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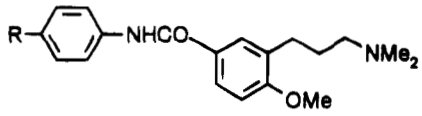
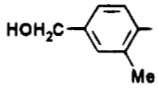
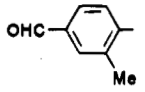
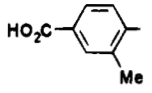
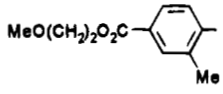
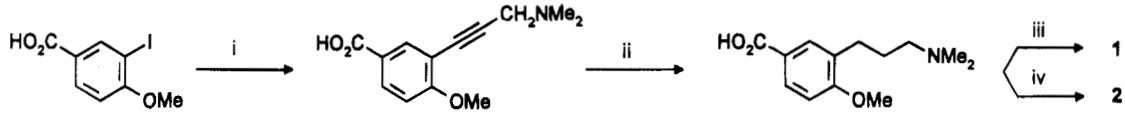
Table 1. *In Vitro* and *in Vivo* Activities of [(*N,N*-dimethylamino)propyl]benzanilides[a]



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)

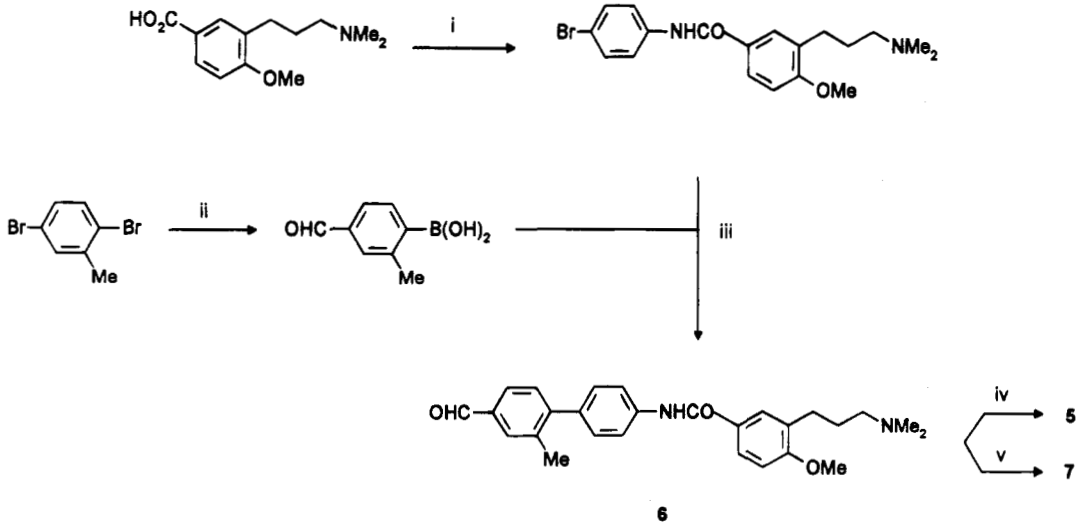
[a] For *in vitro* data, figures quoted are the mean of two independent determinations, each within 0.2 log units of the mean. [b] Antagonism of 5-HT-induced contraction of the dog saphenous vein.[18] [c] Binding affinity, [^{3}H]-5-HT (in the presence of BMY7378 and mesulergine) was used to label 5-HT_{1D} 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%.

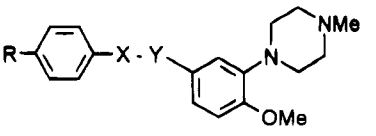
Scheme 2[a]

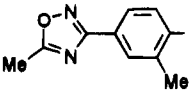
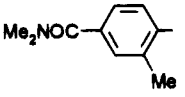
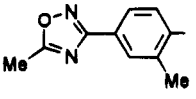


[a] (i) (a) $SOCl_2$, (b) 4-$BrC_6H_4NH_2$, pyridine; 74%; (ii) (a) *n*-BuLi, THF, −78 °C; (b) DMF, −78 °C; (c) $(i\text{-PrO})_3B$, −78 °C room temperature; 76%; (iii) $Pd(PPh_3)_4$, Na_2CO_3, DME–H_2O; 80%; (iv) H_2, Pt–C, EtOH; 40%; (v) $AgNO_3$, NaOH, H_2O, MeOH; 68%.

