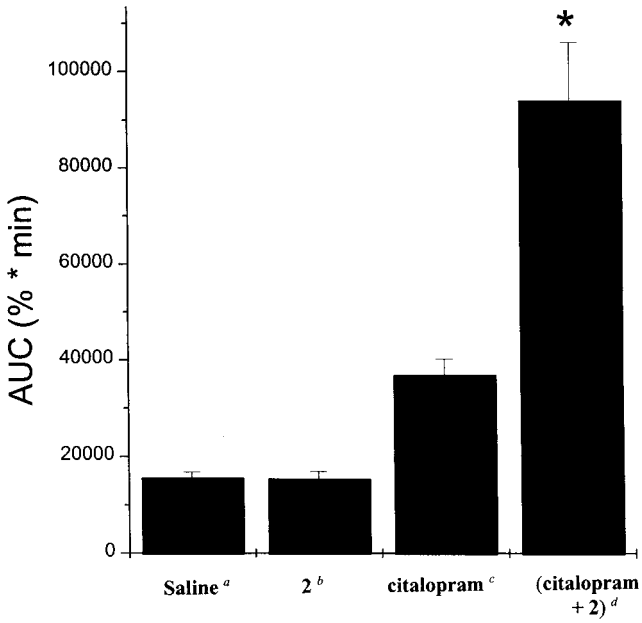


Figure 2. Effect of compound **2** on citalopram-induced increase in hippocampal extracellular 5-HT level (expressed as area under curve, AUC): [a]saline (sc, n = 7), [b]compound **2** (10 μmol/kg sc, n = 4), [c]citalopram (10 μmol/kg sc, n = 7), [d]10 μmol/kg of citalopram and 10 μmol/kg of compound **2** (sc, n = 5); *p < 0.05 (Student's t-test).

In conclusion, a series of new biphenyl derivatives were generated to be selective and even more potent antagonists at either the *r*5-HT$_{1B}$ receptor or both the

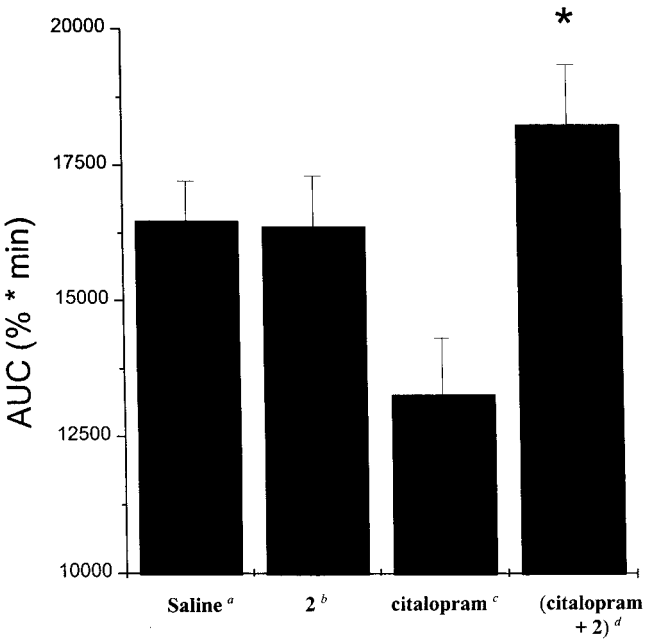


Figure 3. Effect of compound **2** on citalopram-induced decrease in hippocampal extracellular ACh level (expressed as area under curve, AUC; neostigmine 0.1 M): [a]saline (sc, $n = 5$), [b]compound **2** (10 μmol/kg sc, $n = 5$), [c]citalopram (10 μmol/kg sc, $n = 6$), [d]10 μmol/kg of citalopram and 10 μmol/kg of compound **2** (sc, $n = 5$); *$p < 0.05$ (Student's t-test).

r5-HT_{1B} receptor and the c5-HT_{1D} receptor (mainly constituting the homologous bovine 5-HT_{1B} receptor), as compared to GR127935 (**1**) and SB224289 (**3**). Several compounds appear to be interesting for further pharmacological studies to gain SAR information relevant to delineating the *N*-piperazinylphenyl biphenylcarboxamides as selective and potent (partial) agonists or antagonists at the h5-$HT_{1B/1D}$ receptors.

