The Selective 5-HT_{1B} Receptor Inverse Agonist 1′-Methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4′-piperidine] (SB-224289) Potently Blocks Terminal 5-HT Autoreceptor Function Both in Vitro and in Vivo

Laramie M. Gaster,*,‡ Frank E. Blaney,‡ Susannah Davies,‡ D. Malcolm Duckworth,‡ Peter Ham,‡ Sarah Jenkins,‡ Andrew J. Jennings,‡ Graham F. Joiner,‡ Frank D. King,‡ Keith R. Mulholland,‡ Paul A. Wyman,‡ Jim J. Hagan,† Jon Hatcher,† Brian J. Jones,† Derek N. Middlemiss,† Gary W. Price,† Graham Riley,† Claire Roberts,† Carol Routledge,† Julie Selkirk,† and Paula D. Slade†

SmithKline Beecham Pharmaceuticals, Discovery Research, New Frontiers Science Park (North), Third Avenue, Harlow, Essex CM19 5AW, England

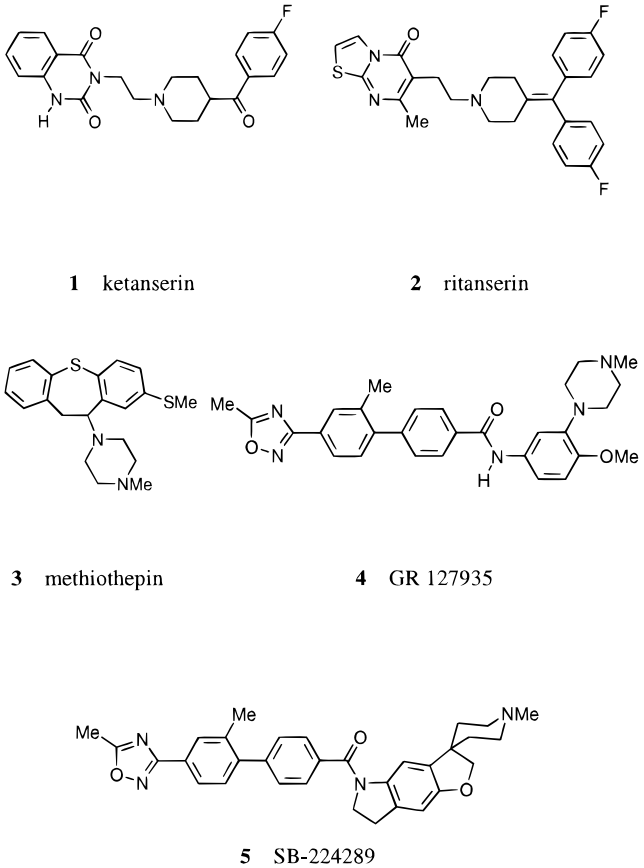
Received July 14, 1997

5-HT_1 receptors are members of the G-protein-coupled receptor superfamily and are negatively linked to adenylyl cyclase activity. The human 5-HT_{1B} and 5-HT_{1D} receptors (previously known as 5-$HT_{1D\beta}$ and 5-$HT_{1D\alpha}$, respectively), although encoded by two distinct genes, are structurally very similar. Pharmacologically, these two receptors have been differentiated using nonselective chemical tools such as ketanserin and ritanserin, but the absence of truly selective agents has meant that the precise function of the 5-HT_{1B} and 5-HT_{1D} receptors has not been defined. In this paper we describe how, using computational chemistry models as a guide, the nonselective 5-HT_{1B}/5-HT_{1D} receptor antagonist **4** was structurally modified to produce the selective 5-HT_{1B} receptor inverse agonist **5**, 1′-methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4′-piperidine] (SB-224289). This compound is a potent antagonist of terminal 5-HT autoreceptor function both in vitro and in vivo.

Introduction

5-HT (5-hydroxytryptamine, serotonin) receptors are classified into seven distinct groups[1] (5-HT_{1-7}). Although 5-HT_3 receptors belong to the family of ligand-gated ion channels, the remainder are all members of the G-protein-coupled receptor (GPCR) superfamily. 5-HT_1 and 5-HT_2 receptors have been further divided into subtypes based on considerations of structural features, functional pharmacology, and secondary messenger coupling systems. The GPCRs of the 5-HT_1 group are negatively coupled to adenylyl cyclase and have been characterized as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}.[2−5] The human 5-HT_{1B} and 5-HT_{1D} receptors (previously known as 5-$HT_{1D\beta}$ and 5-$HT_{1D\alpha}$, respectively) are especially similar in sequence despite being encoded by two distinct genes.[6,7] Although human 5-HT_{1B} and 5-HT_{1D} receptors have been pharmacologically differentiated using nonselective 5-$HT_{1B/D}$ receptor antagonists such as ketanserin (**1**) and ritanserin[8−10] (**2**) (Chart 1), the precise function of these receptors remains undefined, and progress toward this has been hampered by the lack of selective ligands. The 5-HT_{1B} receptor is of particular interest since it has been demonstrated that this receptor is present in the brain in higher densities than 5-HT_{1D}.[11] It has also been shown that the majority of terminal 5-HT autoreceptors in rat, guinea pig, and human are of the 5-HT_{1B} receptor subtype and that these receptors elicit negative feedback control on the release of 5-HT from serotonergic nerve terminals in the central nervous system (CNS).[12−17] Since there is evidence that enhancement of 5-HT

Chart 1



1 ketanserin **2** ritanserin

3 methiothepin **4** GR 127935

5 SB-224289

* To whom correspondence should be addressed.
‡ Department of Medicinal Chemistry.
† Department of Neuroscience Research.

S0022-2623(97)00457-3 CCC: $15.00
Published on Web 03/20/1998

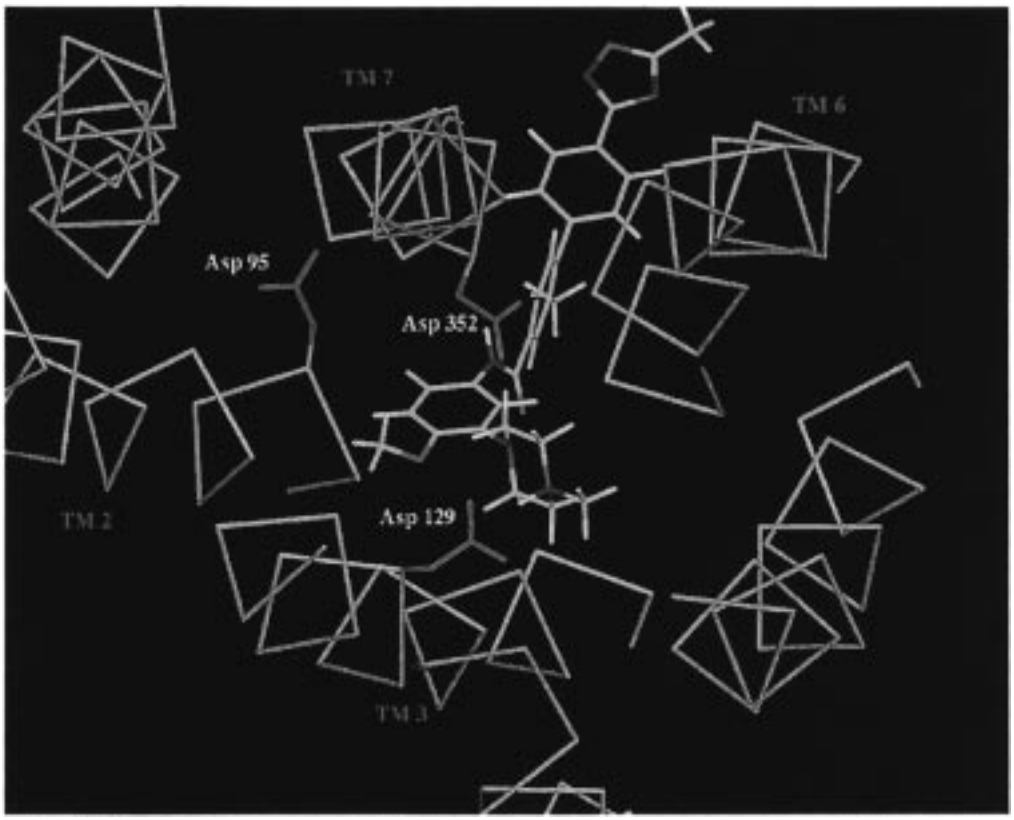


Figure 1. Attempted docking of **4** into model of human 5-HT_{1B} receptor with piperazine binding to Asp 129 on TM3.

neurotransmission underlies the therapeutic response to several types of antidepressant agent,[18,19] it has been postulated that a selective brain-penetrant 5-HT_{1B} receptor antagonist might act as a fast-acting antidepressant.[20] The 5-$HT_{1B/D}$ receptor antagonist methiothepin (**3**) and the 5-$HT_{1B/D}$ receptor antagonist **4** (GR 127935) (Chart 1) have been reported to increase extracellular 5-HT when perfused into the frontal cortex of the guinea pig, although when administered systemically they both decrease extracellular 5-HT in this region.[21−23] Although attempts have been made to rationalize these findings, neither compound is an ideal tool for studying terminal 5-HT autoreceptor function. Methiothepin has affinity for a number of receptors including 5-HT, dopamine, histamine, and adrenoceptors. Compound **4** does not discriminate between 5-HT_{1B} and 5-HT_{1D} receptor subtypes and also has affinity for 5-HT_{1A} and 5-HT_{2A} receptors.[24] It has also been demonstrated that **4** displays partial agonist activity in recombinant cell lines expressing functional human cloned receptors.[25] Using **4** as a lead for chemistry, we have now identified **5**, 1′-methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]-carbonyl]-2,3,6,7-tetrahydro[spiro[furo[2,3-*f*]indole-3,4′-piperidine] (SB-224289) (Chart 1), as the first selective 5-HT_{1B} receptor (terminal 5-HT autoreceptor) antagonist.

Computational models of the 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{2A} receptors were constructed using the GPCR Builder program[26] and a protocol previously described for our model of the 5-HT_{2C} receptor.[27] Studies were then conducted to investigate possible modes of binding of **4** to these receptors. It is generally accepted that 5-HT receptor agonists and antagonists bind their protonated amino sites to the highly conserved aspartate (Asp 129)[28] on transmembrane helix 3 (TM3).[29,30] However attempts to dock **4** into the receptors in this way indicated that this mode of binding was energetically unfavorable and resulted in part of the molecule extending between helices 6 and 7 into the lipid bilayer (Figure 1). As the protonated nitrogen of these ligands is an essential feature for binding, alternative acidic residues within the transmembrane bundle were sought. All members of the rhodopsin GPCR family (which includes the various 5-HT sequences) have a totally conserved aspartate (Asp 95) on transmembrane helix 2 (TM2), and this has been implicated in GPCR receptor activation.[31] On the other hand, sequence analysis of the 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1E} receptors reveals the presence of a third aspartate residue (Asp 352) on transmembrane helix 7 (TM7). This residue is not present in other 5-HT_1 or 5-HT_2 receptor subtypes. Alternative modes of binding, involving these two aspartates, were therefore evaluated. The principal difference between the 5-$HT_{1B/D}$ and 5-HT_{1E} receptors is the presence of a "Lys-Glu" pair at the extracellular side of TM6 in the 5-HT_{1E} receptor instead of the less polar "Ile-Ser" (5-HT_{1B}) or "Val-Ser" (5-HT_{1D}). In our models of these receptors, this lysine forms a salt bridge with the aspartate on TM7, and this has been used to explain the observed approximate 1000-fold lower affinity of the agonist 5-carboxamidotryptamine (5-CT) for the 5-HT_{1E} over the 5-HT_{1B} receptor.[32] Furthermore, it has been reported that mutation of this "Lys-Glu" pair to the corresponding 5-HT_{1B} sequence results in a switch in the pharmacology of these receptors.[33] Compound **4** is known to bind only weakly to 5-HT_{1E} receptors (pK_i 5.5 vs [^{3}H]-5-HT), and this would therefore suggest that it is the aspartate on TM7 which is primarily involved in the binding of this compound. When **4** was docked into

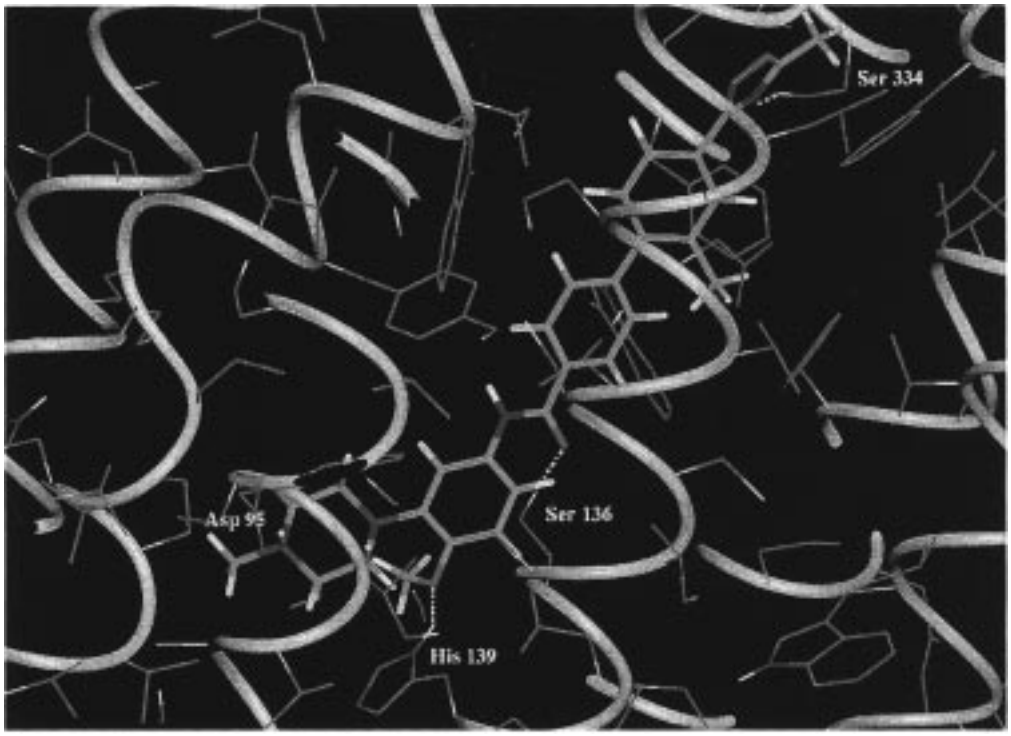


Figure 2. Docking of **4** into model of human 5-HT_{1B} receptor with piperazine binding to Asp 95 on TM2.

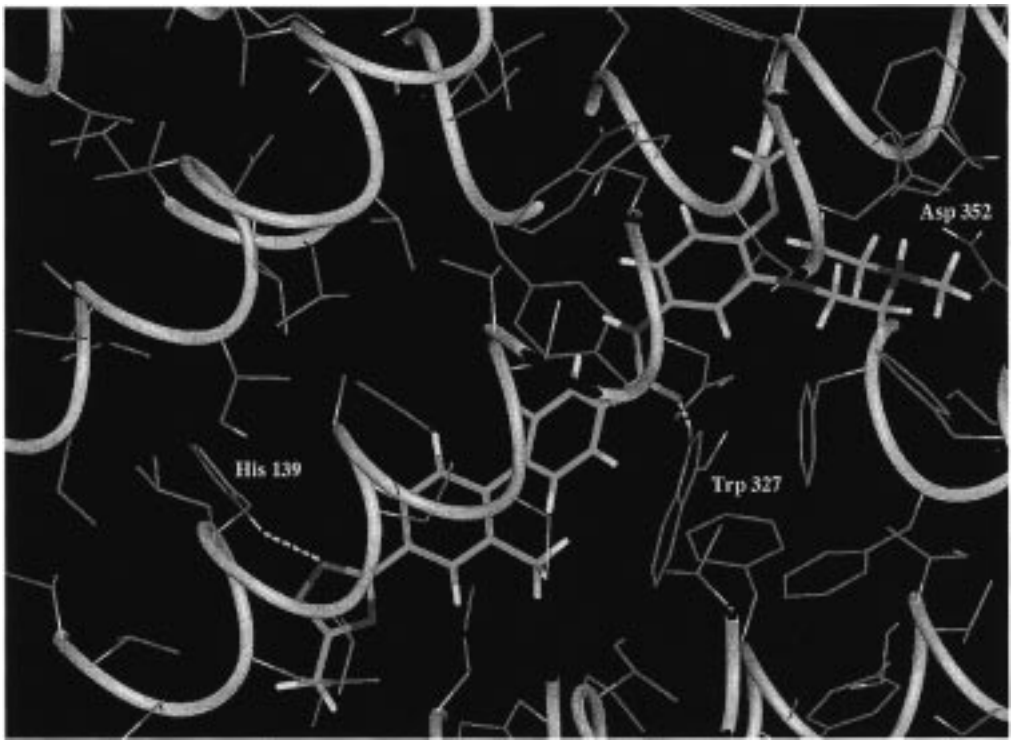
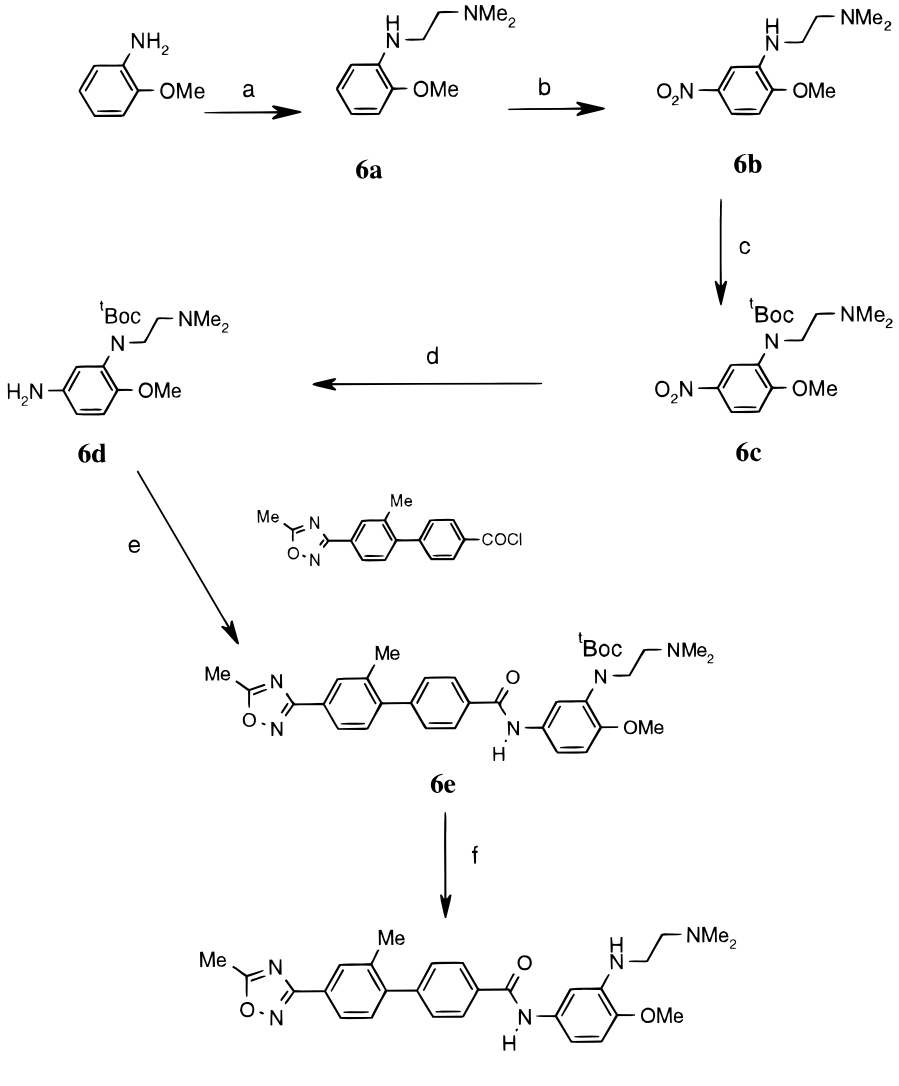