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-

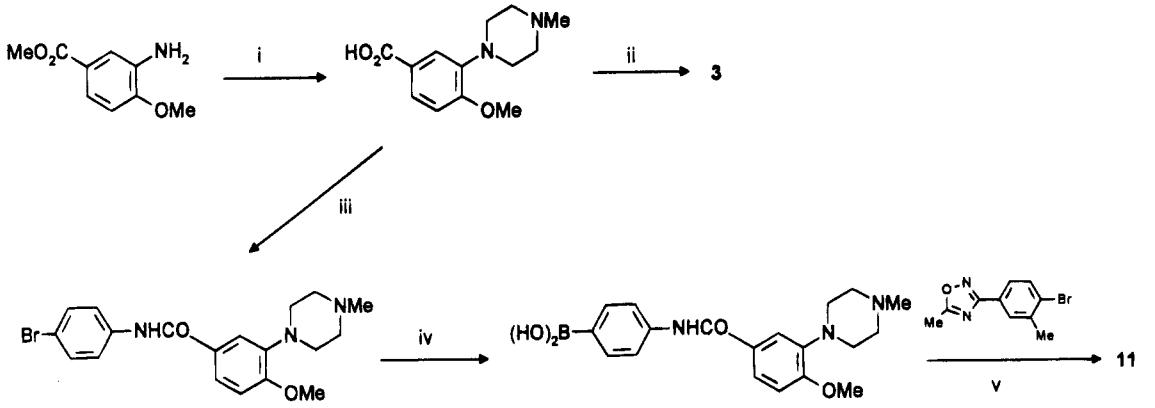
Table 2. *In Vitro* and *in Vivo* Activities of Piperazinylbenzanilides[a]



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)

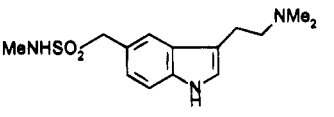
[a] For *in vitro* data, figures quoted are the mean of two independent determinations, each within 0.2 log units of the mean. [b] Antagonism of 5-HT-induced contraction of the dog saphenous vein.[18] [c] Binding affinity, [^{3}H]-5-HT (in the presence of BMY7378 and mesulergine) was used to label 5-HT_{1D} 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]

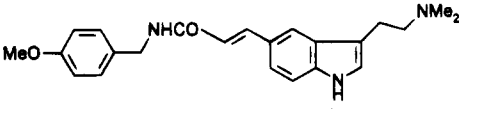


[a] (i) (a) $(ClCH_2CH_2)_2NMe{\cdot}HCl$, Na_2CO_3, *n*-BuOH; 20%; (b) NaOH, H_2O; (ii) (a) $SOCl_2$ (b) 4-(4-pyridinyl)benzenamine, pyridine; 64%; (iii) (a) $SOCl_2$; (b) $4\text{-}BrC_6H_4NH_2$, pyridine, 34%; (iv) (a) *n*-BuLi, THF, −78 °C; (b) $(i\text{-}PrO)_3B$, −78 °C; (c) HCl, H_2O; 90%; (v) $Pd(PPh_3)_4$, 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).