Experimental Section

Chemistry. Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. IR spectra were obtained on a ATI-Mattson spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini 200 and 500 NMR instruments. Chemical shifts are given in δ units (ppm) and relative to TMS or deuterated solvent. Coupling constants (J) are given in Hz. Mass spectra were obtained on a Unicam 610/Automass 150 GC−MS system or on a Finnegan 3300 system. Elemental analyses were performed either in the Analytic Laboratory at Merck KGaA (Darmstadt) or in the Microanalytic Laboratory of the University of Groningen and were within 0.4% of the theoretical values. Merck silica gel (Kieselgel 60, 230−400 mesh) was used for flash chromatography.

***N*-[4-Methoxy-3-(4-methylpiperazin-1-yl)phenyl] 2′-Methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxamide (1) and *N*-[4-Methoxy-3-(4-methylpiperazin-1-yl)phenyl] 2′-Methyl-4′-aminocarbonylbiphenyl-4-carboxamide (9).** 3-(4′-Bromo-3′-methylphenyl)-5-methyl-1,2,4-oxadiazole (**8a**)[22] (1.9 g, 7.5 mmol), the boronic acid intermediate **7** (2.5 g, 6.8 mmol), $Na_2CO_3 \cdot 10H_2O$ (6.3 g), *N*,*N*-dimethylformamide (100 mL), and water (100 mL) were combined and degassed with nitrogen. Tetrakis(triphenylphosphine)-palladium(0) (160 mg) was added to the solution and the mixture was heated to reflux for 1 day, evaporated to remove the solvents, and purified by flash chromatography (CH_2Cl_2/EtOH, 9:1 then 4:1, with adding gradually small amount of 25% aq NH_4OH), affording 0.6 g (18%) of **1** and 2.0 g (67%) of **9**.

1: mp 252−4 °C (EtOAc−EtOH); IR (KBr) 3421, 1663 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.95 (s, 1 H), 8.13 (d, 2 H, J = 8.4 Hz), 7.98 (s, 1 H), 7.93 (dd, 1 H, J = 1.5, 7.7 Hz), 7.70 (dd, 1 H, J = 2.2, 8.8 Hz), 7.44 (d, 2 H, J = 8 Hz), 7.32 (d, 1 H, J = 8 Hz), 7.25 (d, 1 H, J = 2.2 Hz), 6.77 (d, 1 H, J = 9.1 Hz), 3.81 (s, 3 H), 3.60−3.10 (m, 8 H), 2.85 (s, 3 H), 2.67 (s, 3 H), 2.32 (s, 3 H); ^{13}C NMR ($CDCl_3$, 500 MHz, with attached proton tested) δ 176.4 (no H), 168.0 (no H), 165.5 (no H), 148.5 (no H), 144.4 (no H), 143.5 (no H), 138.6 (no H), 135.9 (no H), 133.2 (no H), 131.8 (no H), 130.0 (1 H), 129.2 (1 H), 129.1 (2 C, all with 1 H), 127.4 (2 C, all with 1 H), 125.9 (no H), 124.7 (1 H), 116.8 (1 H), 112.3 (1 H), 111.3 (1 H), 55.6 (3 H), 54.0 (2 C, all with 2 H), 47.3 (2 C, all with 2 H), 43.6 (3 H), 20.3 (3 H), 12.3 (3 H); MS (CI) m/z 498 ($M^+ + 1$). Anal. ($C_{29}H_{31}N_5O_3 \cdot 0.25H_2O$) C, H, N.