Figure 3. Docking of **4** into model of human 5-HT_{1B} receptor with piperazine binding to Asp 352 on TM7.

the 5-HT_{1B} receptor model with the protonated nitrogen bound either to the aspartate on TM7 or to the aspartate on TM2, it was found that both binding modes were predicted to be energetically favorable. In each case the biphenyl moiety was stabilized within a hydrophobic pocket, and in addition a number of favorable hydrogen-bonding interactions were possible (Figures 2 and 3). Since the aspartate on TM2 has been implicated in agonist function, it might be concluded therefore that ligands which interact directly with this residue would function as agonists or partial agonists. Hutchins[34] has suggested that dopamine agonists act by a bridging interaction between the aspartates on TM2 and TM3 rather than the more generally accepted bridging mode between the aspartate on TM3 and the serines on TM5.[28] On the other hand, compounds which bind to the aspartate on TM7 only would be expected to act as antagonists. Following on from this hypothesis, compounds which can bind to either site and sit in an equilibrium between the two binding modes would be

Scheme 1[a]



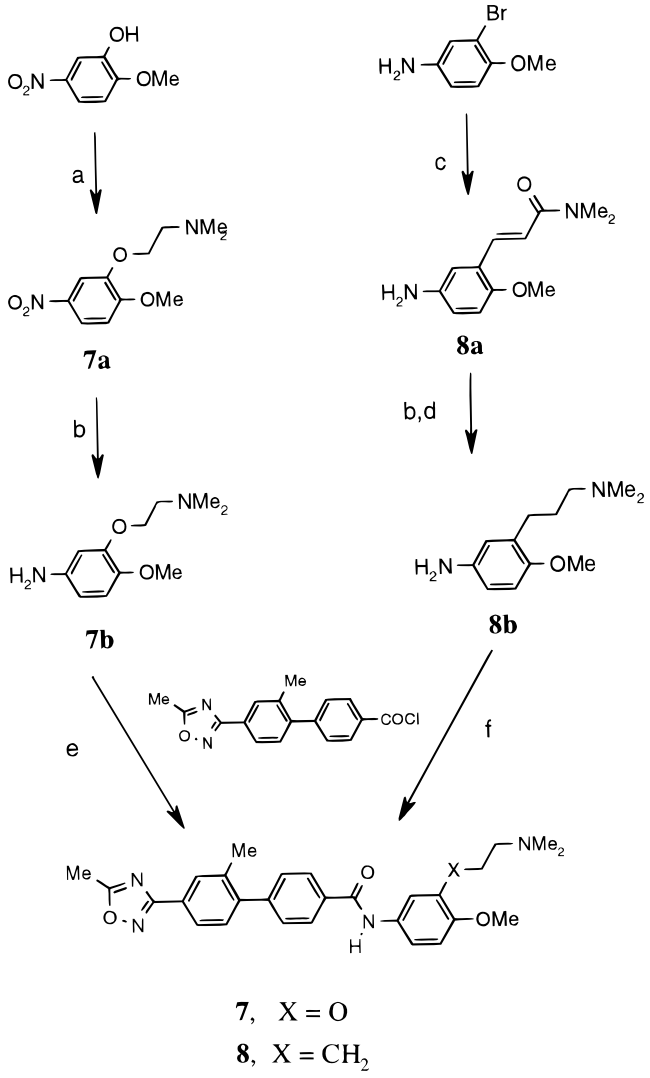
[a] Reagents: (a) $ClCH_2CH_2NMe_2 \cdot HCl$, Na_2CO_3, EtOH, Δ; (b) KNO_3, cH_2SO_4; (c) $(^tBoc)_2O$, Et_3N, CH_2Cl_2; (d) H_2, 10% Pd/C; (e) NaOH, H_2O, THF; (f) TFA, CH_2Cl_2.

expected to be partial agonists with the degree of partial agonism related to the relative binding energies of the two modes. Since our aim was to identify a 5-HT_{1B} receptor-selective compound with no intrinsic activity, our working hypothesis was to identify structural features and optimize these to favor binding with the aspartate on TM7 over that on TM2. It was also rationalized that a compound which binds preferentially to TM7 might have reduced affinity for receptors other than 5-HT_{1B} and 5-HT_{1D} which do not have the third aspartate residue (Asp 352) on TM7 (notably 5-HT_2). However, although the computational models served as a guide for achieving these targets, the close similarity between the 5-HT_{1B} and 5-HT_{1D} receptor models precluded their use for discriminating between these two receptor subtypes. In the model of **4** bound to the aspartate on TM2, the *o*-methoxy group sat out of plane from the phenyl ring and was predicted to be involved in a hydrogen bond with the conserved histidine 139 on TM3. No corresponding role could be found for the methoxy group in the alternative binding mode to the TM7 aspartate. Furthermore in the TM2 binding mode, the carbonyl of the amide could H-bond to the hydroxyl of serine 136 on TM3 provided that the amide was rotated out of plane with the piperazine-containing phenyl ring. In the alternative TM7 binding mode, the in-plane amide carbonyl was stabilized by a strong H-bond with the NH of the highly conserved tryptophan 327 on TM6. In both modes of binding, the 1,2,4-oxadiazole was capable of acting as a hydrogen bond acceptor (with either Ser 334, TM2, or His 139, TM7), so no preference for either binding mode was evident. Areas pinpointed for modification therefore were the arylpiperazine and the amide linker group.

Chemistry

The methods of preparation of test compounds **5**−**15** are shown in Schemes 1−8 and have been previously reported in the patent literature.[35−40] Alkylation of 2-methoxyaniline was effected with *N*,*N*-dimethylaminoethyl chloride hydrochloride and sodium carbonate in ethanol at reflux to give the ethylenediamine **6a** which was nitrated using potassium nitrate in concentrated sulfuric acid to give **6b**. Reaction with di-*tert*-butyl dicarbonate gave the carbamate **6c** which was reduced to the aniline **6d** by catalytic hydrogenation. 2′-Methyl-

Scheme 2[a]



[a] Reagents: (a) $ClCH_2CH_2NMe_2 \cdot HCl$, K_2CO_3, Me_2CO, H_2O; (b) H_2, 10% Pd/C, EtOH; (c) $CH_2{=}CHCONMe_2$, $Pd(OAc)_2$, Et_3N, DMF; (d) $LiAlH_4$, THF, Δ; (e) NaOH, H_2O, THF; (f) Et_3N, CH_2Cl_2.

4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid[41] was converted to the acid chloride and coupled to the aniline **6d** using Schotten−Baumann conditions to give the amide **6e**, which was converted to **6** by treatment with TFA in dichloromethane (Scheme 1).

Compound **7** was prepared using similar methodology from 2-methoxy-5-nitrophenol. *N*,*N*-Dimethyl-3-(5-amino-2-methoxyphenyl)acrylamide (**8a**) was prepared from 4-amino-2-bromoanisole via a Heck reaction and then reduced by catalytic hydrogenation followed by treatment with $LiAlH_4$ to give *N*,*N*-dimethyl-3-(5-amino-2-methoxyphenyl)propylamine (**8b**). Coupling with 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid was effected as before to give the amide **8** (Scheme 2).

2,3-Dihydrobenzofuran-7-carboxylic acid[42] was converted to the corresponding acetyl derivative **9a** by reaction with methyllithium. Nitration of **9a** with potassium nitrate in concentrated sulfuric acid gave **9b** which was converted to the phenol **9c** via Baeyer−Villiger rearrangement and base hydrolysis. Alkylation of **9c** with *N*,*N*-dimethylaminoethyl chloride hydrochloride and potassium carbonate in DME gave the ether **9d** which was reduced to aniline **9e** by catalytic hydrogenation. Coupling as before gave the amide **9** (Scheme 3).

Table 1. 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{2A} Receptor Binding Affinities (pK_i Values) of Compounds **4**−**15**[a,b]

compd	5-HT_{1B}	5-HT_{1D}	5-HT_{2A}
4	9.0 ± 0.07 (10)	8.6 ± 0.05 (5)	7.8 ± 0.05 (6)
5	8.2 ± 0.05 (12)	6.3 ± 0.09 (12)	5.8 ± 0.06 (3)
6	8.6 ± 0.05 (4)	7.4 ± 0.19 (4)	8.3, 8.6
7	9.0 ± 0.04 (23)	7.5 ± 0.03 (23)	7.3, 7.4
8	8.5 ± 0.12 (4)	7.0 ± 0.11 (4)	8.2, 8.5
9	8.8 ± 0.04 (5)	7.6 ± 0.08 (5)	7.3, 7.3
10	8.6 ± 0.07 (9)	7.7 ± 0.07 (9)	8.1, 8.4
11	9.3 ± 0.12 (6)	8.3 ± 0.04 (4)	6.5, 6.6
12	8.2 ± 0.4 (3)	7.9 ± 0.02 (3)	6.7, 6.9
13	6.9 ± 0.11 (3)	6.7 ± 0.06 (3)	6.4, 6.5
14	7.9 ± 0.02 (3)	7.4 ± 0.13 (4)	6.9, 6.9
15	7.5 ± 0.05 (9)	5.7 ± 0.05 (5)	6.0, 6.2

[a] All human cloned receptors. [b] Values are represented as mean ± SEM; *n* is in parentheses. For $n = 2$ determinations, individual values are shown.

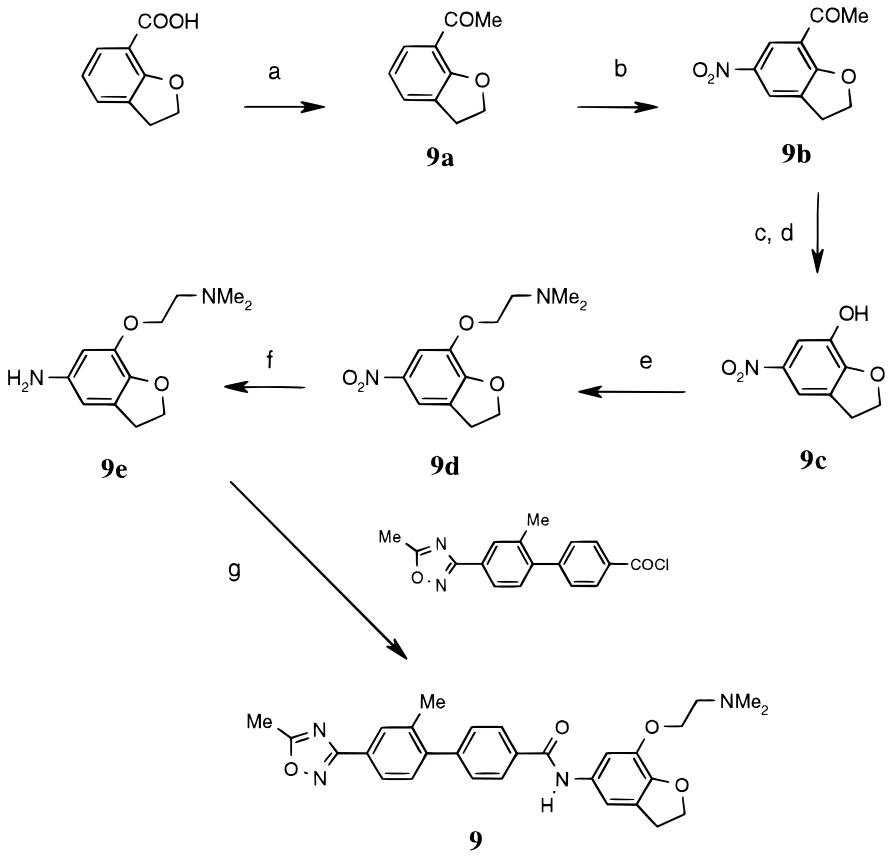
The benzoxazine **10** was prepared according to Scheme 4. 6-Nitro-2*H*-1,4-benzoxazin-3(4*H*)-one[43] was converted to the (dimethylamino)ethyl derivative **10a** by treatment with sodium hydride and 2-(dimethylamino)-ethyl chloride in toluene. Reduction of this amide with diborane gave the nitrobenzoxazine **10b** which was reduced by catalytic hydrogenation to the aniline **10c**. Coupling with 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid chloride using triethylamine in dichloromethane gave **10**. 2,2-Dimethoxyacetaldehyde was converted to the dimethyl acetal **11a** by reductive amination with 3-[2-(dimethylamino)ethoxy]-4-methoxyaniline, **7b**. Cyclization to the indole **11b** was carried out by heating under reflux with trifluoroacetic anhydride in trifluoroacetic acid. Reduction of **11b** to the 2,3-dihydroindole **11c** was effected with sodium cyanoborohydride in acetic acid. Coupling with 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid chloride under Schotten−Baumann conditions gave **11** (Scheme 5).

The aryl piperazines **12**−**14** were prepared in a similar manner from the previously reported anilines[35] (Scheme 6). The ether **15a** was prepared from 2-iodophenol using Mitsunobu conditions. Cyclization to the spiropiperidine **15b** was carried out using tributyltin hydride in benzene in the presence of AIBN. Nitration with copper nitrate in acetic anhydride gave **15c** which was reduced by catalytic hydrogenation to the aniline **15d**. Coupling with 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid gave **15** (Scheme 7). Compound **5** was prepared in a similar way from 1-acetyl-6-bromo-2,3-dihydro-1*H*-indol-5-ol[44] (Scheme 8).

Results and Discussion

Structure−activity relationships were determined from affinity values obtained using radioligand binding in human cloned receptors (summarized in Table 1). In vitro functional data were obtained using [^{35}S]GTPγS radioligand binding in CHO cells expressing human cloned 5-HT_{1B} receptors as previously described.[45,46] (Table 2). Removal of conformational restraint of the piperazine in **4** to give the (dimethylamino)ethylamine **6** resulted in an improvement in 5-HT_{1B} vs 5-HT_{1D} receptor selectivity to 15-fold, although 5-HT_{2A} affinity

Scheme 3[a]



[a] Reagents: (a) MeLi, Et_2O; (b) KNO_3, cH_2SO_4; (c) mCPBA, $CHCl_3$, TsOH; (d) NaOH, H_2O, EtOH; (e) $ClCH_2CH_2NMe_2$·HCl, K_2CO_3, DME, Δ; (f) H_2, 10% Pd/C, EtOH; (g) NaOH, H_2O, THF.