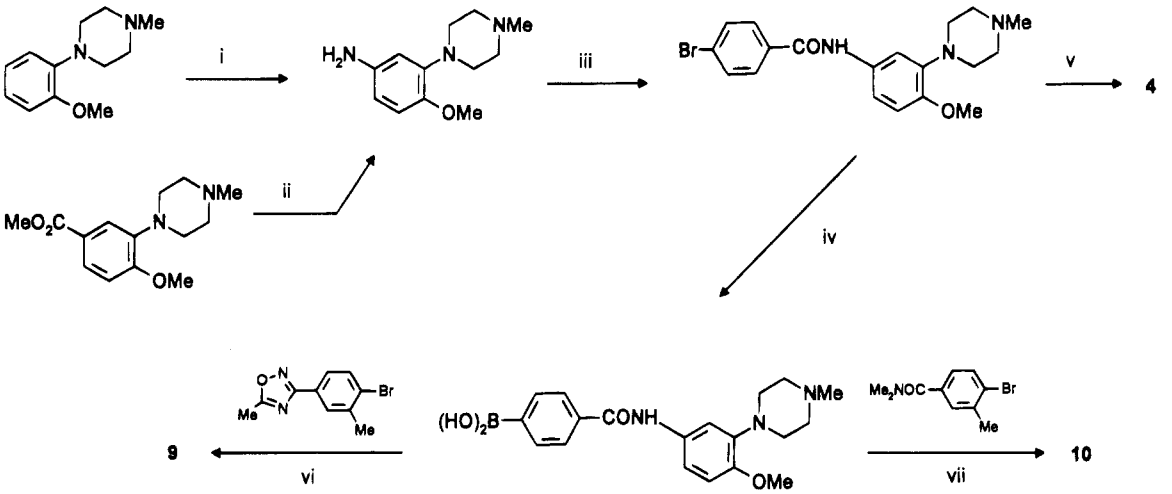
Sumatriptan



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.

References

(1) Presented in part: Scopes, D. I. C.; Clitherow, J. W.; Mitchell, W. L.; Kelly, H. A.; Irving, W.; Campbell, I. B.; Oxford, A. W.; Collington, E. W.; Jordan, C. C.; Skingle, M.; Feniuk, W.; Connor, H.; Tyers, M. B. Selective 5-HT_{1D} antagonists: a novel series of biarylanilides. 207th National Meeting of the American Chemical Society, San Diego, March 13−17, 1994, MEDI164. Scopes, D. I. C.; Clitherow, J. W.; Carter, M. C.; Irving, W.; Collington, E. W.; Skingle, M.; Feniuk, W.; Tyers, M. B. Heterocyclic biarylanilides as potent 5-HT_{1D} antagonists. *Ibid.*, MEDI 165.

(2) For a recent review on 5-HT receptor nomenclature, see: Humphrey, P. P. A.; Hartig, P.; Hoyer, D. A proposed new nomenclature for 5-HT receptors. *Trends Pharmacol. Sci.* **1993**, *14*, 233−236.

(3) Julius, D. Molecular biology of serotonin receptors. *Ann. Rev. Neurosci.* **1991**, *14*, 335−360.

(4) Hartig, P. R.; Kao, H.-T.; Macchi, M.; Adham, N.; Zgombick, J.; Weinshank, R.; Branchek, T. The molecular biology of serotonin receptors: an overview. *Neuropsychopharmacology* **1990**, *3*, 335−347.

(5) Frazier, A.; Maayani, S.; Woolfe, B. B. Subtypes of receptors for serotonin. *Ann. Rev. Pharmacol. Toxicol.* **1990**, *30*, 307−348.

(6) Schmidt, A. W.; Peroutka, S. J. 5-Hydroxytryptamine receptor "families". *FASEB J.* **1989**, *3*, 2242−2249.