9 (converted to hydrochloride): mp 286−8 °C (EtOH); IR (KBr) 3358 (s), 3441−3183 (brs), 1667 (s), 1643 (vs), 1606 (s), 1507 (s), 1231 (s) cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.1 (s, 1 H), 7.90 (d, 2 H, J = 7.7 Hz), 7.72 (s, 1 H), 7.65 (d, 1 H, J = 8 Hz), 7.38 (d, 2 H, J = 7.7 Hz), 7.35 (dd, 1 H, J = 1.8, 7.7 Hz), 7.26 (s, 1H), 7.18 (d, 1 H, J = 8 Hz), 6.83 (d, 1 H, J = 8.8 Hz), 3.65 (s, 3 H), 3.60−2.90 (m, 8 H), 2.67 (s, 3 H), 2.16 (s, 3 H); ^{13}C NMR ($CDCl_3$, 500 MHz, attached proton tested) δ 167.7 (no H), 164.8 (no H), 148.2 (no H), 143.6 (no H), 143.1 (no H), 139.3 (no H), 134.8 (no H), 133.8 (no H), 133.4 (no H), 132.7 (no H), 129.7 (1 H), 129.4 (1 H), 128.9 (1 H), 127.6 (1 H), 125.2 (1 H), 115.1 (1 H), 111.9 (1 H), 111.4 (1 H), 55.7 (3 H), 53.0 (2 C, all with 2 H), 47.4 (2 C, all with 2 H), 42.5 (3 H), 20.3 (3 H); MS (CI) m/z 459 (M + 1). Anal. ($C_{27}H_{30}N_4O_3 \cdot 2HCl \cdot H_2O$) C, H, N.

***N*-[4-Methoxy-3-(4-methylpiperazin-1-yl)phenyl] 2′-Methyl-4′-(5-methyl-1,3,4-oxadiazol-2-yl)biphenyl-4-carboxamide (2).** 3-(4′-Bromo-3′-methylphenyl)-5-methyl-1,3,4-oxadiazole (**8b**)[22] reacted with **7** in the same manner described above, in either 1,2-dimethoxyethane or *N*,*N*-dimethylforamide, affording **2** in 67−72% yield: white crystal, mp 219−22 °C (from EtOAc−EtOH); IR (KBr) 1669 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.96 (d, 2 H, J = 8.3 Hz), 7.93 (s, 1 H), 7.80 (m, 2 H), 7.45 (d, 2 H, J = 8.3 Hz), 7.35 (d, 1 H, J = 8 Hz), 7.31 (m, 1 H), 7.25 (m, 1 H), 6.85 (d, 1 H, J = 8.7 Hz), 3.88 (s, 3 H), 3.15 (brs, 4 H), 2.64 (s + brs, 7 H), 2.37 (s, 3 H), 2.34 (s, 3 H); MS (EI) m/z 497 (M^+). Anal. ($C_{29}H_{31}N_5O_3 \cdot 0.25H_2O$) C, H, N. The crystals for X-ray were developed by slow evaporation from EtOH and collected.

***N*-[4-Methoxy-3-(4-methylpiperazin-1-yl)phenyl] 2′-Methyl-4′-amidinylbiphenyl-4-carboxamide (10).** Compound **1** (200 mg, 0.4 mmol) was dissolved into a mixture of 50 mL of methanol and 10 mL of acetic acid, followed by addition of 50% Raney-Ni slurry in water (100 mg, 1 mL of H_2O was added additionally). The mixture was hydrogenated with 4 atm of H_2 at room temperature in a Parr shaker overnight. Filtration through Celite, washing with acetic acid (10 mL), and evaporation of the filtrate afforded an oily residue. The residue was dissolved into CH_2Cl_2 (50 mL), washed with 2 N aq NaOH (15 mL), dried over $MgSO_4$, filtered, and evaporated to afford the titled compound which was solidified from ethyl acetate: 140 mg (77%), mp 159−62 °C; IR (KBr) 3300, 2935, 2801, 1645, 1607, 1508 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.10 (brs, 1 H), 9.60 (brs, 2 H), 8.31 (s, 1 H), 8.04 (d, 2 H, J = 10 Hz), 7.78 (s, 1 H), 7.71 (d, 1 H, J = 5 Hz), 7.51 (d, 2 H, J = 10 Hz), 7.46 (m, 1 H), 7.38 (m, 2 H), 6.91 (d, 1 H, J = 10 Hz), 3.77 (s, 3 H), 3.31 (brs, 4 H), 2.99 (brs, 2 H), 2.47 (brs, 2 H), 2.32 (s, 3 H), 2.23 (s, 3 H); MS (FAB) m/z 458 ($M^+ + 1$). Anal. ($C_{27}H_{31}N_5O_2 \cdot 1.5H_2O$) C, H, N.