Table 2. Effect of Compounds **4**−**10** and **15** on [^{35}S]GTPγS Binding in CHO Cells Expressing 5-HT_{1B} Receptors[a]

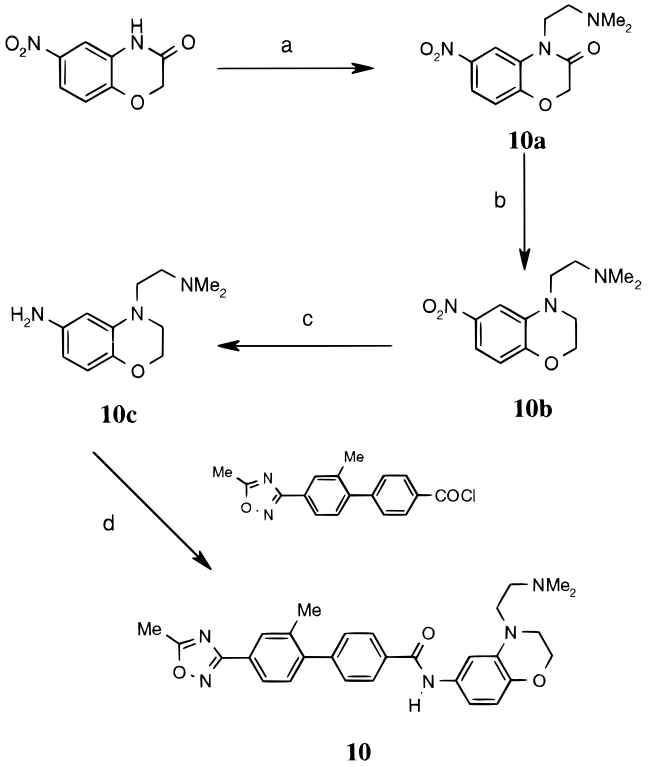
compd	% stimulation (mean ± SEM)	intrinsic activity (mean ± SEM)
5-HT	219 ± 31	1.0
4	143 ± 25	0.64 ± 0.01
5	−62 ± 4	<0
6	108 ± 19	0.49 ± 0.02
7	139 ± 25	0.63 ± 0.02
8	−44 ± 4	<0
9	207 ± 33	0.94 ± 0.02
10	91 ± 20	0.40 ± 0.02
15	−64 ± 4	<0

[a] All values represent the mean of at least three independent determinations. Data are expressed as the percent stimulation above basal when compounds are added at 1 μM. Basal stimulation is defined as the amount of [^{35}S]GTPγS specifically bound in the absence of compounds. The intrinsic activity for each compound is calculated as a percentage of the 5-HT response in the same assay. Thus the intrinsic activity of 5-HT is always 1, for partial agonists between 0 and 1, and for inverse agonists below 0.

was increased 5-fold. Replacement of the anilinic nitrogen of **6** with oxygen to give the ether **7** improved the selectivity for 5-HT_{1B} over 5-HT_{1D} to 30-fold, yet interestingly 5-HT_{2A} affinity was reduced 15-fold over that of **6**. The corresponding methylene derivative **8** was slightly less potent than **7** at 5-$HT_{1B/D}$ receptors and showed increased 5-HT_{2A} affinity, although it retained 30-fold selectivity for the 5-HT_{1B} vs 5-HT_{1D} receptor. However, an equally interesting finding was that compound **8** showed no intrisic activity in the [^{35}S]GTPγS functional model, whereas the two related compounds **6** and **7** were partial agonists in this system (Table 2). In these three compounds the methoxy group could be oriented so as to interact with histidine 139. However it was also feasible that with compounds **6** and **7**, the heteroatom of the (dimethylamino)ethyl-X chain could also be involved in an H-bonding interaction with histidine 139. In compound **6** this would consist of a bond from the NH of the ligand to the unprotonated nitrogen of the histidine; in **7** the histidine NH could donate an H-bond to the oxygen of the chain. Compound **8** had no such possible second interaction. To complicate matters further, the increased flexibility of all these compounds made it possible for the protonated amino group to now interact with the aspartate on TM3 (Asp 129). It was clearly evident however that the orientation of the methoxy oxygen electron lone pairs was important. Interaction with histidine 139 would be favored if they were oriented toward the aminoethyl chain and disfavored if they were oriented in the opposite direction.

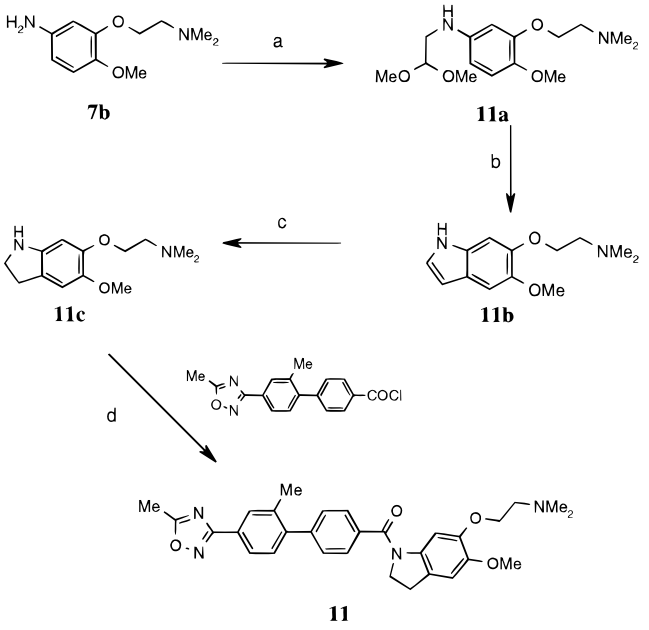
To investigate this further, the methoxy oxygen was incorporated into two different ring systems: the dihydrobenzofuran **9** and the benzoxazine **10**. Both compounds were potent 5-HT_{1B} receptor ligands, but whereas **9** displayed high intrinsic activity in the human cloned receptors, **10** was a weak partial agonist, supporting the idea that the direction of the oxygen electron density was influencing receptor activation by favoring primary binding to the aspartate on TM2 (Table 2). As already mentioned, the computational models suggested that restricting the conformational freedom of the amide linker group within a ring to evoke an in-plane carbonyl

Scheme 4[a]



[a] Reagents: (a) $ClCH_2CH_2NMe_2 \cdot HCl$, NaH, MePh, Δ; (b) BH_3, THF, Δ; (c) H_2, 10% Pd/C, EtOH; (d) Et_3N, CH_2Cl_2.

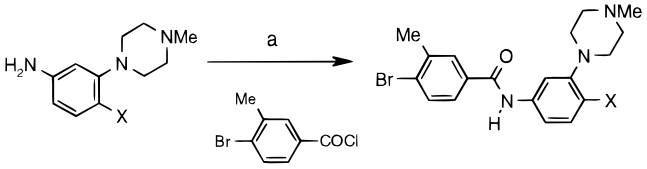
Scheme 5[a]



[a] Reagents: (a) $(MeO)_2CHCHO$, EtOH, H_2, 10% Pd/C; (b) TFA, TFAA; (c) $NaBH_3CN$, AcOH; (d) NaOH, H_2O, THF.

"right-hand side" aromatic might favor the interaction with the TM7 aspartate resulting in improved selectivity over 5-HT_2 receptors. This was due to an interaction with the NH of tryptophan 327 as opposed to the H-bond with serine 136 which arose from the out-of-plane orientation favored for binding to aspartate 95. Cyclization of the amide of compound **7** to give the indoline **11** resulted in a 6-fold loss of 5-HT_{2A} affinity over **7**. This cyclization also resulted in a slight increase in 5-HT_{1B} affinity while retaining 10-fold selectivity for the 5-HT_{1B} over the 5-HT_{1D} receptor. The models also suggested that the piperazine in compound **4** preferred to lie out of plane with the plane of the adjacent aromatic ring. It was therefore feasible that the methoxy group could also be involved in stabilizing this conformation. The role of this substituent was briefly investigated in a model system based on compound **12**. Removal of the methoxy substituent of **12** to give **13** brought about a 20-fold reduction in 5-HT_{1B} affinity while 5-HT_{1D} affinity was reduced 15-fold. The corresponding methyl analogue **14** was only marginally less potent than **12**. This is in keeping with the hypothesis that the primary role of the ortho substituent in the TM7 binding mode is to orientate the protonated amino group, as no hydrogen-bonding role can be found for the methoxy in this pocket.

Scheme 6[a]



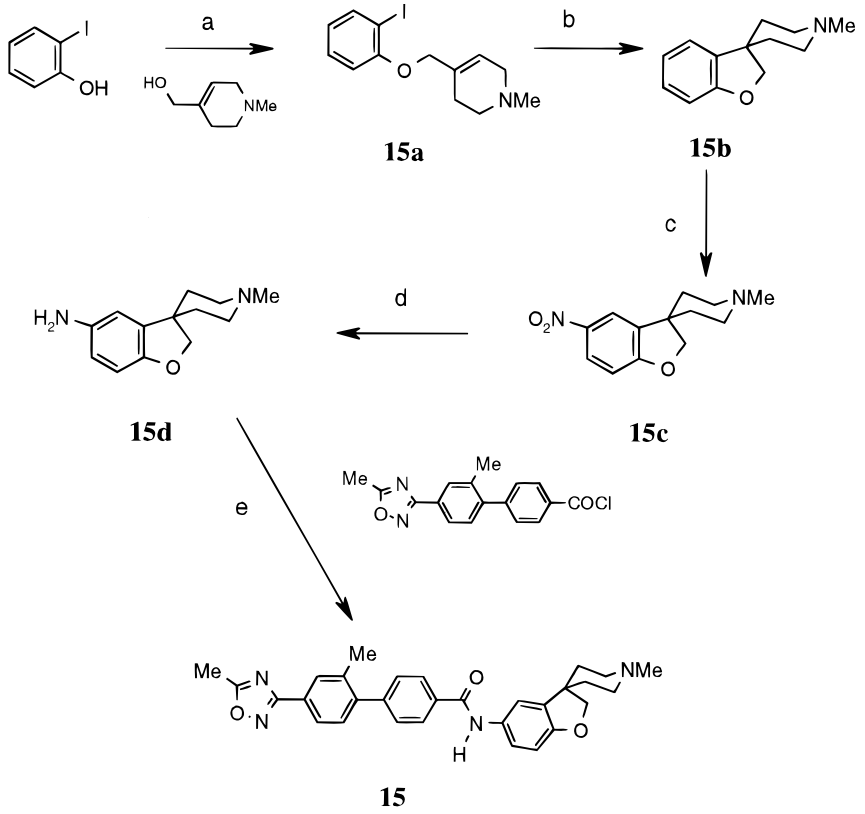
[a] Reagents: (a) NaOH, H_2O, THF.

Given that 5-HT_{1B} affinity could be retained when the anilinic nitrogen was replaced by methylene (compound **8**) and also considering that the orientation of the oxygen lone pairs in the benzoxazine reduced intrinsic activity, it was postulated that the spirocyclic piperidine **15**, in which the basic nitrogen-containing ring lies virtually orthogonal to the adjacent aromatic, could be of interest. Although this compound was less potent than the conformationally less restricted analogue **8** or the parent piperazine **4**, it was surprisingly much more selective for the 5-HT_{1B} vs 5-HT_{1D} receptor and, as expected, showed no intrinsic activity in the [^{35}S]GTPγS model (Table 2). In addition, 5-HT_{2A} affinity was reduced by 50- and 150-fold over **4** and **8**, respectively.

Applying the knowledge that cyclization to the indoline **11** had given improved 5-HT_{1B} potency and reduced 5-HT_{2A} affinity, we targeted compound **5** for synthesis. Computational studies with compound **5** indicated that binding into TM2 would not be favored. In contrast **5** could bind very favorably into TM7. In addition to stabilization evoked by the hydrogen-bonding interactions with His 319 and Trp 327, an interaction between the oxygen of the dihydrobenzofuran and Ser 128 was also favored (Figure 4). Compound **5**, SB-224289, retained high (80-fold) selectivity for the 5-HT_{1B} over 5-HT_{1D} receptor and showed >200-fold selectivity for 5-HT_{1B} over 5-HT_{2A} receptors. In the [^{35}S]GTPγS functional model in human cloned 5-HT_{1B} receptors, **5** also displayed inverse agonist activity. (Table 2, Figure 5). In radioligand binding studies, **5** was found to be highly selective over a range of other receptors (Table 3) and was therefore selected for further evaluation both in vitro and in vivo.

Secondary Evaluation in Vitro. In both human 5-HT_{1B} and 5-HT_{1D} receptor-expressing cell lines, 5-HT produced a concentration-dependent stimulation of basal

Scheme 7[a]



[a] Reagents: (a) DEAD, Ph_3P, THF; (b) Bu_3SnH, AIBN, C_6H_6, Δ; (c) $Cu(NO_2)_2$, Ac_2O; (d) H_2, 10% Pd/C, EtOH; (e) Et_3N, CH_2Cl_2.

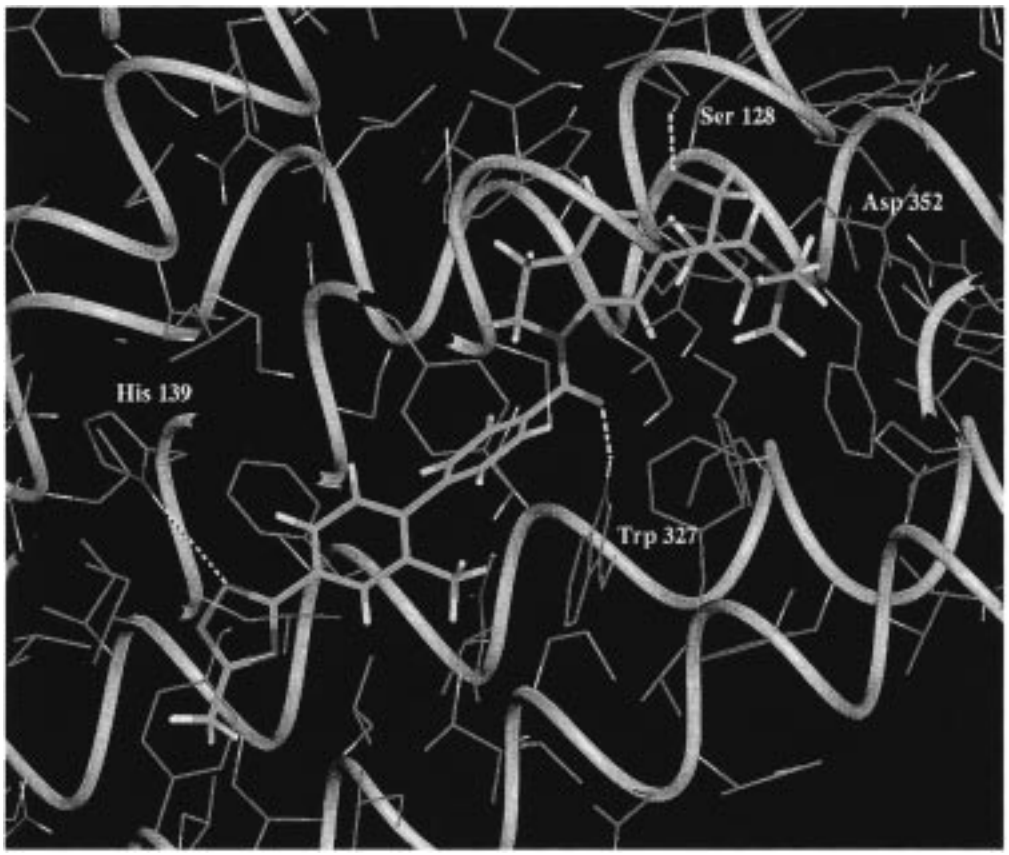
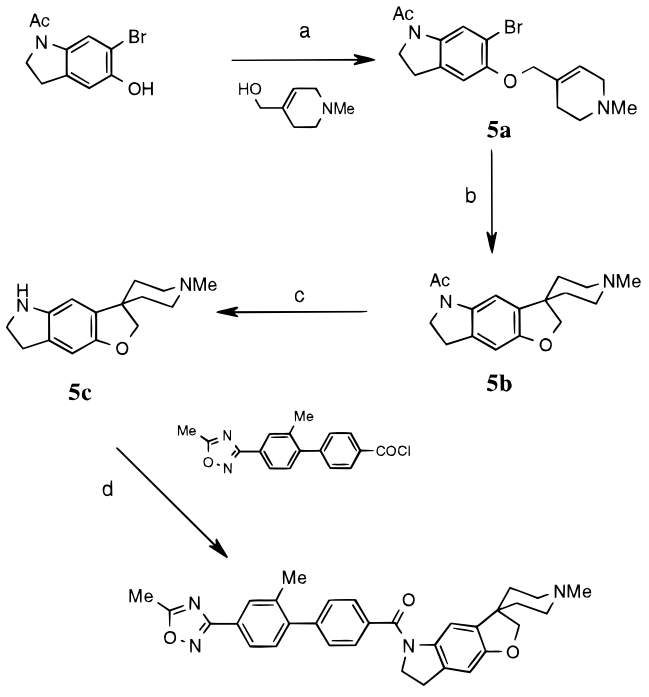


Figure 4. Docking of **5** into model of human 5-HT_{1B} receptor with spiropiperidine binding to Asp 352 on TM7.

[^{35}S]GTPγS binding with a maximal stimulation of 290% and 137% above basal levels, respectively. In contrast, **5** evoked a concentration-dependent inhibition of basal [^{35}S]GTPγS binding in both cell lines (Figures 5 and 6). Data for compound **4** are included for comparison. In antagonist studies at human 5-HT_{1B} and 5-HT_{1D} recep-

Scheme 8[a]



5, SB-224289

[a] Reagents: (a) DEAD, Ph_3P, THF; (b) Bu_3SnH, AIBN, C_6H_6, Δ; (c) HCl, EtOH, Δ; (d) Et_3N, CH_2Cl_2.

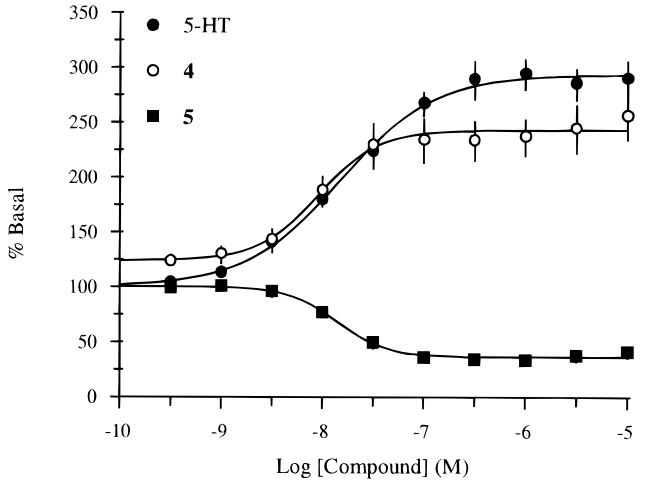


Figure 5. Inverse agonist effect of **5** in [^{35}S]GTPγS radioligand binding in human cloned 5-HT_{1B} receptors expressed in CHO cells (mean ± SEM, $n = 7$).

tors, compound **5** produced a rightward shift of the concentration curve to 5-HT with apparent pA_2 values of 8.4 ± 0.2 and 6.9 ± 0.1, respectively, demonstrating functional selectivity for the 5-HT_{1B} over the 5-HT_{1D} receptor (Figures 7 and 8). To evaluate terminal 5-HT autoreceptor function in vitro, **5** was investigated for its ability to influence 5-HT release in electrically stimulated guinea pig brain cortex slices. At concentrations of 100 nM and 1 μM, **5** evoked an increase in [^{3}H]-5-HT release, indicative of terminal 5-HT receptor antagonist activity (Figure 9).

