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Communications to the Editor

Evolution of a Novel Series of [(*N*,*N*-Dimethylamino)propyl]- and Piperazinylbenzanilides as the First Selective 5-HT$_{1D}$ Antagonists[1]

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Central 5-hydroxytryptamine (5-HT) receptors have been classified into four main families: 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$,[2-7] although others, such as 5-HT$_5$, 5-HT$_6$, and 5-HT$_7$, have been identified from cloning studies.[8-10] The 5-HT$_1$ family comprises subtypes 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$,[11-13] and 5-HT$_{1F}$[14] (the 5-HT$_{1C}$ receptor has been reclassified as a member of the 5-HT$_2$ family). The 5-HT$_{1D}$ receptor has recently attracted considerable attention since radioligand binding studies have shown it to be widely distributed throughout the central nervous system (CNS) where it is the most abundant 5-HT$_1$ receptor subtype[15] playing a role as a presynaptic heteroreceptor or as a terminal autoreceptor. Activation of this receptor in the CNS inhibits neurotransmitter release.[16,17] 5-HT$_1$ receptors, very similar to the 5-HT$_{1D}$ receptor identified in brain tissue, are located in vascular smooth muscle and mediate contraction.[18] Recently, cloning studies have identified a pair of human 5-HT$_{1D}$ gene products which have been designated 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors.[19]

Sumatriptan,[18] which is effective in the treatment of migraine,[20] is an agonist at a vascular 5-HT$_1$ receptor and shows some selectivity for the 5-HT$_{1D}$ receptor.[21,22] However, the lack of selective 5-HT$_{1D}$ antagonists[23] has frustrated efforts to characterize the functional role of 5-HT$_{1D}$ receptors in the CNS. Current pharmacological tools used to antagonize effects at 5-HT$_{1D}$ receptors include metergoline and methiothepin, but these compounds are poorly selective and therefore of limited utility. We now report on a novel series of benzanilides which represent the first examples of selective 5-HT$_{1D}$ antagonists.

As part of a program to identify selective 5-HT$_{1D}$ antagonists we discovered that the benzanilide **1** blocked 5-HT-induced contractile responses in the dog isolated saphenous vein (DSV)[18] (Table 1). Although its level of activity was modest and significant antagonist activity at the 5-HT$_{2A}$ receptor was observed, this compound served as a lead on which to base a potency and selectivity optimization program. Early modifications led to the biaryl anilides which showed a significant increase in antagonist potency. Thus, the 4-pyridinylphenyl derivatives **2** and **3** displayed a 1 order of magnitude greater potency as antagonists in the DSV. More significantly, these compounds were approximately 30-fold more potent in the latter tissue compared to the rabbit aorta which measured antagonist activity at 5-HT$_{2A}$ receptors. The affinity of **2** and **3** for 5-HT$_{1D}$ binding sites in guinea pig striatum was similar to their antagonist activity in the DSV, and it is likely that the 5-HT$_1$ receptor mediating contraction in the DSV bears a close resemblance to the 5-HT$_{1D}$ receptor.[24]

Compounds **2** and **3** were evaluated in our model for CNS activity: blockade of hypothermia in the guinea pig caused by stimulation of central 5-HT$_{1D}$ receptors by the agonist GR46611.[25] Disappointingly, neither of these compounds displayed any activity up to 30 mg/kg following either subcutaneous or oral administration. When the linking amide group orientation was reversed, the resulting compound (**4**) displayed an *in vitro* pharmacological profile closely paralleling that of **2** and **3**. However, in contrast to **2** and **3**, compound **4** was now an effective antagonist (ED_{50} = 5 mg/kg, po) of the hypothermia induced in guinea pigs by GR46611.[26]

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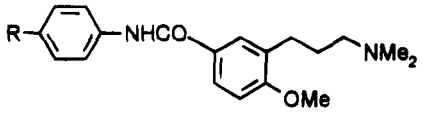
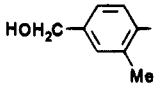
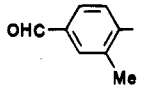
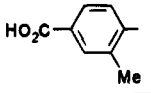
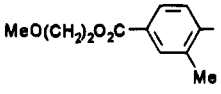
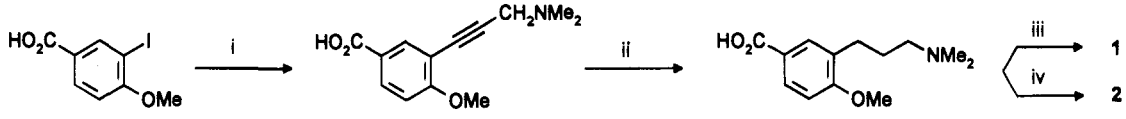
Table 1. *In Vitro* and *in Vivo* Activities of [(*N,N*-dimethylamino)propyl]benzanilides[a]