***N*-[4-Hydroxy-3-(4-methylpiperazin-1-yl)phenyl] 2′-Methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxamide (11a).** Compound **1** (200 mg, 0.40 mmol) in 3 mL of EtSH and 2 mL of CH_2Cl_2 was cooled to 0 °C and treated with $AlCl_3$ (1.0 g) with stirring overnight at room temperature. The resulting solution was quenched with ice−water (20 mL), neutralized with $NaHCO_3$ (powder) until the pH value of the solution was about 8, and extracted with CH_2Cl_2 (4 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under vacuum to give 200 mg of the crude product, which was purified by flash chromatography (SiO_2, THF with gradually adding small amount of 25% aq NH_4OH

as eluent) and recrystallization from 1:2 ethyl acetate/hexane to afford 140 mg (73%) of **11a** as a fine solid: mp 140−2 °C; IR (KBr) 3296 (brs), 1638 (s), 1504 (s), 1425 (s), 1261 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.95 (m, 5 H), 7.55 (s, 1 H), 7.43 (d, 2 H, J = 8.3 Hz), 7.30 (m, 1 H), 6.94 (d, 1 H, J = 8.5 Hz), 2.94 (brs, 4 H), 2.67 (s, 3 H), 2.62 (brs, 4 H), 2.37 (s, 3 H), 2.32 (s, 3 H); MS (EI) m/z 483 (M^+). Anal. ($C_{28}H_{29}N_5O_3 \cdot 0.25H_2O$) C, H, N.

***N*-[4-Hydroxy-3-(4′-methylpiperazin-1′-yl)phenyl] 2′-methyl-4′-(5-methyl-1,3,4-oxadiazol-2-yl)biphenyl-4-carboxamide (11b)** was prepared from **2** in the same manner described above for the preparation of **11a**. **11b**: 85%, white solid, mp 149−52 °C; IR (KBr) 3504, 3350, 3294, 1646, 1505, 1262, 1241 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.47 (s, 1 H), 7.96 (d, 2 H, J = 8.3 Hz), 7.93 (s, 1 H), 7.85 (dd, 1 H, J = 1.4, 7.7 Hz), 7.53 (d, 1 H, J = 2.3 Hz), 7.38 (d, 2 H, J = 8.3 Hz), 7.30 (m, 1 H), 6.89 (d, 1 H, J = 8.6 Hz), 2.96 (m, 4 H), 2.65 (brs, 4 H), 2.61 (s, 3 H), 2.38 (s, 3 H), 2.29 (s, 3 H); MS (EI) m/z 483 (M^+, 56), 425 (80), 277 (90), 71 (100). Anal. ($C_{28}H_{29}N_5O_3 \cdot 0.5H_2O$) C, H, N.