Secondary Evaluation in Vivo. Compound **5** was evaluated in the previously reported SK&F 99101H-induced hypothermia model of central 5-$HT_{1B/D}$ receptor function in the guinea pig.[47] Compound **5** (2.2−22.0 mg/kg po) had no effect on body temperature alone (Table 4) but reversed SK&F 99101H-induced hypothermia giving an ED_{50} of 3.62 mg/kg po (Table 5). When dosed

Table 3. Receptor Binding Profile of **5**[a]

receptor	affinity (pK_i)	receptor	affinity (pK_i)
5-HT_{1A}	<5.5	α_{2A}	<6.0
5-HT_{1B}	8.2 ± 0.05 (12)	α_{2B}	<6.0
5-HT_{1D}	6.3 ± 0.09 (12)	β_1	<6.0
5-HT_{1E}	<5.0	β_2	<6.0
5-HT_{1F}	<5.0	D_2	<6.0
5-HT_{2A}	<6.0	D_3	<6.0
5-HT_{2C}	6.2 ± 0.06 (9)	H_1	<6.0
5-HT_4	<6.0	H2	<6.0
α_{1A}	<6.0	M_1	<6.0
α_{1B}	<6.0	M_2	<6.0

[a] All human cloned receptors except 5-HT_4 (piglet hippocampus, [^{125}I]SB 207710), α_{1A} (rat submaxillary gland, [^{3}H]prazosin), α_{1B}, (rat liver, [^{3}H]prazosin), α_{2B} (NG 108-15 cells, [^{3}H]RX821002), H_1 (guinea pig cerebellum, [^{3}H]pyrilamine), and H_2 (guinea pig striatum, [^{125}I]APT).

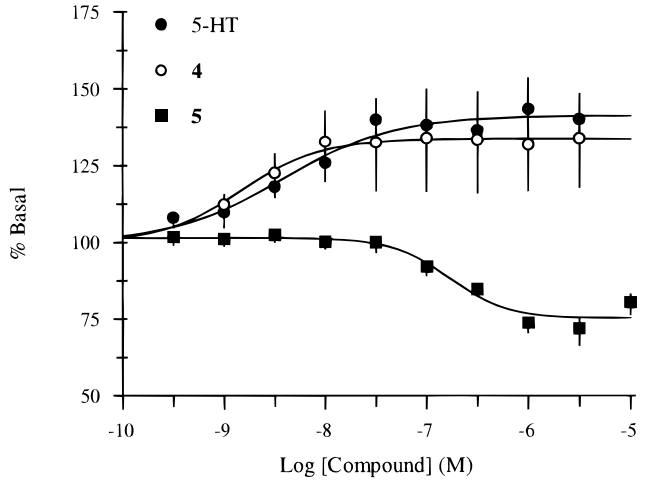


Figure 6. Inverse agonist effect of **5** in [^{35}S]GTPγS radioligand binding in human cloned 5-HT_{1D} receptors expressed in CHO cells (mean ± SEM, $n = 3$).

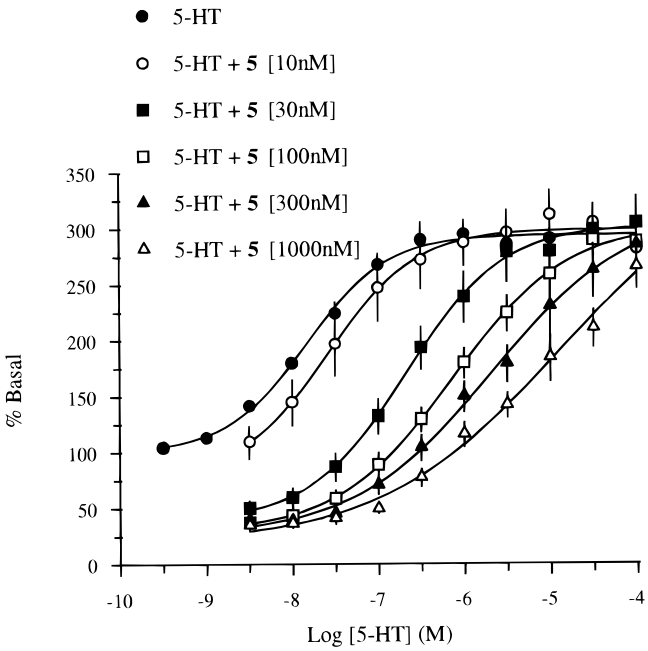


Figure 7. Effect of **5** on dose response to 5-HT in [^{35}S]GTPγS radioligand binding in human cloned 5-HT_{1B} receptors expressed in CHO cells (mean ± SEM, $n = 16$).

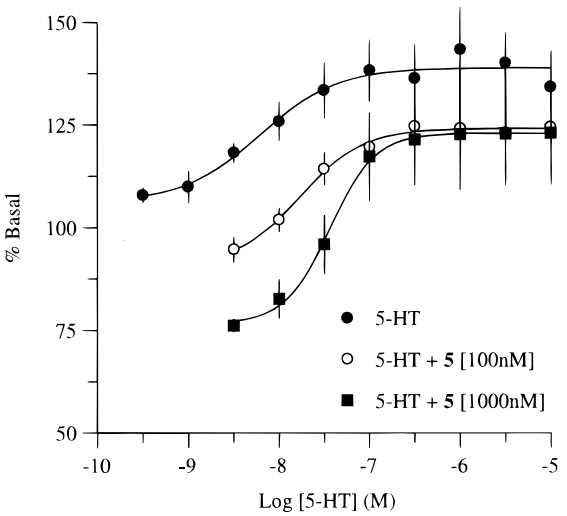


Figure 8. Effect of **5** on dose response to 5-HT in [^{35}S]GTPγS radioligand binding in human cloned 5-HT$_{1D}$ receptors expressed in CHO cells (mean ± SEM, $n = 6$).

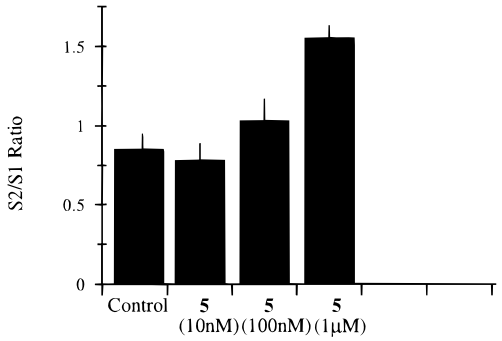


Figure 9. Effects of **5** on electrically evoked 5-HT release in guinea pig frontal cortex slices (mean ± SEM, $n = 4$).

Table 4. Lack of Effects of **5** (po) Alone on Body Temperature of the Guinea Pig[a]

treatment (dose, mg/kg)	body temp drop (°C, ±SEM)
vehicle	−0.07 ± 0.08
SK&F 99101H[b]	−2.00 ± 0.44**
5 (2.2)	−0.23 ± 0.11
5 (4.6)	−0.05 ± 0.11
5 (10)	−0.03 ± 0.07
5 (22)	−0.03 ± 0.11

[a] $n = 6$/group. [b] SK&F 99101H used as positive control. **$p < 0.01$ vs vehicle/vehicle controls.

Table 5. Effects of **5** (po) on Hypothermia Induced by the 5-HT$_{1B/D}$ Receptor Agonist SK&F 99101H (30 mg/kg ip)[a]

treatment (dose, mg/kg)	body temp drop (°C ±SEM)	% vehicle/SK&F 99101H controls
vehicle/vehicle	−0.12 ± 0.07	
vehicle/SK&F 99101H	−1.92 ± 0.23	100
5 (0.3)/SK&F 99101H	−1.77 ± 0.27	93
5 (1.0)/SK&F 99101H	−1.63 ± 0.18	85
5 (3.0)/SK&F 99101H	−1.00 ± 0.38*	52
5 (10.0)/SK&F 99101H	−0.67 ± 0.19**	35
ED_{50} (95% confidence limits)		3.62 (1.62−19.88)

[a] $n = 6$/group. *$p < 0.05$ vs vehicle/SK&F 99101H controls. **$p < 0.01$ vs vehicle/SK&F 99101H controls.

at 5 mg/kg po, the antagonist effects of **5** were still evident at 24 h postdose, indicating that this compound has a long pharmacodynamic duration of action (Table 6). Experimental details have been published.[48] Compound **5** was therefore evaluated for its effects on

Table 6. Effects of **5** (5.0 mg/kg po) on SK&F 99101H (30 mg/kg ip)-Induced Hypothermia (2−24 h Postdose)[a]

treatment (h postdose)	mean maximum temp drop (°C) (SEM) SK&F 99101H	**5**	% of SK&F 99101H control
2	−1.72 (0.09)	−0.38 (0.19)**	22
4	−1.73 (0.17)	−0.27 (0.13)**	15
8	−1.22 (0.27)	−0.53 (0.35)*	43
8[b]	−2.23 (0.13)	−1.10 (0.37)**	48
24[b]	−2.60 (1.37)	−1.31 (0.57)**	50

[a] $n = 6$/group. [b] Data from second, independent experiment. *$p < 0.05$ and **$p < 0.01$ vs SK&F 99101H controls.

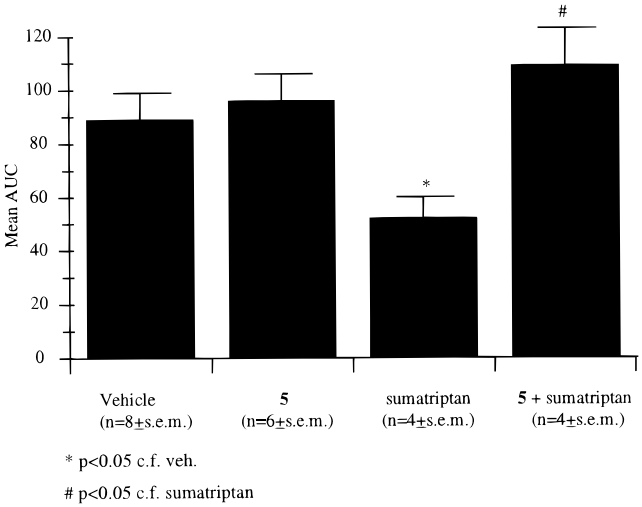


Figure 10. Effects of **5** on sumatriptan-induced inhibition of 5-HT release in the frontal cortex of the freely moving guinea pig.

cortical 5-HT release using microdialysis in the freely moving guinea pig as an in vivo model of terminal 5-HT$_{1B}$ autoreceptor function. The 5-HT$_{1B/1D}$ receptor agonist sumatriptan (1 μM), administered locally via the dialysis probe, significantly reduced 5-HT levels to 53% of controls, indicative of presynaptic terminal 5-HT$_{1B}$ receptor stimulation. Compound **5** (4 mg/kg po) significantly reversed this inhibition of 5-HT demonstrating its ability to act as a terminal 5-HT autoreceptor antagonist in vivo (Figure 10). Full experimental details are described elsewhere.[49] Recently we have also shown that compound **5** (4 mg/kg po) is able to significantly increase 5-HT levels in a different brain region, the guinea pig dentate gyrus, and this work is described in a separate publication. [50]

Conclusion

Using molecular modeling as a basis for stuctural modification of the nonselective 5-HT$_{1B/D}$ receptor partial agonist **4**, we have identified the selective 5-HT$_{1B}$ receptor ligand **5**. This compound behaves as an inverse agonist in CHO cells expressing human cloned 5-HT$_{1B}$ receptors and shows excellent oral activity with a long duration of action in a pharmacodynamic model of central 5-HT$_{1B}$ receptor-mediated function. In addition, **5** has been shown to act as a potent terminal 5-HT autoreceptor antagonist both in vitro and in vivo. As such, compound **5** provides an excellent new chemical tool for the investigation of 5-HT$_{1B}$ autoreceptor function in the CNS.

Experimental Section

Biological Assays. 1. Receptor Binding. Affinities of compounds were determined using competition binding assays to determine pK_i values at the various receptors. Human cloned 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors were expressed in HEK 293 cells. Human 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, and dopamine D_2 and D_3 receptors were all expressed in CHO cells. Affinities were measured as the displacement of [^{3}H]-5-HT from 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} receptors, [^{3}H]-8-OH-DPAT from 5-HT_{1A} receptors, [^{3}H]ketanserin from 5-HT_{2A} receptors, [^{3}H]mesulergine from 5-HT_{2C} receptors, and [^{3}H]-spiperone from dopamine D_2 and D_3 receptors. The incubation buffer used for 5-HT_1 receptors was Tris-Mg^{2+} (50 mM Trizma-HCl, pH 7.7 at 25 °C; 6 mM L-ascorbic acid; 10 mM $MgCl_2$; 0.5 μM pargyline), and 50 mM Tris-HCl (pH 7.7 at 25 °C) was used for 5-HT_{2A} and 5-HT_{2C} receptors. For dopamine D_2 and D_3 receptors the incubation buffer was 50 mM Tris (pH 7.7 at 25 °C), 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$. Affinity for the 5-HT_4 receptor was evaluated in piglet hippocampus, by displacement of [^{125}I]SB-207710 according to the method of Brown et al.[51] Affinities for α-adrenergic, β-adrenergic, histamine H_1 and H_2, and muscarinic M_1 and M_2 receptors were determined using literature procedures.[52−56] Briefly, the appropriate radioligand and a range of concentrations of test compound were incubated with the receptor preparation for between 30 and 45 min at 37 °C. At the end of the incubation period, samples were rapidly filtered and washed with ice-cold incubation buffer. Filters were dried, and specifically bound radiolabel was measured on a scintillation counter. Results are expressed as mean values (±SEM, n) or as individual values where $n = 2$.

2. [^{35}S]GTPγS Radioligand Binding. [^{35}S]GTPγS binding studies in CHO cells expressing human cloned 5-HT_{1B} or 5-HT_{1D} receptors were performed as previously described.[45,46] In brief, membranes from 1×10^6 cells were preincubated at 30 °C for 30 min, in HEPES buffer (20 mM HEPES, 3 mM $MgCl_2$, 100 mM NaCl, 0.2 mM ascorbate), containing 10 μM GDP, with or without test compounds. The reaction was started by the addition of 10 μL of [^{35}S]GTPγS (100 pM) followed by a further 30-min incubation at 30 °C. Nonspecific binding was determined by addition of unlabeled GTPγS (10 μM), prior to the addition of cells. The reaction was stopped by rapid filtration using Whatman GF/B grade filters followed by washing with ice-cold HEPES buffer. Radioactivity was determined by liquid scintillation spectrometry. Basal stimulation is the amount of [^{35}S]GTPγS specifically bound in the absence of compounds and was taken as 0% in each assay. Compounds were added at 1 μM, and the percent stimulation above basal was determined. The intrinsic activity for each compound was calculated as a percentage of the 5-HT response in each assay. Thus the intrinsic activity of 5-HT is always 1, for partial agonists between 0 and 1, and for inverse agonists below 0. Percent stimulation and intrinsic activity values are expressed as the mean (±SEM) of three or more separate determinations.

3. Effects on Electrically Stimulated 5-HT Release in Guinea Pig Frontal Cortex. Methodology has previously been described by Roberts et al.[57] Cerebral cortices were removed from male guinea pigs, cross-chopped into slices, and then incubated with [^{3}H]-5-HT. Slices were placed in a Brandel Superfusion System and superfused with oxygenated Kreb's solution. Transmitter release was evoked twice (S1 and S2), through electrical stimulation using a Brandel stimulator (2-ms biphasic square wave pulses, 20 mA in amplitude at a frequency of 3 Hz, to optimize the biphase concentration of endogenous 5-HT). Compounds were superfused prior to and during the S2 stimulation period. Released radioactivity in each sample was measured, and after basal release was subtracted, the S2/S1 ratio was calculated. Results represent the mean (±SEM) of four determinations.