Compd	R	DSV[b] (pK_B)	5-HT_{1D}[c] (pKi)	5-HT_{2A}[d] (pK_B)	5-HT_{1A}[e] (pKi)	Guinea-pig hypothermia[f] %Inhibn. (dose/route)
1	EtO	6.9	NT[g]	6.2	5.2	NT
2		7.8	8.0	6.3	4.7	<30 (50 mg/kg, po)
5		8.5	NT	5.9	NT	52 (45 mg/kg, po)
6		8.4	8.2	6.3	5.3	<30 (45 mg/kg, po)
7		8.7	7.5	5.7	<5.0	NT
8		8.4	8.5	6.2	NT	55 (45 mg/kg, po) 66 (3 mg/kg, sc)

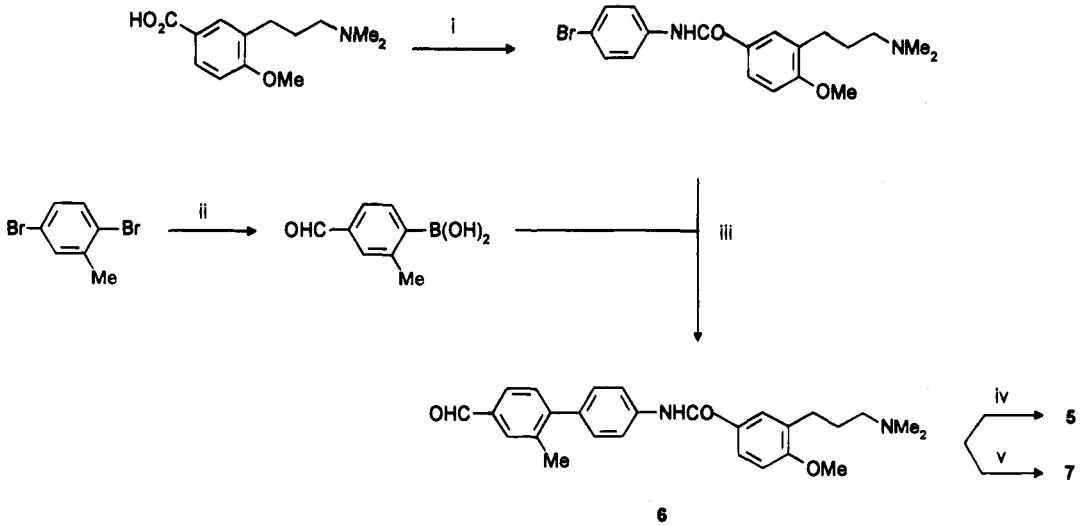
[a] For *in vitro* data, figures quoted are the mean of two independent determinations, each within 0.2 log units of the mean. [b] Antagonism of 5-HT-induced contraction of the dog saphenous vein.[18] [c] Binding affinity, [^{3}H]-5-HT (in the presence of BMY7378 and mesulergine) was used to label 5-HT_{1D} sites in guinea pig striatum, cf. ref 25. [d] Antagonism of 5-HT-induced contraction of rabbit isolated aorta.[18] [e] Binding affinity, [^{3}H]-8-OH-DPAT was used to label 5-HT_{1A} sites in rat hippocampus, cf. ref 25. [f] See ref 25. [g] Not tested.

Scheme 1[a]



[a] (i) *N,N*-Dimethyl-2-propynamine, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF; 25%; (ii) H_2, Pd–C, EtOH–DMF; 66%; (iii) (a) $SOCl_2$, (b) 4-$EtOC_6H_4NH_2$, pyridine, 48%; (iv) (a) $SOCl_2$, (b) 4-(4-pyridinyl)benzenamine, pyridine; 83%.