***N*-[4-Methylsulfonyloxy-3-(4-methylpiperazin-1-yl)phenyl] 2′-Methyl-4′-(5-methyl-1,3,4-oxadiazol-2-yl)biphenyl-4-carboxamide (13a)**. Compound **11b** (40 mg, 0.083 mmol) in 3 mL of CH_2Cl_2 and 0.1 mL of triethylamine was treated with methanesulfonyl chloride (0.06 mL) at −78 °C with stirring for 1 h (−78 °C to room temperature). To the mixture were added 0.1 mL of 2 N NaOH and 2 g of SiO_2. The mixture was evaporated and the residue was purified by flash chromatography (SiO_2, 9:1 CH_2Cl_2/EtOH with gradually adding small amount of 25% aq NH_4OH as eluent) to afford 40 mg (86%) of **13a**, which was recrystallized from EtOAc−hexane as a white solid: mp 194−7 °C; IR (KBr) 3420 (brm), 2977 (s), 2941 (s), 2603 (vs), 2496 (vs), 1655 (s), 1476 (s), 1397 (s), 1186 (s), 1037 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.25 (s, 1 H), 8.13 (d, 2 H, J = 8 Hz), 7.94 (s, 1 H), 7.86 (d, 1 H, J = 8 Hz), 7.75 (s, 1 H), 7.56 (d, 1 H, J = 8.3 Hz), 7.41 (d, 2 H, J = 8.3 Hz), 7.30 (d, 1 H, J = 8.3 Hz), 7.19 (d, 1 H, J = 8.3 Hz), 3.20 (brs, 4 H), 3.15 (s, 3 H), 2.80 (brs, 4 H), 2.60 (s, 3 H), 2.49 (s, 3 H), 2.30 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 165.4, 164.6, 163.6, 144.7, 144.6, 143.7, 138.9, 137.9, 136.2, 133.3, 130.2, 129.3, 128.6, 127.2, 124.2, 123.2, 115.0, 112.2, 54.8, 49.7, 45.0, 38.4, 20.3, 11.1; MS (EI) m/z 561 (M^+); HRMS for $C_{29}H_{31}N_5SO_5$: calcd, 561.2045; found, 561.2038.

***N*-[4-Trifluoromethylsulfonyloxy-3-(4-methylpiperazin-1-yl)phenyl] 2′-Methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-biphenyl-4-carboxamide (13b).** Compound **11a** (101 mg, 0.21 mmol) in 10 mL of CH_2Cl_2 and 0.1 mL of triethylamine was treated with the solution of Tf_2O (0.05 mL) in 2 mL of CH_2Cl_2 dropwise at −78 °C. The resulting solution was stirred overnight at room temperature, quenched with water, and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were dried over $MgSO_4$, filtered, evaporated, and purified by flash chromatography (SiO_2, EtOAc then THF as eluent) and recrystallization from EtOAc/hexane to afford 80 mg (62%) of **13b**: mp 140 °C dec; IR (KBr) 3416 (brm), 1676 (m), 1628 (m), 1595 (s), 1422 (s), 1212 (brs), 1139 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (s, 1 H), 7.90 (m, 4 H), 7.50 (m, 3 H), 7.35−7.17 (m, 2 H), 6.95 (d, 1 H, J = 8.6 Hz), 2.95 (m, 4 H), 2.70 (m, 4 H), 2.66 (s, 3 H), 2.40 (s, 3 H), 2.22 (s, 3 H); MS (EI) m/z 615 (M^+, 1.3). Anal. ($C_{29}H_{28}N_5O_5SF_3$) C, H, N.

***N*-[4-(2′-Fluoroethoxy)-3-(4-methylpiperazin-1-yl)phenyl] 2′-Methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxamide (13c).** Compound **11a** (120 mg, 0.25 mmol), acetonitrile (15 mL), $BrCH_2CH_2F$ (52 mg, 0.4 mmol), and $CsCO_3$ (600 mg, 1.8 mmol) were combined and heated to reflux for 3 h. The mixture was evaporated, quenched with water (10 mL), and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by flash chromatography (SiO_2, 10:1 CH_2Cl_2/EtOH) to afford 94 mg (71%) of **13c**: mp 128−30 °C (liquidization); IR (KBr) 3417 (br m), 1654 (m), 1643 (m), 1607 (m), 1507 (s), 1257 (s), 1230 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.99−7.92 (m, 5 H), 7.50−7.20 (m, 5 H), 6.85 (d, 1 H, J = 8.5 Hz), 4.88 (m, 1 H), 4.62 (m, 1 H), 4.30 (m, 1 H), 4.18 (m, 1 H), 3.20 (brs, 4 H), 2.67 (s, 3 H), 2.60 (m, 4 H), 2.38 (s, 3 H), 2.32 (s, 3 H); MS (EI) m/z 529 (M^+); HRMS for $C_{30}H_{32}N_5O_3F$: calcd, 529.2488; found, 529.2471.