4. SK&F 99101H-Induced Hypothermia Model of Central 5-$HT_{1B/D}$ Receptor Function in the Guinea Pig.[47] Male Dunkin−Hartley guinea pigs (Charles River UK Ltd.) weighing between 270 and 400 g were used. Animals were housed in groups of five or six, in a temperature-controlled environment (20 ± 1 °C), and maintained on a 12-h light/dark cycle for at least 5 days prior to use with free access to food and water. On test days animals were either housed in pairs in opaque observation cages (24 cm × 45 cm × 20 cm) at least 2 h prior to the start of the experiment for intraperitoneal (ip) dosing or food-deprived overnight in pairs in clear grid-bottomed starving cages for perioral (po) dosing. Rectal temperature was monitored using an electric thermometer (Comark, model 9001) coupled to a rectal probe (Comark, model BS4937). Animals were manually restrained and temperatures measured by inserting the probe approximately 4−5 cm into the rectum for a period of 15 s or until a stable reading was obtained. Compound **5** was administered perorally (po) at 45 min prior to injection of SK&F 99101H. SK&F 99101H or vehicle was administered ip at time 0. Temperature measurements were taken at 60 min (−60), 45 min (−45), and immediately prior to administration of SK&F 991091H. Further measurements were taken 45, 60, and 90 min after injection of SK&F 99101H. Temperature at each time point was expressed as a function of the temperature at time 0. To simplify the data analysis the peak temperature drop was then calculated for each animal. These peak drops provide a simple measure of the maximal drug effect and were averaged for each treatment group. A time course for each drug was also plotted. Changes in temperature were assessed using a one-way analysis of variance (ANOVA) with post hoc analysis carried out using Dunnett's t-tests. ED_{50} values and 95% confidence limits were calculated using a linear fit procedure in RS1.[58]

5. Microdialysis in the Freely Moving Guinea Pig. Male Dunkin−Hartley guinea pigs weighing between 350 and 450 g were used in all experiments. Animals were maintained on a 12-h light/dark cycle at 22 °C and given free access to food and water. Guinea pigs were anesthetized with 2% methoxyfluorane delivered with O_2 (1 L/min) and N_2O (3 L/min) in an induction chamber. On attaining surgical anesthesia the guinea pigs were transferred to a stereotaxic frame which had been adapted to accommodate an anesthetic mask and scavenging unit.[59] Anesthesia was maintained on 1−2% methoxyfluorane with O_2 (1 L/min), NO_2 (2 L/min). Dialysis probes were implanted into the frontal cortex (coordinates from the intra-aural zero: AP+15.0 mm, ML+2.0 mm, and 3.0 mm vertical from the dura, using the atlas of Rapisarda and Bacchelli).[60] Probes were secured with two skull screws and dental acrylic cement, and the wound was sealed. Animals were allowed 24 h for recovery, after which time the probes were perfused with artificial CSF at a rate of 2 μL/min. After 2-h perfusion, samples were collected every 20 min into 10 μL of 0.4 M perchloric acid. Three samples were taken to measure basal extracellular levels of 5-HT before drug treatment. Following drug treatments, 5-HT levels were measured for at least nine samples. Guinea pigs were dosed orally with vehicle or **5** after the third dialysis sample (i.e., 60 min from the start of the experiment), and samples were collected for 360 min in total; 45-μL dialysis samples were injected onto an HPLC system using center loop filling, and 5-HT was separated from other substances using reverse-phase ion pair chromatography. Separation was achieved at a flow rate of 1 mL/min with a 4.6-mm × 7.5-mm ODS2, 3-μm column (HPLC Technology Ltd.) and a mobile phase consisting of 0.15 M NaH_2PO_4, 0.8 mM sodium octylsulfate, 0.4 mM EDTA (pH 3.8), and 14% MeOH. The mobile phase was filtered through a 0.22-μm GS filter and degassed with helium. Data from experiments are reported as area under chromatogram peaks. The first three samples were averaged to yield a basal level of extracellular 5-HT. All samples were expressed as a percentage of basal levels. Percentages of basal levels for the individual time points posttreatment were accumulated and averaged, producing a value for the mean percent basal, which was an estimate of the mean area under the curve (AUC). Statistical comparisons of mean AUC following drug administration were calculated on an SAS-Research Scientist Application (v.1.4, release 6.08 (1992); SAS Institute Inc., Cary, NC 27513). Analyses were performed using a one-way ANO-

VA followed by a post hoc Tukey−Kramer *t*-test. Significance was taken at the 5% level.

Chemistry. ^{1}H NMR spectra were recorded on a Bruker AC-200 or AC-250 instrument using tetramethylsilane as internal standard. High-resolution mass spectra were obtained using a JEOL DX303 double focusing mass spectrometer with 5000 resolution using manual peak matching. Chromatography was performed on Merck Art. 7734 silica gel. Where required, tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone prior to use. HPLC analysis of test compounds was carried out on a Gilson 712 HPLC system, using a model 231 sample injector and 306 pump with 806 manomeric module detector. HPLC method A—a Hypersil BDS C18 3-μm (100- × 3-mm i.d.) column was used with elution using the following conditions: eluant A 0.1% TFA/H_2O v/v, eluant B 0.1% TFA/CH_3CN v/v; flow rate 0.7 mL/min. The eluation gradient was 0% B held for 0.5 min, then linearly increased to 75% B over 24.5 min, then held for 5 min. A detection wavelength of 218 nm was used. HPLC method B—a Merck RP-select B (125 × 4.6 mm) column was used, with elution using A 0.1% v/v TFA in H_2O, eluant B 0.1% v/v TFA in MeCN, with a flow rate of 1.0 mL/min. The elution gradient increase was from 5% B to 80% B over 40 min, then held at 80% B for 40 min. A detection wavelength of 218 nm was used. HPLC method C—a Symmetry C8 5-μm (150- × 3.9-mm i.d.) column was used with elution using eluant A 0.1% TFA v/v in H_2O, eluant B 0.1% TFA v/v in MeCN, with a flow rate of 1.0 mL/min. The elution gradient was held at 0% B for 0.5 min, then linearly increased to 90% over 24.5 min, then held for 5 min. The UV detection wavelength was 218 nm. Elemental analysis was carried out using a Control Equipment Corp. (CEC) 440 elemental analyzer. Where reported only by symbols, values were within 0.4% of theoretical limits.

***N*,*N*-Dimethyl-*N*′-(2-methoxyphenyl)ethylenediamine (6a).** A mixture of 2-methoxyaniline (6.20 g, 0.05 mol), *N*,*N*-dimethylaminoethyl chloride hydrochloride (8.6 g, 0.06 mol), and Na_2CO_3 (7.42 g, 0.07 mol) in EtOH (75 mL) was heated under reflux for 7 h. After cooling, the mixture was filtered and the filtrate concentrated in vacuo. The residue was dissolved in H_2O and the pH adjusted to 12 by the addition of 10% aqueous NaOH. The mixture was extracted into Et_2O, dried (Na_2SO_4), concentrated in vacuo, and purified by flash chromatography, eluting with EtOAc to give **6a** as a pale-orange oil (3.28 g, 48%). ^{1}H NMR ($CDCl_3$): δ 6.87 (1H, dt, H4), 6.75 (1H, dd, H6), 6.68 (1H, dd, H5), 6.60 (1H, dd, H3), 4.62 (1H, brs, NH), 3.82 (3H, s, OCH_3), 3.15 (2H, m, $NHCH_2$), 2.58 (2H, t, CH_2NMe_2), 2.25 (6H, s, $N(CH_3)_2$).

***N*,*N*-Dimethyl-*N*′-(2-methoxy-5-nitrophenyl)ethylenediamine (6b).** *N*,*N*-Dimethyl-*N*′-(2-methoxyphenyl)ethylenediamine, **6a** (1.5 g, 0.008 mol), was dissolved in 5 N H_2SO_4 (0.86 mL) and the resulting solution concentrated to dryness in vacuo. Concentrated H_2SO_4 (6.5 mL) was added, and the mixture was stirred until homogeneous and then cooled to 0 °C. Potassium nitrate (1.0 g, 0.01 mol) was added portionwise, maintaining the temperature below 10 °C; then the mixture was stirred for 4 h at room temperature. The reaction mixture was poured onto ice (150 mL) and made slightly alkaline by the addition of Na_2CO_3. The product was extracted into EtOAc and the extract dried (Na_2SO_4) and concentrated in vacuo to give **6b** (0.90 g, 49%) as an orange oil. ^{1}H NMR ($CDCl_3$): δ 7.62 (1H, dd, H4), 7.36 (1H, d, H6), 6.75 (1H, d, H3), 4.95 (1H, brs, NH), 3.94 (3H, s, OCH_3), 3.23 (2H, m, $NHCH_2$), 2.63 (2H, t, CH_2NMe_2), 2.29 (6H, s, $N(CH_3)_2$).

***N*,*N*-Dimethyl-*N*′-(*tert*-butyloxycarbonyl)-*N*′-(2-methoxy-5-nitrophenyl)ethylenediamine (6c).** *N*,*N*-Dimethyl-*N*′-(2-methoxy-5-nitrophenyl)ethylenediamine, **6b** (0.90 g, 0.0038 mol), in CH_2Cl_2 (50 mL) was treated with Et_3N (0.42 g, 0.004 mol) and di-*tert*-butyl dicarbonate (1.0 g, 0005 mol), and the reaction mixture stirred at room temperature for 24 h. The reaction mixture was washed with H_2O, dried (Na_2SO_4), and concentrated in vacuo to give **6c** (0.50 g, 39%) as an orange oil. ^{1}H NMR ($CDCl_3$): δ 8.28−8.10 (2H, m, H4, H5), 6.98 (1H, d, H3), 3.96 (3H, s, OCH_3), 3.72−3.28 (2H, br, NCH_2), 2.45 (2H, t, CH_2NMe_2), 2.24 (6H, s, $N(CH_3)_2$), 1.45−1.35 (9H, br, $C(CH_3)_3$).

***N*,*N*-Dimethyl-*N*′-(*tert*-butyloxycarbonyl)-*N*′-(5-amino-2-methoxyphenyl)ethylenediamine (6d).** A solution of *N*,*N*-dimethyl-*N*′-(*tert*-butyloxycarbonyl)-*N*′-(2-methoxy-5-nitrophenyl)ethylenediamine, **6c** (0.50 g, 0.0015moL), in EtOH (25 mL) was hydrogenated over 10% Pd/C at room temperature and pressure until H_2 uptake was complete. The catalyst was removed by filtration through Kieselguhr and the filtrate concentrated in vacuo to give **6d** (0.37 g, 80%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ 6.65 (1H, d, H6), 6.50 (2H, d, H3, H4), 3.68 (3H, s, OCH_3), 3.60−3.02 (4H, m, NH_2, NCH_2), 2.49, (2H, t, CH_2NMe_2), 2.16 (6H, s, NMe_2), 1.45−1.28 (9H, br, $C(CH_3)_3$).

***N*-[3-[*N*-[2-(Dimethylamino)ethyl]-*N*-(*tert*-butyloxycarbonyl)amino]-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxamide (6e).** A stirred solution of 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid (0.34 g, 0.0012 mol) in $SOCl_2$ (15 mL) was heated under reflux for 1 h. After cooling to room temperature, the excess $SOCl_2$ was removed in vacuo to give the acid chloride. A solution of *N*,*N*-dimethyl-*N*′-(*tert*-butyloxycarbonyl)-*N*′-(5-amino-2-methoxyphenyl)ethylenediamine, **6d** (0.37 g, 0.0012 mol), in THF (15 mL) was treated with a solution of NaOH (0.4 g, 0.1 mol) in H_2O (15 mL), and a solution of the acid chloride in THF (15 mL) was added. The reaction mixture was stirred overnight at room temperature. After removal of the solvent in vacuo, the residue was dissolved in CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), and concentrated in vacuo to give **6e** (0.40 g, 57%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ 8.10−7.61 (6H, m, biphenyl, H2, H3, H5, H6, H3′, H5′), 7.53−7.20 (4H, m, biphenyl H6′, H4, H6, CONH), 6.97−6.62 (1H, m, H3), 3.82 (3H, s, OCH_3), 3.51 (2NH, br, NCH_2), 2.70 (3H, s, CH_3), 2.62−2.41 (2H, m, CH_2NMe_2), 2.41−2.11 (9H, m, NMe_2, CH_3), 1.58−1.35 (9H, br, $C(CH_3)_3$).

***N*-[3-[[2-(Dimethylamino)ethyl]amino]-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxamide (6).** *N*-[3-[*N*-[2-(Dimethylamino)ethyl]-*N*-(*tert*-butyloxycarbonyl)amino]-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxamide, **6e** (0.40 g, 0.0007 mol), was dissolved in CH_2Cl_2 (20 mL) and TFA (5 mL). The solution was stirred at room temperature for 2.5 h, and the solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ solution, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel, eluting with CH_2Cl_2 with increasing concentrations of MeOH. The product **6** was isolated by conversion to its oxalate salt (0.25 g, 75%) as an off-white solid. ^{1}H NMR ($CDCl_3$): δ 10.08 (1H, s, COOH), 8.05 (2H, d, biphenyl, H2, H6), 8.00 (1H, s, NH), 7.92 (1H, d, biphenyl, H′5), 7.57 (2H, d, biphenyl, H3, H5), 7.44 (1H, d, biphenyl, H3′), 7.11 (2H, m, biphenyl, H6, H6), 6.82 (2H, d, H3, H4), 5.51−3.90 (1H, brs, NH), 3.78 (3H, s, OCH_3), 3.41 (2H, t, $NHCH_2$), 3.27 (2H, t, CH_2NMe_2), 2.79 (6H, s, $N(CH_3)_2$), 2.70 (3H, s, CH_3), 2.36 (3H, s, CH_3). HRMS (FAB): calcd for 486.250 (M^+), found 486.253. Purity was determined as 96.8% by HPLC (method A), retention time 17.35 min.

3-[2-(Dimethylamino)ethoxy]-4-nitroanisole (7a). A stirred solution of of 2-methoxy-5-nitrophenol (5.0 g, 0.03 mol) and K_2CO_3 (8.3 g, 0.06 mol) in acetone (200 mL) and H_2O (60 mL) was treated with *N*,*N*-dimethylaminoethyl chloride hydrochloride (8.64 g, 0.60 mol). The reaction mixture was heated under reflux for 10 h and then concentrated in vacuo to approximately 80-mL volume. The residue was acidified with 2 M HCl (150 mL) and washed with EtOAc. The acid solution was basified with K_2CO_3 and extracted with EtOAc (2 × 100 mL). These extracts were combined, dried (Na_2SO_4), and concentrated in vacuo to give **7a** (4.87 g, 70%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.92 (1H, dd, H3), 7.77 (1H, d, H5), 6.91 (1H, d, H2), 4.18 (2H, t, OCH_2), 3.96 (3H, s, OCH_3), 2.82 (2H, t, CH_2NMe_2), 2.37 (6H, s, NMe_2).

3-[2-(Dimethylamino)ethoxy]-4-methoxyaniline (7b). A solution of 3-[2-(dimethylamino)ethoxy]-4-nitroanisole, **7a** (4.8 g, 0.02 mol), in EtOH (200 mL) was hydrogenated over 10%

Pd/C at room temperature and pressure until hydrogen uptake was complete. The catalyst was removed by filtration through Kieselguhr and the filtrate concentrated in vacuo to afford **7b** (4.0 g, 95%) as a pink solid. ^{1}H NMR ($CDCl_3$): δ 6.71 (1H, d, H2), 6.33 (1H, d, H6), 6.24 (1H, d, H5), 4.07 (2H, t, OCH_2), 3.78 (3H, s, OCH_3), 3.46 (2H, brs, NH_2), 2.76 (2H, t, CH_2NMe_2), 2.33 (6H, s, NMe_2).

***N*-[3-[2-(Dimethylamino)ethoxy]-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxamide (7).** A stirred suspension of 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid (0.13 g, 0.0005 mol) in $SOCl_2$ (5 mL) was heated under reflux for 1 h. After cooling to room temperature, the solvent was removed in vacuo. A solution of 3-[2-(dimethylamino)ethoxy]-4-methoxyaniline, **7b** (0.10 g, 0.0005 mol), in THF (5 mL) was treated with a solution of NaOH (0.04 g) in H_2O (0.6 mL), and a solution of the acid chloride in THF (15 mL) was added. The reaction mixture was stirred overnight at room temperature. After removal of the solvent in vacuo, the residue was dissolved in CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography on silica gel eluting with CH_2Cl_2 with increasing concentrations of MeOH gave **7** (0.10 g, 43%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.07 (5H, m, biphenyl H2, H6, H′3, H′5, NH), 7.55−7.40 (3H, m, biphenyl H3, H5, H′6), 7.35 (1H, d, H2), 7.08 (1H, dd, H6), 6.87 (1H, d, H5), 4.18 (2H, t, OCH_2), 3.87 (3H, s, OCH_3), 2.82 (2H, t, CH_2NMe_2), 2.70 (3H, s, CH_3), 2.38 (9H, s, CH3, $N(CH_3)_2$). HRMS (FAB): calcd for 486.227 (M^+), found 486.227. Purity was determined as >99% by HPLC (method A), retention time 23.21 min.

***N*,*N*-Dimethyl-3-(5-amino-2-methoxyphenyl)acrylamide (8a).** 4-Amino-2-bromoanisole (1.70 g, 0.008 mol), tri-*o*-tolylphosphine (0.21 g, 0.007 mol), *N*,*N*-dimethylacrylamide (0.95 mL, 0.009 mol), Et_3N (2.9 mL, 0.002 mol), $Pd(OAc)_2$ (0.04 g, 0.002 mol), and dry DMF (4 mL) were heated together with stirring, under argon at 110 °C. After 4 h, the reaction mixture was allowed to cool and was partitioned between EtOAc and H_2O. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The residual oil was purified by column chromatography on silica using EtOAc increasing polarity to 10% MeOH/EtOAc to give **8a** (0.36 g, 20%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.83 (1H, d, H6), 6.95 (1H, d, H4), 6.87 (1H, d, H3), 6.75 (1H, d, =CH), 6.68 (1H, dd, =CH), 3.80 (3H, s, OCH_3), 3.48 (2H, s, NH_2), 3.18 (3H, s, NCH_3), 3.05 (3H, s, NCH_3).