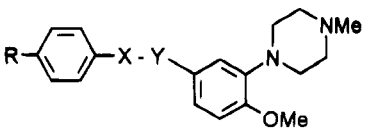
Scheme 2[a]

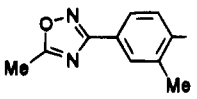
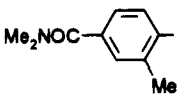
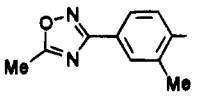


[a] (i) (a) $SOCl_2$, (b) 4-$BrC_6H_4NH_2$, pyridine; 74%; (ii) (a) *n*-BuLi, THF, −78 °C; (b) DMF, −78 °C; (c) (*i*-PrO)$_3$B, −78 °C room temperature; 76%; (iii) $Pd(PPh_3)_4$, Na_2CO_3, DME–H_2O; 80%; (iv) H_2, Pt–C, EtOH; 40%; (v) $AgNO_3$, NaOH, H_2O, MeOH; 68%.

Concurrent with this discovery, it was found that a range of substituted biaryl analogues (**5**–**8**) possessed potent and selective antagonist activity in the DSV with greater than 100-fold selectivity over the 5-HT_{2A} recep-

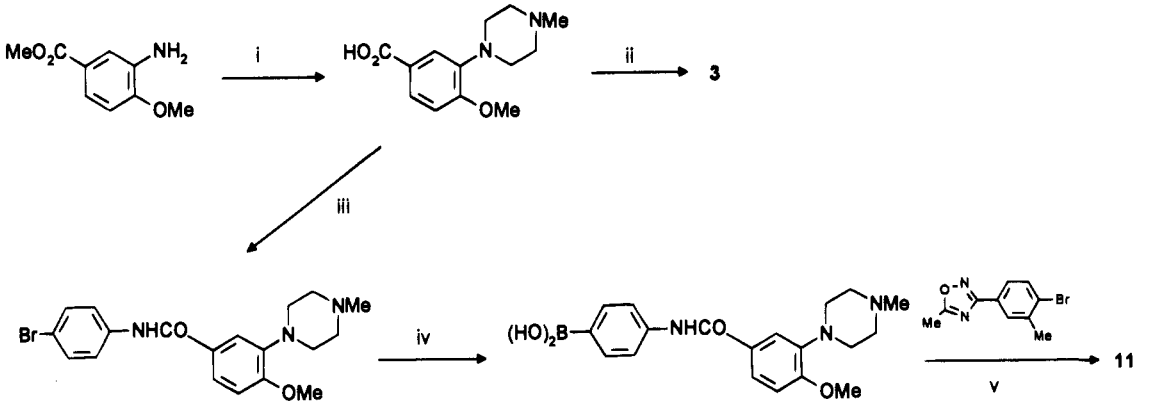
Table 2. *In Vitro* and *in Vivo* Activities of Piperazinylbenzanilides[a]



Compd	R	X - Y	DSV[b] (pK_B)	5-HT_{1D}[c] (pKi)	5-HT_{1B}[d] (pKi)	5-HT_{2A}[e] (pK_B)	5-HT_{1A}[f] (pKi)	Guinea-pig hypothermia[g] ED_{50} (mg/kg, po)
3		NHCO	7.9	8.5	NT[h]	6.5	NT	> 45
4		CONH	8.0	8.3	NT	6.5	5.9	5.0 (2.0- 9.0)
9		CONH	i	8.5	8.5	6.4	6.5	0.3 (0.2-0.4)
10		CONH	9.2	8.3	8.2	4.9	6.6	0.67 (0.2-1.6)
11		NHCO	8.2	8.2	8.2	7.8	5.9	0.5 (0.2-1.2)

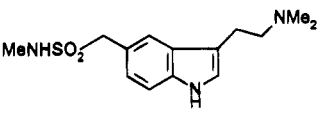
[a] For *in vitro* data, figures quoted are the mean of two independent determinations, each within 0.2 log units of the mean. [b] Antagonism of 5-HT-induced contraction of the dog saphenous vein.[18] [c] Binding affinity, [^{3}H]-5-HT (in the presence of BMY7378 and mesulergine) was used to label 5-HT_{1D} sites in guinea-pig striatum, cf. ref 25. [d] Binding affinity, [^{125}I]iodocyanopindolol was used to label 5-HT_{1B} sites in rat striatal membranes, cf. ref 25. [e] Antagonism of 5-HT-induced contraction of rabbit isolated aorta.[18] [f] Binding affinity, [^{3}H]-8-OH-DPAT was used to label 5-HT_{1A} sites in rat hippocampus, cf. ref 25. [g] See ref 25. [h] Not tested. [i] Reduced maximum effect, slowly dissociating antagonist.