***N*-[4-Methoxy-3-(4-methylpiperazin-1-yl)phenyl] 4-Bromobenzenesulfonamide (14)**. A mixture of 4-bromobenzenesulfonyl chloride (511 mg, 2.0 mmol), 4-methoxy-3-(*N*-methylpiperazinyl)aniline dihydrochloride (588 mg, 2.0 mmol), 10 mL of dry THF, and 2 mL of triethylamine was stirred overnight at room temperature, filtered to remove the white precipitate, and evaporated. The residue was purified by flash chromatography (SiO_2, EtOAc and THF as eluents) and recrystallization from ethyl acetate to afford 0.85 g (96%) of **14** as a white solid: mp 180−2 °C; IR (KBr) 3005, 2946, 2830, 1594, 1574, 1510, 1334, 1153, 998 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.55 (s, 4 H), 6.70 (s, 3 H), 6.50 (s, 1 H), 3.82 (s, 3 H), 2.85 (m, 4 H), 2.60 (m, 4 H), 2.40 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 151.5, 142.0, 138.9, 132.4, 129.2, 129.0, 127.9, 120.1, 116.3, 111.9, 99.5, 56.0, 55.4, 50.6, 46.4; MS (EI) m/z 440 (M^+). Anal. ($C_{18}H_{22}N_3SO_3Br$) C, H, N.

4-{*N*-[4′-Methoxy-3′-(4-methylpiperazin-1-yl)phenyl]-aminosulfonyl}benzeneboronic Acid (15). Triisopropyloxyboron (2.1 mL, 9 mmol) was added to a solution of **14** (329 mg, 0.75 mmol) in 15 mL of dry THF at −100 °C under nitrogen. The solution was treated with *n*-BuLi (3.8 mL in 2.5 M in hexane, 9 mmol) dropwise during 20 min. The resulting mixture was stirred for 3 h at the temperature in a range of −100 to −78 °C and then overnight at room temperature, quenched by adding dropwise 5 mL of water, stirred for 1 h, and evaporated to dryness under vacuum. The residue was preadsorbed onto 10 mL of silica gel and purified by flash chromatography (SiO_2, CH_2Cl_2/EtOH, 9:1 to 7:3 with adding gradually small amount of 25% aq NH_4OH as eluent) to afford 220 mg (73%) of **15** as a white foam, which was used in the following reaction without further purification.

***N*-[4-Methoxy-3-(4-methylpiperazin-1-yl)phenyl] 2′-Methyl-4′-(5-methyl-1,3,4-oxadiazol-2-yl)biphenyl-4-sulfonamide (16).** Intermediate **15** (180 mg, 0.44 mmol) reacted with **8b** (189 mg, 0.75 mmol) in the same manner described above for the preparation of **2** to afford 200 mg (84%) of **16** as a white solid, which was recrystallized from ethyl acetate: mp 242−4 °C; IR (KBr) 2984, 2824, 1584, 1503, 1339, 1236, 1166 cm^{-1}; ^1H NMR ($CDCl_3$, little CD_3OD) δ 7.86 (s, 1 H), 7.80 (dd, 1 H, J = 1.7, 7.9 Hz), 7.74 (d, 2 H, J = 8.2 Hz), 7.32 (d, 2 H, J = 8.3 Hz), 7.23 (d, 1 H, J = 8.1 Hz), 6.86 (dd, 1 H, J = 2.4, 8.6 Hz), 6.68 (d, 1 H, J = 7.8 Hz), 6.60 (d, 1 H, J = 2.4 Hz), 3.74 (s, 3 H), 3.27 (brs, 8 H), 2.77 (s, 3 H), 2.56 (s, 3 H), 2.21 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 164.9, 164.1, 149.9, 145.3, 143.6, 139.3, 138.7, 136.5, 130.5, 130.4, 129.6, 128.9, 127.4, 124.5, 123.3, 118.3, 114.2, 111.9, 55.8, 54.1 (2C), 47.5 (2C), 43.7, 20.4, 11.1; MS (EI) m/z 533 (M^+). Anal. ($C_{28}H_{31}N_5O_4S$) C, H, N.