***N*,*N*-Dimethyl-3-(5-amino-2-methoxyphenyl)propylamine (8b).** A solution of *N*,*N*-dimethyl-3-(5-amino-2-methoxyphenyl)acrylamide, **8a** (0.22 g, 0.001 mol), in EtOH (30 mL) was hydrogenated over 10% Pd/C until H_2 uptake was complete. The catalyst was removed by filtration through Kieselguhr and the filtrate concentrated in vacuo. The residue was dissolved in dry THF (15 mL) under an argon atmosphere and treated with $LiAlH_4$ (0.05 g, 0.0014 mol). The reaction mixture was heated under reflux for 4 h. After cooling, H_2O (0.05 mL) was added, followed by 10% aqueous NaOH (0.08 mL), followed by H_2O (0.13 mL). After stirring for a further 30 min, the mixture was filtered. Evaporation of the filtrate under reduced pressure gave **8b** (0.15 g, 80%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ 6.69 (1H, d, H6), 6.52 (2H, m, H3, H4), 3.75 (3H, s, OCH_3), 3.40 (2H, s, NH_2), 2.53 (2H, t, CH_2), 2.30 (2H, t, CH_2NMe_2), 2.20 (6H, s, $N(CH_3)_2$), 1.75 (2H, m, $CH_2CH_2CH_2$).

***N*-[3-[3-(Dimethylamino)propyl]-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxamide (8).** 2′-Methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-biphenyl-4-carboxylic acid (0.21 g, 0.007 mol) was suspended in CH_2Cl_2 (5 mL) and treated with oxalyl chloride (0.09 mL, 0.001 mol), followed by 1 drop of dry DMF with stirring. After 4 h, the reaction mixture was concentrated in vacuo to give the crude acid chloride as a yellow solid. A solution of *N*,*N*-dimethyl-3-(5-amino-2-methoxyphenyl)propylamine, **8b** (0.014 g 0.007 mol), in CH_2Cl_2 (10 mL) was treated with Et_3N (0.1 mL, 0.007 mol); then the crude acid chloride in CH_2Cl_2 (4 mL) was added. The reaction mixture was stirred for 1 h and then washed with H_2O followed by $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4) and concentrated in vacuo to give a brown oil, which was purified by column chromatography on silica gel using 7.5% MeOH in CH_2Cl_2 as eluant to give **8** (0.052 g, 16%), isolated as the oxalate salt. ^{1}H NMR (DMSO-d_6): δ 10.20 (1H, s, COOH), 8.08 (2H, d, biphenyl H2, H6), 7.98 (1H, brs, CONH), 7.91 (2H, d, biphenyl H3′, H5′), 7.65−7.58 (4H, m, biphenyl H6′, H3, H5, H2), 7.45 (1H, d, H6), 7.00 (1H, d, H5), 3.80 (3H, s, OCH_3), 3.08 (2H, t, CH_2), 2.75 (6H, s, $N(CH_3)_2$), 2.65 (3H, s, CH_3), 2.58 (2H, t, CH_2NMe_2), 2.32 (3H, s, CH_3), 1.90 (2H, m, $CH_2CH_2CH_2$). HRMS (FAB): calcd for 485.255 (M^+), found 485.254. Purity was determined as >99% by HPLC (method A), retention time 18.04 min.

7-Acetyl-2,3-dihydrobenzofuran (9a). To a cooled suspension of 2,3-dihydrobenzofuran-7-carboxylic acid (2 g, 0.012 mol), in dry Et_2O (100 mL) under argon, was added methyllithium (16.2 mL of a 1.5 M solution in Et_2O, 0.02 mol). The mixture was stirred at room temperature for 18 h and then added to 5 M HCl (50 mL). The product was extracted into EtOAc and the organic extract washed with aqueous K_2CO_3 and then H_2O and dried (Na_2SO_4). Evaporation of solvent gave **9a** (1.5 g, 76%) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.69 (1H, d, H6), 7.35 (1H, d, H4), 6.89 (1H, t, H5), 4.70 (2H, t, OCH_2), 3.25 (2H, t, CH_2), 2.61 (3H, s, $COCH_3$).

7-Acetyl-5-nitro-2,3-dihydrobenzofuran (9b). Potassium nitrate (0.50 g, 0.005 mol) was added portionwise over 0.5 h to a solution of 7-acetyl-2,3-dihydrobenzofuran, **9a** (0.50 g, 0.003 mol), in concentrated H_2SO_4 (5 mL) at 5−10 °C. The resulting solution was stirred for a further 2 h, then added to ice, basified with 40% aqueous NaOH, and extracted with EtOAc. The organic extract was dried (Na_2SO_4) and concentrated in vacuo to give **9b** (0.52 g, 81%) as a cream powder. ^{1}H NMR ($CDCl_3$): δ 8.65 (1H, s, 1H), 8.20 (1H, s, H4), 4.91 (2H, t, OCH_2), 3.36 (2H, t, CH_2), 2.65 (3H, s, $COCH_3$).

7-Hydroxy-5-nitro-2,3-dihydrobenzofuran (9c). A solution of 7-acetyl-5-nitro-2,3-dihydrobenzofuran, **9b** (0.50 g, 0.0024 mol), in $CHCl_3$ (3 mL) was added to *m*-chloroperoxybenzoic acid (0.83 g, 55%, 0.008 mol) in $CHCl_3$ (8 mL) containing a few crystals of *p*-toluenesulfonic acid. A further quantity of *m*-chloroperoxybenzoic acid (0.83 g, 0.008 mol) was added after 24 h and a further 0.40 g (0.004 mol) after another 24 h. After stirring at room temperature for 3 days under argon, the reaction mixture was washed with aqueous $NaHCO_3$ solution, dried (Na_2SO_4), and concentrated in vacuo. The residue was dissolved in EtOH (50 mL) containing 40% aqueous NaOH (5 mL) and stirred for 30 min at room temperature. The mixture was acidified by the addition of 5 M HCl and evaporated to dryness. The residue was partitioned between $CHCl_3$ and H_2O and the organic layer separated, dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography on silica eluting with 60−80 °C petroleum ether with increasing concentrations of EtOAc gave **9c** (0.29 g, 67%) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.80−7.67 (2H, m, H4, H6), 5.59 (1H, brs, OH), 4.80 (2H, t, OCH_2), 3.22 (2H, t, CH_2).

7-[2-(Dimethylamino)ethoxy]-5-nitro-2,3-dihydrobenzofuran (9d). A solution of 7-hydroxy-5-nitro-2,3-dihydrobenzofuran, **9c** (280 mg, 0.0015 mol), in DME (20 mL) was treated with *N*,*N*-dimethylaminoethyl chloride hydrochloride (0.49 g, 0.0033 mol) and K_2CO_3 (3 g, 0.02 mol), and the mixture was heated to reflux under argon for 18 h. The solvent was removed in vacuo and the residue partitioned between H_2O and EtOAc. The organic phase was removed and extracted with 5 M HCl. The acid layer was separated, washed with EtOAc, and then basified by the addition of K_2CO_3. The product was extracted into EtOAc, the resulting solution washed with H_2O and dried (Na_2SO_4), and the solvent removed in vacuo to give **9d** (0.28 g, 73%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 7.80 (1H, s, H6), 7.71 (1H, s, H4), 4.80 (2H, t, OCH_2), 4.2 (2H, t, $OCH_2CH_2NMe_2$), 3.31 (2H, t, CH_2), 2.8 (2H, t, CH_2NMe_2), 2.36 (6H, s, $N(CH_3)_2$).

5-Amino-7-[2-(dimethylamino)ethoxy]-2,3-dihydrobenzofuran (9e). A solution of 7-[2-(dimethylamino)ethoxy]-5-

nitro-2,3-dihydrobenzofuran, **9d** (0.27 g, 0.001 mol), in EtOH was hydrogenated over 10% Pd/C at room temperature and atmospheric pressure until hydrogen uptake ceased. The catalyst was removed by filtration and the filtrate concentrated in vacuo to give **9e** (0.28 g, 94%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 6.25 (1H, s, H6), 6.17 (1H, s, H4), 4.51 (2H, t, OCH_2), 4.19 (2H, t, $OCH_2CH_2NMe_2$), 3.95 (2H, brs, NH_2), 3.12 (2H, t, CH_2), 2.89 (2H, t, CH_2NMe_2), 2.44 (6H, s, $N(CH_3)_2$).

***N*-[7-[2-(Dimethylamino)ethoxy]-2,3-dihydrobenzofuran-5-yl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-biphenyl-4-carboxamide (9).** A stirred solution of 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid (0.17 g, 0.0006 mol) in $SOCl_2$ (5 mL) was heated under reflux for 1 h. After cooling to room temperature, the solvent was removed in vacuo. The resulting acid chloride was redissolved in THF (15 mL) and the solution treated with 5-amino-7-[2-(dimethylamino)ethoxy]-2,3-dihydrobenzofuran, **9e** (0.13 g, 0.0006 mol), in THF (10 mL) and a solution of NaOH (0.045 g, 0.0011 mol) in H_2O (0.5 mL). The mixture was stirred at room temperature overnight and the solvent removed in vacuo. The residue was partitioned between H_2O and CH_2Cl_2 and the organic phase separated, dried (Na_2SO_4), and concentrated in vacuo to give the crude product which was chromatographed on silica eluting with CH_2Cl_2 with increasing quantities of methanol to give **9** (0.12 g, 45%) as an off-white solid. ^{1}H NMR ($CDCl_3$): δ 8.07 (1H, s, CONH), 8.02−7.90 (4H, m, biphenyl, H2, H6, H3′, H5′), 7.45 (2H, d, biphenyl H3, H5), 7.33 (1H, d, biphenyl H6′), 7.25 (1H, s, H4), 7.12 (1H, s, H6), 4.60 (2H, t, OCH_2), 4.23 (2H, t, $OCH_2CH_2NMe_2$), 3.23 (2H, t, CH_2), 2.92 (2H, t, CH_2NMe_2), 2.70 (3H, s, CH_3), 2.48 (6H, s, $N(CH_3)_2$), 2.32 (3H, s, CH_3). HRMS (FAB): calcd for 499.234 (M^+), found 499.236. Purity was determined as 93.1% by HPLC (method A), retention time 17.45 min.

4-[2-(Dimethylamino)ethyl]-6-nitro-2*H*-1,4-benzoxazin-3(4*H*)-one (10a). To a suspension of 6-nitro-2*H*-1,4-benzoxazin-3(4*H*)-one (1.0 g, 0.0057 mol) in dry THF (20 mL) at 0 °C under argon was added NaH (0.2 g, 80% dispersion in mineral oil, 0.0057 mol). A solution of *N*,*N*-dimethylaminoethyl chloride (2.3 g, 0.028 mol) in dry toluene (15 mL) was added, and the reaction mixture was heated under reflux for 19 h. After cooling, H_2O was added dropwise until effervescence ceased; then the mixture was separated and the aqueous further extracted with EtOAc. The organic layers were combined, dried (Na_2SO_4), and evaporated under reduced pressure to give **10a** (1.10 g, 79%) as a pale-brown solid. ^{1}H NMR ($CDCl_3$): δ 8.03 (1H, d, H5), 7.94 (1H, dd, H7), 7.04 (1H, d, H8), 4.73 (2H, s, OCH_2CO), 4.11 (2H, t, NCH_2), 2.60 (2H, t, CH_2NMe_2), 2.35 (6H, s, $N(CH_3)_2$).

3,4-Dihydro-4-[2-(dimethylamino)ethyl]-6-nitro-2*H*-1,4-benzoxazine (10b). Boron trifluoride diethyl etherate (2 mL, 0.0016 mol) was added dropwise to a suspension of sodium borohydride (0.46 g, 0.0012 mol) in dry THF (30 mL) at 0 °C under argon. After 1 h, a solution of 4-[2-(dimethylamino)-ethyl]-6-nitro-2*H*-1,4-benzoxazin-3(4*H*)-one, **10a** (1.10 g, 0.004 mol), in dry THF (20 mL) was added, and the reaction mixture was heated under reflux for 2 h. After cooling in ice, aqueous $NaHCO_3$ was added dropwise until effervescence ceased; then the solvent was removed under reduced pressure and the residue dissolved in a mixture of EtOH (10 mL) and 5 M HCl (10 mL) and heated under reflux for 1 h. After cooling, the solvent was removed under reduced pressure. The residue was treated with saturated K_2CO_3 solution to pH 8 and then extracted with EtOAc. The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give **10b** (0.94 g, 92%) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.52 (2H, m, H5, H7), 6.78 (1H, d, H8), 4.30 (2H, t, $NCH_2CH_2NMe_2$), 3.42 (4H, m, OCH_2CH_2N), 2.56 (2H, t, CH_2NMe_2), 2.31 (6H, s, $N(CH_3)_2$).

6-Amino-3,4-dihydro-4-[2-(dimethylamino)ethyl]-2*H*-1,4-benzoxazine (10c). A solution of 3,4-dihydro-4-[2-(dimethylamino)ethyl]-6-nitro-2*H*-1,4-benzoxazine, **10b** (0.94 g, 0.004 mol), in EtOH (100 mL) was hydrogenated over 10% Pd/C at room temperature and atmospheric pressure until hydrogen uptake ceased. The catalyst was removed by filtration and the filtrate concentrated in vacuo to give **10c** (0.84 g, 100%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ 6.58 (1H, d, H5), 6.08 (1H, d, H7), 5.98 (1H, dd, H8), 4.13 (2H, t, $NCH_2CH_2NMe_2$), 3.35 (6H, m, OCH_2CH_2N, NH_2), 2.50 (2H, t, CH_2NMe_2), 2.30 (6H, s, $N(CH_3)_2$).

***N*-[4-[2-Dimethylamino)ethyl]-3,4-dihydro-2*H*-1,4-benzoxazin-6-yl)-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-biphenyl-4-carboxamide (10).** 2′-Methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid (0.19 g, 0.007 mol) was suspended in CH_2Cl_2 (15 mL) and treated with oxalyl chloride (0.065 mL, 0.074 mol), followed by 1 drop of dry DMF with stirring. After 4 h, the reaction mixture was concentrated in vacuo to give the crude acid chloride as a yellow solid. The solid was redissolved in CH_2Cl_2 (10 mL) and added to a solution of 6-amino-3,4-dihydro-4-[2-(dimethylamino)ethyl]-2*H*-1,4-benzoxazine, **10c** (0.14 g, 0.007 mol), in CH_2Cl_2 (10 mL) containing Et_3N (0.19 mL, 0.0014 mol) under argon. After 19 h at room temperature, the reaction mixture was treated with H_2O (20 mL) and extracted with CH_2Cl_2, and the combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified using flash chromatography on silica and CH_2Cl_2 to give **10** (0.90 g, 30%) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.02 (1H, s, CONH), 7.98 (3H, m, biphenyl H2, H6, H5′), 7.78 (1H, brs, biphenyl H3′), 7.48 (2H, d, biphenyl H3, H5), 7.35 (1H, d, biphenyl H6′), 7.22 (1H, s, H5), 6.77 (2H, brs, H7, H8), 4.23 (2H, t, OCH_2), 3.45 (4H, m, CH_2NCH_2), 2.70 (3H, s, CH_3), 2.59 (2H, t, CH_2NMe_2), 2.35 (3H, s, CH_3), 2.31 (6H, s, $N(CH_3)_2$). HRMS *m/e* 497.255 (M^+), found 497.256. Purity was determined as 95.5% by HPLC (method B), retention time 24.15 min.

***N*-[3-[2-(Dimethylamino)ethoxy]-4-methoxyphenyl]-aminoacetaldehyde Dimethyl Acetal (11a).** A solution of 3-[2-(dimethylamino)ethoxy]-4-methoxyaniline, **7b** (5.7 g, 0.027 mol), in EtOH (120 mL) was treated with a solution of 2,2-dimethoxyacetaldehyde in methyl tert-butyl ether (9.5 g, 40% solution, 0.036 mol), and the mixture was allowed to stand at room temperature for 16 h. The solution was then hydrogenated over 10% Pd/C at room temperature and atmospheric pressure for 7 h. The catalyst was removed by filtration through Kieselguhr and the filtrate concentrated in vacuo. The residue was dissolved in EtOAc and the solution washed with H_2O, dried (Na_2SO_4), and concentrated in vacuo. The product was purified by column chromatography on silica gel eluting with 5% MeOH, 95% $CHCl_3$ to give **11a** (5.4 g, 67%) as a red oil. ^{1}H NMR ($CDCl_3$): δ 6.75 (1H, d, H6), 6.30 (1H, d, H2), 6.19 (1H, dd, H5), 4.56 (1H, t, NH), 4.08 (2H, t, OCH_2), 3.78 (3H, s, OCH_3), 3.61 (1H, brt, $CH(OMe)_2$), 3.42 (6H, s, $O(CH_3)_2$), 3.20 (2H, t, $NHCH_2CH$), 2.78 (2H, t, CH_2NMe_2), 2.34 (6H, s, $N(CH_3)_2$).

6-[2-(Dimethylamino)ethoxy]-5-methoxy-1*H*-indole (11b). A stirred solution of *N*-[3-[2-(dimethylamino)ethoxy]-4-methoxyphenyl]aminoacetaldehyde dimethyl acetal, **11a** (5.3 g, 0.018 mol), in TFA (22 mL) at −5 °C under argon was treated dropwise over 40 min with TFAA (22 mL). After a further 30 min, TFA (33 mL) was added and the reaction mixture was heated at reflux for 7 h. The solvent was removed in vacuo and the residue basified with 10% aqueous Na_2CO_3 solution and extracted with EtOAc. The extract was dried (Na_2SO_4) and concentrated in vacuo and the residue dissolved in MeOH (100 mL), treated with K_2CO_3 (10 g), and stirred at room temperature for 3 h. The mixture was concentrated in vacuo and the residue treated with H_2O (60 mL) and extracted with EtOAc. The organic solution was dried (Na_2SO_4) and concentrated in vacuo to give **11b** (3.5 g, 83%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 7.14 (1H, brt, NH), 7.05 (1H, s, H7), 6.96 (1H, s, H4), 6.27 (1H, brs, H2), 4.03 (2H, t, OCH_2), 3.75 (3H, s, OCH_3), 3.45 (1H, brs, H3), 2.70 (2H, t, CH_2NMe_2), 2.27 (6H, s, $N(CH_3)_2$).