Scheme 3[a]

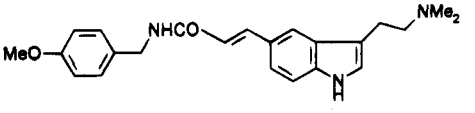


[a] (i) (a) $(ClCH_2CH_2)_2NMe{\cdot}HCl$, Na_2CO_3, *n*-BuOH; 20%; (b) NaOH, H_2O; (ii) (a) $SOCl_2$ (b) 4-(4-pyridinyl)benzenamine, pyridine; 64%; (iii) (a) $SOCl_2$; (b) 4-$BrC_6H_4NH_2$, pyridine, 34%; (iv) (a) *n*-BuLi, THF, −78 °C; (b) (*i*-PrO)$_3$B, −78 °C; (c) HCl, H_2O; 90%; (v) $Pd(PPh_3)_4$, Na_2CO_3, DME−H_2O; 70%.

tor and only weak affinity for the 5-HT_{1A} receptor. However, both **5** and **6** were poorly active in the hypothermia test: the former with an ED_{50} of 45 mg/kg after oral administration, the latter only showing activity when given parenterally (ED_{50} = 45 mg/kg, sc). The low level of *in vivo* activity for **6** was rationalized by the fact that this compound is observed to undergo rapid metabolism to the corresponding carboxylic acid **7** which is unlikely to cross the blood−brain barrier. However, the derived methoxyethoxy ester **8**, itself a potent and selective 5-HT_{1D} antagonist *in vitro*, did display modest oral activity in the hypothermia test (ED_{50} = 45 mg/kg).



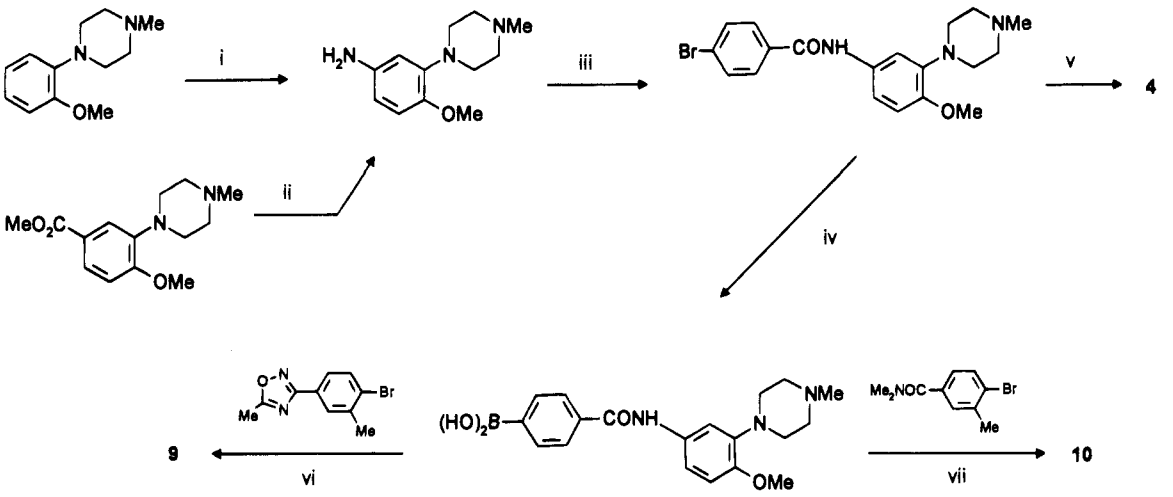
Sumatriptan



GR46611

This last observation led us to evaluate bioisosteric replacements for the potentially labile ester function

Scheme 4[a]



[a] (i) (a) KNO_3, H_2SO_4; 83%; (b) Raney Ni, $N_2H_4{\cdot}H_2O$, EtOH; 53%; (ii) (a) $N_2H_4{\cdot}H_2O$ (b) $NaNO_2$, HCl, H_2O; (c) Δ; 12%; (iii) 4-BrC_6H_4COCl, pyridine; 77%; (iv) (a) *n*-BuLi, THF, −100 °C, (b) $(i\text{-PrO})_3B$, −100 °C → −78 °C; 76%; (v) 4-pyridinylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME−H_2O; 57%; (vi) $Pd(PPh_3)_4$, Na_2CO_3, DME−H_2O; 70%; (vii) $Pd(PPh_3)_4$, Na_2CO_3, DME−H_2O; 46%.