(7) Peroutka, S. J. 5-Hydroxytryptamine receptor subtypes: molecular biochemical and physiological characterization. *Trends Neurosci.* **1988**, *11*, 496–500.
(8) Matthes, H.; Boschert, U.; Amlaiky, N.; Grailhe, R.; Plassat, J. L.; Muscatelli, F.; Mattei, M. G.; Hen, R. The mouse 5-HT_{5A} and 5-HT_{5B} receptors define a new family of serotonin receptors: cloning, functional expression and chromosomal localization. *Mol. Pharmacol.* **1993**, *43*, 313–319.
(9) Monsma, F. J.; Shen, Y.; Ward, R. P.; Hamblin, M. W.; Sibley, D. R. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.* **1993**, *43*, 320–327.
(10) Shen, Y.; Monsma, F. J.; Metcalf, M. A.; Jose, P. A.; Hamblin, M. W.; Sibley, D. R. Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype. *J. Biol. Chem.* **1993**, *268*, 18200–18204.
(11) McAllister, G.; Charlesworth, A.; Snodin, C.; Beer, M. S.; Noble, A. J.; Middlemiss, D. N.; Iversen, L. L.; Whiting, P. Molecular cloning of a serotonin receptor from human brain (5-HT_{1E}): A fifth 5-HT_1-like subtype. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5517–5521.
(12) Zgombik, J. M.; Schechter, L. E.; Macchi, M.; Hartig, P. R.; Branchek, T. A.; Weinshank, R. L. Human gene S31 encodes the pharmacologically defined serotonin 5-hydroxytryptamine$_{1E}$ receptor. *Mol. Pharmacol.* **1992**, *42*, 180–185.
(13) Guderman, T.; Levy, F. O.; Birnbaumer, M.; Birnbaumer, L.; Kaumann, A. J. Human S31 serotonin receptor clone encodes a 5-hydroxytrypamine$_{1E}$-like serotonin receptor. *Mol. Pharmacol.* **1993**, *43*, 412–418.
(14) Adham, N.; Kae, H.-T.; Schechter, L. E.; Bard, J.; Olsen, M.; Urquhart, D.; Durkin, M.; Hartig, P. R.; Weinshank, R. L.; Branchek, T. A. Cloning of another human serotonin receptor (5-HT_{1F}): A fifth 5-HT_1 receptor subtype coupled to the inhibition of adenylate cyclase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 408–412.
(15) Waeber, C.; Schoeffer, P.; Hoyer, D.; Palacios, J. M. The serotonin 5-HT_{1D} receptor: a progress review. *Neurochem. Res.* **1990**, *15*, 573–588.
(16) Hoyer, D.; Middlemiss, D. N. Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. *Trends Pharmacol. Sci.* **1989**, *10*, 130–132.
(17) Maura, G.; Thellung, S.; Andrioli, G. C.; Ruelle, A.; Raiteri, M. Release-regulating serotonin 5-HT_{1D} autoreceptors in human cerebral cortex. *J. Neurochem.* **1993**, *60*, 1179–1182.
(18) Humphrey, P. P. A.; Feniuk, W.; Perren, M. J.; Connor, H. E.; Oxford, A. W.; Coates, I. H.; Butina, D. GR43175, a selective agonist for the 5-HT_1-like receptor in dog isolated saphenous vein. *Br. J. Pharmacol.* **1988**, *94*, 1123–1132.
(19) For a review see: Hartig, P. R.; Branchek, T. A.; Weinshank, R. L. A subfamily of 5-HT_{1D} receptor genes. *Trends Pharmacol. Sci.* **1992**, *13*, 152–159.
(20) Doenicke, A.; Brand, J.; Perrin, V. L. Possible benefit of GR43175, a novel 5-HT_1-like receptor agonist, for the acute treatment of migraine. *Lancet* **1988**, No. 1, 1309–1311.
(21) Schoeffter, P.; Hoyer, D. How selective is GR43175? Interactions with functional 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} binding sites. *Naunyn-Schmiedeberg Arch. Pharmacol.* **1989**, *340*, 135–138.
(22) Peroutka, S. J.; McCarthy, B. G. Sumatriptan (GR43175) interacts selectively with 5-HT_{1B} and 5-HT_{1D} binding sites. *Eur. J. Pharmacol.* **1989**, *163*, 133–136.
(23) Glennon, R. A.; Dukat, M. Serotonin receptors and their ligands: a lack of selective agents. *Pharmacol. Biochem. Behav.* **1991**, *40*, 1009–1017.
(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.
(25) Skingle, M.; Higgins, G. A.; Feniuk, W. Stimulation of central 5-HT_{1D} receptors causes hypothermia in the guinea-pig. *J. Psychopharmacol.* **1994**, *8*, 14–21.
(26) The reason for this quite dramatic effect of the amide link orientation is not clear and, interestingly, was not reflected in other series of structures (see **9** and **11**). In the pyridinyl series this may relate to greater lability of the amide link of **3** *in vivo*.
(27) Skingle, M.; Scopes, D. I. C.; Feniuk, W.; Tyers, M. B.; Connor, H. E.; Carter, M. C.; Clitherow, J. W. GR127935: A potent orally active 5-HT_{1D} receptor antagonist. *Br. J. Pharmacol.* **1993**, *110*, 9P.
(28) Skingle, M.; Cole, N.; Higgins, G. A.; Feniuk, W. Inhibition of DOI-induced wet dog shakes in the guinea-pig by 5-HT_2 receptor antagonists. *J. Psychopharmacol.* **1991**, *5*, 196–201.