2,3-Dihydro-6-[2-(dimethylamino)ethoxy]-5-methoxy-1*H*-indole (11c). A stirred solution of 6-[2-(dimethylamino)-ethoxy]-5-methoxy-1*H*-indole, **11b** (0.50 g, 0.002 mol), in AcOH (10 mL) at room temperature was treated portionwise over 15 min with sodium cyanoborohydride (0.25 g, 0.004 mol).

After 2 h the solution was diluted with H_2O (50 mL), basified by addition of Na_2CO_3, and then extracted with EtOAc. The organic extract was dried (Na_2SO_4) and concentrated in vacuo to give **11c** (0.37 g, 73%) as a brown oil. ^{1}H NMR (DMSO-d_6): δ 6.75 (1H, s, H7), 6.37 (1H, s, H4), 4.05 (2H, t, OCH_2), 3.90 (1H, brs, NH), 3.77 (3H, s, OCH_3), 3.52 (2H, t, $NHCH_2$), 2.96 (2H, t, CH_2), 2.73 (2H, t, CH_2NMe_2), 2.32 (6H, s, $N(CH_3)_2$).

2,3-Dihydro-6-[2-(dimethylamino)ethoxy]-5-methoxy-1-[4-[2-methyl-4-(5-methyl-1,2,4-oxadiazol-3-yl)phenyl]-benzoyl]-1*H*-indole (11). A stirred suspension of 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid (0.17 g, 0.0055 mol) in thionyl chloride (5 mL) was heated under reflux for 2 h. The solution was concentrated in vacuo to leave the acid chloride as a yellow solid. This was redissolved in THF (5 mL) and added to a stirred solution of 2,3-dihydro-6-[2-(dimethylamino)ethoxy]-5-methoxy-1*H*-indole, **11c** (0.12 g, 0.0005 mol), in a mixture of H_2O (5 mL) and THF (5 mL) containing NaOH (0.045 g, 0.0011 mol). The mixture was kept at room temperature for 18 h, then concentrated to approximately 50% volume, diluted with 10% Na_2CO_3 solution (20 mL), and extracted with EtOAc. The organic extract was dried (Na_2SO_4) and concentrated in vacuo and the product purified by column chromatography on silica gel eluting with 5% MeOH, 95% $CHCl_3$. Crystallization from Et_2O afforded **11** (0.055 g, 21%) as a white solid. ^{1}H NMR (DMSO-d_6 at 80 °C): δ 7.96 (1H, s, H3′), 7.90 (1H, d, biphenyl H5′), 7.65 (2H, d, biphenyl H2, H6), 7.50 (2H, d, biphenyl H3, H5), 7.43 (1H, 1H, biphenyl H6′), 7.35 (1H, br, H7), 6.94 (1H, s, H4), 4.09 (2H, t, OCH_2), 3.93 (2H, brt, NCH_2), 3.76 (3H, s, OCH_3), 3.10−3.00 (2H, m, CH_2), 2.68 (3H, s, CH_3), 2.61 (2H, t, CH_2NMe_2), 2.36 (3H, s, CH_3), 2.22 (6H, s, $N(CH_3)_2$). HRMS *m/e* 512.239 (M^+), found 512.238. Purity was determined as 95.3% by HPLC (method B), retention time 22.85 min.

***N*-[3-(4-Methyl-1-piperazinyl)phenyl]-4-bromo-3-methylbenzamide (13).** A stirred suspension of 4-bromo-3-methylbenzoic acid (3.5 g, 0.016 mol) in thionyl chloride (100 mL) was heated under reflux for 2 h and then concentrated to dryness in vacuo. The resultant acid chloride was redissolved in THF (25 mL) and the solution added to a mixture of 3-(4-methyl-1-piperazinyl)aniline (2.0 g, 0.01 mol) in THF containing NaOH (0.56 g, 0.0014 mol) and H_2O (25 mL). The reaction mixture was stirred at room temperature for 4 h, then poured into H_2O, and extracted with CH_2Cl_2. The organic extract was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel using 1% MeOH, 99% CH_2Cl_2 as eluant to give **13** (1.1 g 28%) as an off-white solid. ^{1}H NMR ($CDCl_3$): δ 7.80 (1H, brs, CONH), 7.72 (1H, d, H6 adjacent to CO), 7.63 (1H, d, H2 adjacent to CO), 7.40−7.52 (2H, m, H5 adjacent to Br, H5), 7.22 (1H, d, H6), 6.95 (1H, d, H2), 6.71 (1H, dd, H4), 3.25 (4H, t, CH_2NCH_2), 2.58 (4H, t, CH_2NMeCH_2), 2.44 (3H, s, CH_3), 2.32 (3H, s, NCH_3). HRMS (FAB): calcd for 388.102 (M^+), found 388.103. Purity was determined as 96.0% by HPLC (method A), retention time 15.63 min.

***N*-[4-Methyl-3-(4-methyl-1-piperazinyl)phenyl]-4-bromo-3-methylbenzamide (14).** A stirred suspension of 4-bromo-3-methylbenzoic acid (0.56 g, 0.0026 mol) in thionyl chloride (25 mL) was heated under reflux for 2 h and then concentrated to dryness in vacuo. The resultant acid chloride was redissolved in THF (3 mL) and the solution added to a mixture of 4-methyl-3-(4-methyl-1-piperazinyl)aniline (0.36 g, 0.0018 mol) in THF containing NaOH (0.1 g, 0.0025 mol) and H_2O (3 mL). The reaction mixture was stirred at room temperature for 4 h, then poured into H_2O, and extracted with CH_2Cl_2. The organic extract was dried (Na_2SO_4) and concentrated in vacuo to give a beige foam which was purified by column chromatography on silica gel using 1% MeOH, 99% CH_2Cl_2 as eluant to give **14** (0.44 g, 63%) as a pale-cream solid. ^{1}H NMR ($CDCl_3$): δ 7.78 (1H, brs, CONH),7.72 (1H, d, H6 adjacent to CO), 7.66 (1H, d, H2, adjacent to CO), 7.52 (1H, dd, H5 adjacent to Br), 7.25 (2H, m, H5, H6), 7.14 (1H, d, H2), 3.00 (4H, t, CH_2NCH_2), 2.62 (4H, 4H, CH_2NMeCH_2), 2.50 (3H, s, CH_3), 2.38 (3H, s, CH_3), 2.29 (3H, s, NCH_3). HRMS calcd for 402.118 (M^+), found 402.119. Purity was determined as 95.2% by HPLC (method C), retention time 13.99 min.

4-[(2-Iodophenoxy)methyl]-1-methyl-1,2,3,6-tetrahydropyridine (15a). A stirred soluton of 2-iodophenol (0.5 g, 0.002 mol), triphenylphosphine (0.53 g, 0.002 mol), and 1-methyl-1,2,3,6-tetrahydropyridine-4-methanol (0.32 g, 0.0025 mol) in THF (20 mL) at 0 °C under argon was treated with a solution of diethyl azodicarboxylate (0.4 mL, 0.0025 mol) in THF (3 mL). The reaction mixture was stirred at room temperature for 4 h and then concentrated in vacuo and the residue basified with 10% aqueous Na_2CO_3 solution and extracted with EtOAc. The EtOAc layer was extracted with 1 M HCl and the acid layer basified by addition of K_2CO_3 and extracted with EtOAc. The organic extract was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel using 15% EtOAc, 85% Et_2O to give **15a** (0.50 g, 74%) as a yellow oil. ^{1}H NMR: δ 7.76 (1H, dd, H3), 7.27 (1H, m, H5), 6.81 (1H, dd, H4), 6.68 (1H, td, H6), 5.84 (1H, m, =CH), 4.47 (2H, s, OCH_2), 2.99 (2H, m, CHNCH), 2.58 (2H, t, $=CHCH_2CH_2$), 2.40−2.25 (5H, m, CHNMeCH, NCH_3).

2,3-Dihydro-1′-methylspiro[benzofuran-3,4′-piperidine] (15b). A stirred solution of 4-[(2-iodophenoxy)methyl]-1-methyl-1,2,3,6-tetrahydropyridine, **15a** (0.50 g, 0.0015 mol), and AIBN (0.005 g) in benzene (75 mL) was heated to reflux under argon and then treated dropwise over 1 h with a solution of tributyltin hydride (1 mL, 0.0035 mol) in benzene (10 mL). The reaction mixture was heated under reflux for a further 3 h, then more tributyltin hydride (0.5 mL, 0.0017 mol) and AIBN (0.005 g) were added, and heating was continued for 4 h. The reaction mixture was allowed to cool and then concentrated in vacuo and the residue treated with 2 M HCl, washed with EtOAc, basified with K_2CO_3, and extracted with EtOAc. This organic extract was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel using 2% MeOH, 98% $CHCl_3$ to give **15b** (0.21 g, 69%) as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.15 (2H, m, H4, H6), 6.88 (1H, t, H5), 6.79 (1H, d, H7), 4.35 (2H, s, OCH_2), 2.87 (2H, m, CHNMeCH), 2.33 (3H, s, NCH_3), 2.02 (4H, m, CHNMeCH, CHCH), 1.78 (2H, m, CHCH).

2,3-Dihydro-1′-methyl-5-nitrospiro[benzofuran-3,4′-piperidine] (15c). A stirred solution of 2,3-dihydro-1′-methylspiro[benzofuran-3,4′-piperidine], **15b** (0.20 g, 0.0013 mol), in acetic anhydride (5 mL) at 0 °C under argon was treated portionwise over 15 min with copper(II) nitrate hemipentahydrate (0.35 g, 0.0015 mol). The reaction mixture was kept at 0 °C for a total of 1.5 h and then allowed to warm to room temperature over 0.5 h. The mixture was poured into water/ice (100 mL), basified by the careful addition of K_2CO_3, and then extracted with EtOAc. The organic extract was dried (Na_2SO_4) and concentrated in vacuo to afford **15c** (0.25 g, 79%) as a pale-brown oil. ^{1}H NMR: δ 8.11 (1H, dd, H4), 8.03 (1H, d, H6), 6.83 (1H, d, H7), 4.53 (2H, s, OCH_2), 2.91 (2H, m, CHNMeCH), 2.34 (3H, s, NCH_3), 2.02 (4H, m, CHNMeCH, CHCH), 1.81 (2H, m, CHCH).

5-Amino-2,3-dihydro-1′-methylspiro[benzofuran-3,4′-piperidine] (15d). A solution of 2,3-dihydro-1′-methyl-5-nitrospiro[benzofuran-3,4′-piperidine], **15c** (0.25 g, 0.001 mol), in EtOH (30 mL) was hydrogenated over 10% Pd/C at room temperature and atmospheric pressure until uptake of hydrogen ceased. The catalyst was removed by filtration through Kieselguhr and the filtrate concentrated in vacuo to give **15d** (0.13 g, 60%) as a brown oil. ^{1}H NMR (DMSO-d_6): δ 6.42 (2H, m, H4, H6), 6.33 (1H, dd, H7), 4.22 (2H, s, OCH_2), 2.84 (2H, d, CHCHNMe), 2.29 (3H, s, NCH_3), 2.12 (2H, t, CHNMeCH), 1.82 (2H, m, CHCCH), 1.61 (2H, d, CHCCH).

***N*-(2,3-Dihydro-1′-methylspiro[benzofuran-3,4′-piperidine]-5-yl)-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenylcarboxamide (15).** A suspension of 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid (0.20 g, 0.0007 mol) in $SOCl_2$ (3 mL) was heated under reflux for 1 h. After cooling to room temperature, the solvent was removed in vacuo. This was dissolved in CH_2Cl_2 (2 mL) and added to a stirred solution of 5-amino-2,3-dihydro-1′-methylspiro[ben-

zofuran-3,4′-piperidine], **15d** (0.13 g, 0.0006 mol), and Et_3N (0.17 mL, 0.0013 mol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 20 h, then treated with 10% aqueous Na_2CO_3 solution, and extracted with CH_2Cl_2. The organic extracts were dried (Na_2SO_4) and concentrated in vacuo and the residue chromatographed on silica gel eluting with 15% MeOH, 85% CH_2Cl_2, to give **15** isolated as the oxalate salt (0.28 g, 80%). ^{1}H NMR (DMSO-d_6): δ 10.31 (1H, s, COOH), 8.15−7.90 (4H, m, biphenyl H2, H6, H3′, H5′), 7.70−7.55 (4H, m, biphenyl H3, H5, H6′, H5), 7.45 (1H, d, H4), 6.83 (1H, d, H7), 4.50 (2H, s, OCH_2), 3.41 (2H, d, CHNMeCH), 3.02 (2H, t, CHNMeCH), 2.75 (3H, s, CH_3), 2.70 (3H, s, CH_3), 2.37 (3H, s, NCH_3), 2.20−1.85 (4H, m, CH_2CCH_2). HRMS (FAB): calcd for 495.239 (M^+), found 495.237. Purity was determined as >99% by HPLC (method C), retention time 14.45 min.

1-Acetyl-6-bromo-2,3-dihydro-5-[(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)methoxy]-1*H*-indole (5a). A stirred suspension of powdered 1-acetyl-6-bromo-2,3-dihydro-1*H*-indol-5-ol (19.0 g, 0.074 mol) in dry THF (1700 mL) at room temperature under argon was treated with triphenylphosphine (19.6 g, 0.074 mol) and 1-methyl-1,2,3,6-tetrahydropyridine-4-methanol (9.5 g, 0.075 mol) followed by the dropwise addition over 15 min of a solution of diethyl azodicarboxylate (11.8 mL, 0.075 mol) in THF (40 mL). A mild exotherm occurred, and the insoluble material dissolved. The solution was warmed at 32 °C for 1 h and then concentrated in vacuo to approximately 500-mL volume. The solid which had formed was collected by filtration and dried affording 16.1 g of solid. The filtrate was concentrated in vacuo, the residue treated with EtOAc (700 mL) and 1 M HCl (500 mL) and shaken well, and the acid layer separated. This was washed with EtOAc, basified with 40% NaOH, and extracted with EtOAc, followed by $CHCl_3$. The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to leave a solid, which was crystallized from EtOAc giving a total yield of 19.9 g (75%) of **5a**, isolated as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.42 (1H, s, H7), 6.72 (1H, s, H4), 5.80 (1H, brs, =CH), 4.41 (2H, s, OCH_2), 4.04 (2H, t, CH_2), 3.12 (2H, t, NCH_2), 2.97 (2H, brs, =CHCH_2NMe), 2.58 (2H, t, =CHCH_2CH_2NMe), 2.38 (3H, s, $COCH_3$), 2.28 (2H, brs, CHNMeCH), 2.18 (3H, s, NCH_3).

5-Acetyl-1′-methyl-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]-indole-3,4′-piperidine] (5b). A stirred suspension of 1-acetyl-6-bromo-2,3-dihydro-5-[(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)methoxy]-1*H*-indole, **5a** (20.8 g, 0.057 mol), in benzene (1500 mL) was treated with AIBN (400 mg) and heated to 75 °C under argon and then treated dropwise over 0.5 h with a solution of tributyltin hydride (23 mL, 0.085 mol) in benzene (200 mL). The mixture was heated under reflux for 6 h and then concentrated in vacuo. The residue was treated with 2 M HCl (900 mL) and EtOAc (600 mL) and then shaken well and the acid layer separated, washed with EtOAc, and then basified with 40% aqueous NaOH solution, keeping the temperature below 20 °C. A precipitate was formed which was filtered off, washed with H_2O, and dried to afford **5b** (14.8 g, 91%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.11 (1H, s, H4), 6.60 (1H, s, H8), 4.36 (2H, s, OCH_2), 4.03 (2H, t, CH_2), 3.10 (2H, t, NCH_2), 2.92−2.78 (2H, m, CHNMeCH), 2.30 (3H, s, CH_3), 2.18 (3H, s, NCH_3), 2.15−1.90 (4H, m, CHNMeCH, CHCCH), 1.80−1.63 (2H, m, CHCCH).

1′-Methyl-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4′-piperidine] (5c). A stirred solution of 5-acetyl-1′-methyl-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4′-piperidine], **5b** (14.5 g, 0.051 mol), in a mixture of 5 M HCl (250 mL) and EtOH (100 mL) was heated under reflux under argon for 2 h and then stirred for 16 h at room temperature. The EtOH was removed in vacuo and the remaining solution cooled in an ice bath and basified to pH 12 by addition of 40% aqueous NaOH. The resultant white precipitate was collected by filtration, washed with H_2O, and dried to afford 6.0 g of **5c**. The filtrate was extracted with EtOAc, followed by $CHCl_3$. The combined extract was dried (Na_2SO_4) and concentrated in vacuo leaving a beige solid (6.2 g) and affording a total of 12.2 g (99%) of compound **5c** as a beige solid. ^{1}H NMR ($CDCl_3$): δ 6.61 (1H, s, H4), 6.46 (1H, s, H8), 4.31 (2H, s, OCH_2), 3.53 (2H, t, CH_2), 2.96 (2H, t, NCH_2), 2.90 (3H, m, 3H, CHNMeCH, NH), 2.33 (3H, s, NCH_3), 1.99 (4H, m, CHNMeCH, CHCCH), 1.74 (2H, m, CHCCH).