Pharmacology. 1. In Vitro Receptor Binding Assays. Affinities for *r*5-HT_{1B} receptors were determined in rat frontal cortex using [^{125}I]iodocynopindolol as radioligand, final concentration 0.1 nM.[24] The *c*5-HT_{1D} assays were performed according to Peroutka et al.,[25] using membranes prepared from calf striatum and 1.7 nM [^{3}H]serotonin in the presence of 100 nM 8-OH-DPAT and 100 nM mesulergine to mask 5-HT_{1A} and 5-HT_{2C} binding sites. Nonspecific binding was determined in the presence of 10 μM serotonin.[25] Inhibition by drugs of the binding of 0.5 nM [^{3}H]8-OH-DPAT to 5-HT_{1A} receptors in membranes from rat hippocampus was determined according to the reported method.[26]

2. Rabbit Saphenous Vein (RSV) Model. The inhibition of the contractile responses elicited by sumatriptan in RSV, challenged by the test compound, was determined according to the published method.[27] Segments of the isolated RSV were suspended in organ baths and subjected to two concentration−response curves to sumatriptan, the second challenge being performed in the presence of the antagonist or control vehicle (incubated for 60 min). Apparent pA_2 values were calculated using a single antagonist concentration in at least three preparations.

3. [^{3}H]5-HT Release. The experiments were performed according to a method described by Ormandy et al.[28] Cerebral cortices were removed from male Dunkin−Hartley guinea pigs,

cross-chopped into slices, and then incubated 30 min with [^{3}H]5-HT. Slices were placed into chambers of a superfusion apparatus and superfused with oxygenated Krebs's solution. Superfusate was collected in 4-min periods beginning 60 min after the slices were placed in the chambers. The slices were exposed to two periods (4 min) of 30 mM K^+ at 68 and 108 min superfusion (S1 and S2, respectively). Sumatriptan at 3 μM (controls) and sumatriptan plus test compounds (30 nM, 300 nM, and 3 μM, respectively) were superfused prior to and during the S2 stimulation period. The percentage release rate of each sample was calculated as the radioactivity in that fraction multiplicated by 100 and divided by the total amount of radioactivity present in the tissue at the start of the collection. The K^+-stimulated release for S1 and S2 was calculated as the total overflow during the collection periods after the onset of the stimulation minus basal outflow. Potassium ion-mediated stimulation of release increased 3H efflux by a total amount of 7.71 ± 0.69% (n = 28) of tissue 3H (S1). The addition of 3 μM sumatriptan to the superfusion buffer before S2 produced an inhibition of the stimulated release to 4.61 ± 0.42% (n = 28) of tissue 3H (S2). The effects of drugs on stimulated release were determined by calculating S2/S1 ratios in control and treated slices, expressing the drug response as a percent of the control S2/S1 ratio, which was 0.59 ± 0.01 (n = 28). Results represent the mean (±SEM) of 3−4 determinations. Statistical differences between ratios were analyzed using ANOVA followed by Dunnett test. Data of test compound at 300 nM are shown in Table 1.

4. [^{3}H]5-HT Reuptake Inhibition (5-HTru). Inhibition of [^{3}H]5-HT reuptake by test compound was measured according to the method described by Wong et al.[29]

5. In Vivo Microdialysis. Microdialysis probes were implanted bilaterally into ventral hippocampus of male Wistar rats (280−340 g) (Harlen Zeist, The Netherlands), perfused with artificial cerebrospinal fluid (aCSF). Acetylcholine (ACh) experiments were performed with neostigmine (0.1 M) added to the aCSF, to obtain detectable amounts of ACh. Serotonin and ACh were analyzed online using HPLC with electrochemical detection. Postcolumn conversion into hydrogen peroxide was used for ACh measurements. After data were converted into percentage of basal level, the area under the curve (AUC) from 0 to 150 min was determined (Students t-test, $p < 0.05$). The experiments and results in more detail were described in another paper by Cremers et al. (submitted to *Eur. J. Pharm.*).

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Supporting Information Available: Single-crystal X-ray diffraction data of compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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