and to combine this with the knowledge that reversal of amide orientation (cf. **4**) gave improved activity in the CNS following oral administration. This strategy provided the oxadiazole derivative **9**. *In vitro*, this compound has 100-fold selectivity for 5-HT_{1D} receptors over 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} (pK_i = 6.4) receptors and, significantly, has pK_i values of 9.9 and 8.9 at 5-$HT_{1D\beta}$ and 5$HT_{1D\alpha}$ receptors, respectively.[25,27] Furthermore, it had little or no affinity (pK_i) at 5-HT_3 (5.2), 5-HT_4 (<5.0), 5-HT uptake (<5.0), α_1- and α_2-adrenoceptor (<6.0), dopamine D_{1-4} (<5.0), and muscarinic M1−3 (<6.0) binding sites. Sumatriptan-induced contractions of the DSV were potently antagonized by low concentrations (1−10 nM) of **9** with reduced maximum effect. This antagonism was reversible following extensive washing, and it is likely that the high lipophilicity of **9** is responsible for the slow dissociation. In contrast 1−**8** are competitive antagonists in this tissue. *In vivo*, compound **9** is a potent inhibitor of GR46611 in the hypothermia test with an ED_{50} of 0.3 mg/kg after oral administration. In marked contrast, over a dose range of 0.1−10 mg/kg sc, **9** failed to attenuate 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced (3 mg/kg sc) wet dog shakes in the guinea pig, an effect which is potently inhibited by 5-HT_2 receptor antagonists,[28] thus underlining its *in vivo* selectivity.

The terminal *N*,*N*-dimethylcarboxamide **10** is a potent, competitive antagonist in the DSV and shows >10 000-fold selectivity with respect to its antagonist activity at 5-HT_{2A} receptors. It also shows potent antagonist activity in the guinea pig hypothermia test. Interestingly, the alternative amide-linked analogue **11**, although a potent antagonist in the DSV and potent after oral administration in the guinea pig hypothermia test showed reduced 5-HT_{1D}/5-HT_{2A} selectivity. For selected compounds (**9**−**11**) we have shown that 5-HT_{1D} binding affinity correlates well with affinity at the 5-HT_{1B} receptor, a rodent homologue of the 5-$HT_{1D\beta}$ receptor.

The compounds listed in Tables 1 and 2 were, for the most part, prepared by standard modifications of benzenoid systems with two key aspects of the synthetic strategy relying on palladium(0) chemistry. First, in the synthesis of **1** and **2** (Scheme 1), the (dimethylamino)-propyl side chain was constructed via a Sonogashira reaction followed by hydrogenation. Second, palladium(0)-catalyzed boronic acid coupling provided a versatile means of accessing the biaryl systems, either by utilizing a simple arylboronic acid derivative (Scheme 2) or *via* a functionalized anilide system (Schemes 3 and 4).

In summary, we have discovered a novel series of potent and selective 5-HT_{1D} receptor antagonists based upon a benzanilide pharmacophore. Several of these compounds display good CNS activity. In particular **9** (GR127935) and **10** (GR133867) are likely to be useful tools in determining the role of this receptor subtype in the CNS. 5-HT_{1D} receptor antagonists could also have useful therapeutic applications. For example, selective blockade of central 5-HT_{1D} autoreceptors should facilitate 5-HT transmission and may therefore offer a novel antidepressant therapy. In addition, since 5-HT_{1D} receptors are present in high density in basal ganglia,[15] selective antagonists may also have potential in the treatment of movement disorders.

Supplementary Material Available: Representative synthetic procedures (4 pages). Ordering information is given on any current masthead page.

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(24) A correlation analysis performed between antagonist potency (in the dog saphenous vein) and affinity (5-HT_{1D} binding site, guinea pig striatum) for a series of our benzanilides (pK_i range 6–9) shows a significant correlation ($r = 0.72$, slope = 0.73, $N = 35$) (Skingle, M., unpublished data). See also: Hamel, E.; Bouchard, D. Contractile 5-HT_1 receptors in human isolated pial arterioles: correlation with 5-HT_{1D} binding sites. *Br. J. Pharmacol.* **1991**, *102*, 227–233.
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