1′-Methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydrospiro[furo-[2,3-*f*]indole-3,4′-piperidine] (5). A stirred suspension of 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid (0.036 g, 0.0012 mol) in thionyl chloride (3 mL) was heated under reflux for 1 h. After cooling to room temperature, the solvent was removed in vacuo to leave the acid chloride as a yellow solid. This was dissolved in CH_2Cl_2 (2 mL) and added to a stirred solution of 1′-methyl-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4′-piperidine], **5c** (0.030 g, 0.0012 mol), and Et_3N (0.06 mL, 0.0043 mol) in CH_2Cl_2 and kept at room temperature for 3 days. The mixture was washed with aqueous K_2CO_3 solution and brine, then dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on silica gel eluting with CH_2Cl_2 with increasing concentrations of MeOH to afford **5** (0.019 g, 30%) which was converted to the hydrochloride salt, isolated as a white solid, mp >280 °C. ^{1}H NMR (DMSO-d_6): δ 7.95−7.80 (3H, m, biphenyl H2, H6, H3′), 7.62 (2H, m, biphenyl H5′, H6′), 7.47 (2H, d, biphenyl H3, H5), 7.39 (1H, d, H4), 6.72 (1H, s, H8), 4.44 (2H, s, OCH_2), 4.01 (2H, t, CH_2), 3.42−3.22 (4H, m, NCH_2, CHNMeCH), 2.92 (2H, brt, CHNMeCH), 2.66 (3H, brs, NCH_3), 2.62 (3H, s, CH_3), 2.31 (3H, s, CH_3), 2.02−1.84 (4H, m, CH_2CCH_2). HRMS (FAB): calcd for 521.255 (M^+), found 521.255. Purity was determined as >99% by HPLC (method A), retention time 18.86 min. Anal. ($C_{32}H_{32}N_4O_3 \cdot HCl \cdot H_2O$) C, H, N.

Computational Methods. Molecular mechanics and dynamics calculations on the protein structures with and without bound ligands were performed using the CHARMm program. Geometries for the ligands were generated by optimization with the COSMIC molecular mechanics force field or, where parameters were unavailable, with the semiempirical molecular orbital program VAMP, using the AM1 Hamiltonian. Natural atomic orbital charges were used for the docking calculations, and these were generated from single-point Hartree−Fock calculations on the optimized structures, using the Spartan suite of programs (Wave Function Inc.) with a 3-21G* basis set. The results for the CHARMm calculations were visualized using the program QUANTA (Molecular Simulations, Inc.). All calculations were performed on either a Silicon Graphics 4D-380 Powerserver or a DEC alpha 2100 and the results visualized on a Silicon Graphics Indigo-2 Extreme workstation.

References

(1) Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylechrane, E. J.; Saxena, P. R.; Humphrey, P. P. A. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.* **1994**, *46*, 157−203.

(2) Leonhardt, S.; Herrick-Davis, K.; Titeler, M. Detection of a novel serotonin receptor subtype (5-HT_{1E}) in human brain: interaction with a GTP binding protein. *J. Neurochem.* **1989**, *53*, 465−471.

(3) Schoeffter, P.; Hoyer, D. 5-Hydroxytryptamine 5-HT_{1B} and 5-HT_{1D} receptors mediating inhibition of adenylate cyclase activity: pharmacological comparison with special reference to the effects of yohimbine, rauwolscine and some β-adrenoreceptor antagonists. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1989**, *340*, 285−292.

(4) Hamon, M.; Emerit, M. B.; El Mestikawy, S.; Gallisot, M. C.; Gozlan, H. Regional differences in the transduction mechanisms of 5-HT receptors in the mammalian brain. In *Cardiovascular pharmacology of 5-HT: prospective therapeutic applications*; Sanea, P. R., Walis, D. I., Wouters, W., Bevan, P., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1991; pp 41−59.

(5) Adham, N.; Borden, L. A.; Schechter, L. E.; Gustafson, E. L.; Cochran, T. L.; Vaysse, P. J. J.; Weinshank, R. L.; Branchek, T. A. Cell specific coupling of the cloned human 5-HT_{1F} receptor to multiple signal transduction pathways. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1993**, *348*, 566−575.

(6) Hartig, P. R.; Hoyer, D.; Humphrey, P. P. A.; Martin, G. R. Alignment of receptor nomenclature with the human genome: classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes. *Trends Pharmacol. Sci.* **1996**, *17*, 103−105.

(7) Weinshank, R. L.; Zgombick, J. M.; Macchi, M. J.; Branchek, T. A.; Hartig, P. R. Human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3630−3634.
(8) Kaumann, A. J.; Frenken, M.; Posival, H.; Brown, A. M. Variable participation of 5-HT_1-like receptors and 5-HT_2 receptors in serotonin-induced contraction of human isolated coronary arteries. *Circulation* **1994**, *90*, 1141−1153.
(9) Peroutka, S. J. Pharmacological differentiation of human 5-HT_{1B} and 5-HT_{1D} receptors. *Biol. Signals* **1994**, *3*, 217−222.
(10) Pauwels, P. J.; Reihsaus, E.; Palmier, C.; Journot, L.; Lyons, J.; Colpaert, F. C. Ketanserin differentiates between cloned human 5-$HT_{1D\alpha}$ receptors and cloned human 5-$HT_{1D\beta}$, sheep and bovine caudate nucleus 5-HT_{1D} receptor sites. *Cell Pharmacol.* **1995**, *2*, 183−191.
(11) Beer, M. S.; Middlemiss, D. N. Serotonin-5-O-carboxymethylglycyl[^{125}I]tyrosin-amide labels the 5-$HT_{1D\beta}$ receptor subtype in human cortex. *Eur. J. Pharmacol.* **1993**, *242*, 195−198.
(12) Engel, G.; Göthert, M.; Hoyer, D.; Schlocker, E.; Hillenbrand, K. Identity of inhibitory pre-synaptic 5-HT autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1986**, *332*, 1−7.
(13) Fink, K.; Zentner, J.; Gothert, M. Subclassification of presynaptic 5-HT autoreceptors in the human cerebral cortex as 5-$HT_{1D\beta}$ receptors. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1995**, *352*, 451−454.
(14) Middlemiss, D. N.; Bremer, M. E.; Smith, S. M. A pharmacological analysis of the 5-HT receptors mediating inhibition of 5-HT release in the guinea pig frontal cortex. *Eur. J. Pharmacol.* **1988**, *157*, 101−107.
(15) Davidson, C.; Stamford, J. A. Evidence that 5-HT release in rat dorsal raphé nucleus is controlled by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors. *Br. J. Pharmacol.* **1995**, *114*, 1107−1109.
(16) Buhlen, M.; Fink, K.; Boing, C.; Göthert, M. Evidence for presynaptic location of inhibitory 5-$HT_{1D\beta}$-like receptors in the guinea pig cortex. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1996**, *353*, 281−289.
(17) Roberts, C.; Watson, J.; Burton, M.; Price, G. W.; Jones, B. J. Functional characterisation of the 5-HT terminal autoreceptor in the guinea pig brain cortex. *Br. J. Pharmacol.* **1996**, *117*, 384−388.
(18) Pinder, R. M.; Wieringa, J. H. Third generation antidepressants. *Med. Res. Rev.* **1993**, *13*, 259−325.
(19) Norman, T. R.; Leonard, B. E. Fast-acting antidepressants. Can the need be met? *CNS Drugs* **1994**, *2*, 120−131.
(20) Blier, P.; De Montigny, C. Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci.* **1994**, *15*, 220−226.
(21) Roberts, C.; Thorn, L.; Price, G. W.; Middlemiss, D. N.; Jones, B. J. Effect of the selective 5-HT_{1D} receptor antagonist GR 127935 on in vivo 5-HT release, synthesis and turnover in the guinea pig frontal cortex. *Br. J. Pharmacol.* **1994**, *112*, 489P.
(22) Skingle, M.; Sleight, A. J.; Fenuik, W. Microdialysis study investigating the effects of GR 127935, a potent 5-HT_{1D} receptor antagonist, on cortical levels of 5-HT in the guinea pig. *Br. J. Pharmacol.* **1994**, *112*, C57.
(23) Skingle, M.; Sleight, A. J.; Fenuik, W. Effects of the 5-HT_{1D} receptor antagonist GR 127935 on extracellular levels of 5-HT in the guinea pig frontal cortex as measured by microdialysis. *Neuropharmacology* **1995**, *34*, 377−382.
(24) Skingle, M.; Beattie, D. T.; Scopes, D. I. C.; Starkey, S. J.; Connor, H. E.; Feniuk, W.; Tyers, M. B. GR 127935: a potent and selective 5-HT_{1D} receptor antagonist. *Behav. Brain Res.* **1996**, *73*, 157−161.
(25) Watson, J.; Burton, M.; Price, G. W.; Jones, B. J.; Thomas, D.; Faruq, A.; Middlemiss, D. N. GR 127935 acts as a partial agonist at recombinant human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors. *Br. J. Pharmacol.* **1995**, *114*, 362P.
(26) Blaney, F. E.; Tennant, M. Computational tools and results in the construction of G protein-coupled receptor models. In *Membrane protein models*; Findlay, J., Ed.; Bios. Scientific Publishing: Oxford, U.K., 1996.
(27) Forbes, I. T.; Dabbs, S.; Duckworth, D. M.; Ham, P.; Jones, G. E.; King, F. D.; Saunders: D. V.; Blaney, F. E.; Naylor, C. B.; Baxter, G. S.; Blackburn, T. P.; Kennett, G. A.; Wood, M. D. Synthesis, biological activity and molecular modeling studies of selective 5-$HT_{2C/B}$ receptor antagonists. *J. Med. Chem.* **1996**, *39*, 4966−4977.
(28) The numbers refer to the corresponding residue in the human 5-HT_{1B} sequence.
(29) Trumpp-Kallmeyer, S.; Hoklack, J.; Bruinvels, A.; Hibert, M. Three-dimensional models of neurotransmitter G-binding protein-coupled receptors. *Mol. Pharmacol.* **1991**, *40*, 8−15.
(30) Kristiansen, K.; Dahl, S. G. Molecular modeling of serotonin ketanserin, ritanserin and their 5-HT_{2C} receptor interactions. *Eur. J. Pharmacol.* **1996**, *306*, 195−210.
(31) Strader, C. D.; Sigal, I. S.; Candelore, M. R.; Rands, E.; Hill, W. S.; Dixon, R. A. F. Conserved aspartate residues 79 and 113 of the β-adrenergic receptor have different roles in receptor function. *J. Biol. Chem.* **1988**, *263*, 10267−10271.
(32) Leonhardt, S.; Herrick-Davis, K.; Titeler, M. Detection of a novel serotonin receptor subtype (5-HT_{1E}) in human brain: interaction with a GTP-binding protein. *J. Neurochem.* **1989**, *53*, 465−471.
(33) Parker, E. M.; Izzarelli, D. G.; Lewis-Higgins, L.; Palmer, D.; Shapiro, R. A. Two amino acid differences in the sixth transmembrane domain are partially responsible for the pharmacological differences between the 5-$HT_{1D\beta}$ and 5-HT_{1E} 5-hydroxytryptamine receptors. *J. Neurochem.* **1996**, *67*, 2096−2103.
(34) Hutchins, C. Three-dimensional models of the D1 and D2 dopamine receptors. *Endocrine J.* **1994**, *2*, 7−23.
(35) Oxford, A. W.; Mitchell, W. L.; Bradshaw, J.; Clitherow, J. W. Benzanilide derivatives as 5-HT_{1D} antagonists. Eur. Pat. Appl. 0 533 267 A1, March 1993; *Chem. Abstr.* **1993**, *119*, 49414*c*.
(36) Gaster, L. M.; King, F. D.; Wyman, P. A. Heterocyclic biphenylamides usefuls as 5-HT_{1D} antagonists. Int. Pat. Appl. WO 95/15954, June 1995; *Chem. Abstr.* **1995**, *123*, 313977*n*.
(37) Gaster, L. M. Dihydrobenzofuranyl-biphenyl carboxamides having 5-HT_{1D} antagonist activity. Int. Pat. Appl. WO 95/17401, June 1995; *Chem. Abstr.* **1995**, *123*, 340139*x*.
(38) Ham, P.; Gaster, L. M.; King, F. D.; Duckworth, D. M. Amide derivatives having 5-HT_{1D} antagonist activity. Int. Pat. Appl. WO95/32967, December 1995; *Chem. Abstr.* **1995**, *124*, 232466*p*.
(39) Wyman, P. A.; Gaster, L. M.; Jennings, A. J. Tricyclic spiro compounds, process for their preparation and their use as 5-HT_{1D} receptor antagonists. Int. Pat. Appl. WO 96/11934, April 1996; *Chem. Abstr.* **1996**, *125*, 114514*a*.
(40) Gaster, L. M.; King, F. D.; Wyman, P. A. Tetracyclic spiro compounds, process for their preparation and their use as 5-HT_{1D} receptor antagonists. Int. Pat. Appl. WO 96/19477, June 1996; *Chem. Abstr.* **1996**, *125*, 142702*d*.
(41) Oxford, A. W.; Mitchell, W. L.; Bradshaw, J.; Clitherow, J. W.; Carter, M. Azolylpiperazine benzamide derivatives as 5-HT_{1D} antagonists. Eur. Pat. Appl. 0 533 268 A1, March 1993; *Chem. Abstr.* **1993**, *119*, 117270*e*.
(42) Murphy, R. A.; Kung, H. F.; Kung, M.-P.; Billings, J. Synthesis and characterisation of iodobenzamide analogues: Potential D-2 dopamine receptor imaging agents. *J. Med. Chem.* **1990**, *33*, 171−178.
(43) Chapleo, C. B.; Butler, R. C. M.; England, D. C.; Myers, P. L.; Roach, A. G.; Smith, C. F.; Stillings, M. R.; Tulloch, I. F. Heteroaromatic analogues of the α2-adrenoceptor partial agonist clonidine. *J. Med. Chem.* **1989**, *32*, 1627−1630.
(44) Svensson, K. G.; Selander, H.; Karlsson, M.; Nilsson, J. L. G. The directing effect of annelated rings in aromatic systems − XI. Bromination of bicyclic phenols with heterocyclic annelated rings. *Tetrahedron* **1973**, *29*, 1115−1118.
(45) Thomas, D. R.; Faruq, S. A.; Balcarek, J. M.; Brown, A. M. Pharmacological characterisation of [^{35}S]-GTPγS binding to chinese hamster ovary cell membranes stably expressing cloned human 5-HT_{1D} receptor subtypes. *J. Rec. Sig. Trans. Res.* **1995**, *15*, 199.
(46) Watson, J. M.; Burton, M. J.; Price, G. W.; Jones, B. J.; Middlemiss, D. N. GR 127935 acts as a partial agonist at recombinant human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors. *Eur. J. Pharmacol.* **1996**, *314*, 365−372.
(47) Hatcher, J. P.; Slade, R., C.; Hagan, J. J. 5-HT_{1D} receptors mediate SK&F 99101H induced hypothermia in the guinea pig. *J. Psychopharmacol.* **1995**, *9*, 234−241.
(48) Hagan, J. J.; Slade, P. D.; Gaster, L. M.; Jeffrey, P. M.; Hatcher, J. P.; Middlemiss, D. N. Stimulation of 5-HT_{1B} receptors causes hypothermia in the guinea pig. *Eur. J. Pharmacol.* **1997**, *331*, 169−174.
(49) Roberts, C.; Price, G. W.; Gaster, L. M.; Jones, B. J.; Middlemiss, D. N.; Routledge, C. Importance of 5-HT_{1B} receptor selectivity for 5-HT terminal autoreceptor activity: an in vivo microdialysis study in the freely moving guinea pig. *Neuropharmacology* **1997**, *36*, 549−557.
(50) Roberts, C.; Belenguer, A.; Middlemiss, D. N.; Routledge, C. Comparison of 5-HT autoreceptor control in guinea pig dorsal and median raphe innervated brain regions. *Br. J. Pharmacol.* **1997**, *331*, 207P.
(51) Brown, A. M.; Young, T. J.; Patch, T. L.; Cheung, C. W.; Kaumann, A. J.; Gaster, L. M.; King, F. D. [125I]-SB-207710, A potent selective radioligand for 5-HT_4 receptors. *Br. J. Pharmacol.* **1993**, *110*, 10P.
(52) Michel, A. D.; Loury, D. N.; Whiting, R. L. Identification of a single α1-adrenoceptor corrresponding to the α_{1A} subtype in rat submaximillary gland. *Br. J. Pharmacol.* **1989**, *98*, 883−889.
(53) Bylund, D. B.; Ray-Prenger, C.; Murphy, T. J. Alpha-2A and alpha-2B adrenergic receptor subtypes: antagonist binding in tissues and cell lines containing only one subtype. *J. Pharmacol. Exp. Ther.* **1988**, *245*, 600−607.

(54) Dini, S.; Caselli, G. F.; Ferrari, M. P.; Giani, R.; Clavenna, G. Heterogeneity of [^{3}H]-mepyramine binding sites in guinea pig cerebellum and lung. *Agents Actions* **1991**, *33*, 181−184.
(55) Ruat, M.; Traiffort, E.; Bouthenet, M. L.; Schwartz, J. C.; Hirschfeld, J.; Buschauer, A.; Schunack, W. Reversible and irreversible labeling and autobiographic localization of the cerebral histamine H_2 receptor using [^{125}I]iodinated probes. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1658−1662.
(56) Dorje, F.; Wess, J.; Labrecht, G.; Tacke, R.; Mutschler, E.; Brann, M. R. Antagonist binding profiles of five cloned human muscarinic receptor subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 727−733.
(57) Roberts, C.; Watson, J. M.; Burton, M. J.; Price, G. W.; Jones, B. J. Functional characterisation of the 5-HT terminal autoreceptor in guinea pig brain cortex. *Br. J. Pharmacol.* **1996**, *117*, 384−388.
(58) Draper, N. R.; Smith, H. *Applied regression analysis*; John Wiley and Sons: New York, 1966.
(59) Klapwyk, J. C.; Pallett, M. J.; Roberts, C. An anaesthetic mask for use on guinea pigs (*Cavia porcellus*) in a stereotaxic frame. *Lab. Anim. Sci.* **1995**, *29*, 407−410.
(60) Rapisadra, C.; Bacchelli, B. The brain of the guinea pig in stereotaxic coordinates. *Arch. Sci. Biol.* **1977**, *61*, 1−37.

JM970457S