Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 5298–5302
Bioorganic & Medicinal Chemistry Letters

Binding of amine-substituted N_1-benzenesulfonylindoles at human 5-HT$_6$ serotonin receptors

Manik Pullagurla,[a] Uma Siripurapu,[a] Renata Kolanos,[a] Mikhail L. Bondarev,[a] Małgorzata Dukat,[a] V. Setola,[b] B. L. Roth[b,c] and Richard A. Glennon[a,*]

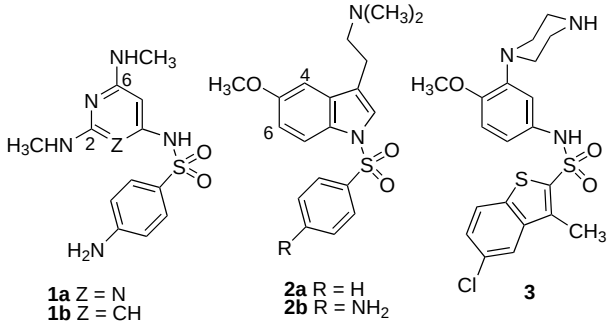
[a]*Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, VA 23298-0540, USA*
[b]*Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA*
[c]*Department of Psychiatry and Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA*

Received 11 July 2005; revised 9 August 2005; accepted 10 August 2005
Available online 23 September 2005

Abstract—An examination of several amine-substituted analogs of N_1-benzenesulfonylindoles reveals that although they bind at human 5-HT$_6$ serotonin receptors with high affinity, they are likely to bind in a dissimilar manner.

5-HT$_6$ receptors represent one of seven (5-HT$_1$–5-HT$_7$) major families of serotonin receptors, and are of interest because of their possible involvement in certain neurological and neuropsychiatric disorders.[1–3] The first 5-HT$_6$ antagonists to be reported included Ro 04-6790 (**1a** K_i ca. 50 nM),[4] Ro 63-0563 (**1b** K_i ca. 12 nM),[4] MS-245 (**2a** K_i ca. 2 nM),[5,6] and SB-271046 (**3** K_i ca. 1 nM).[7] Although these agents were independently identified, there are some conspicuous structural similarities amongst them in that all of them possess an amine-bearing bis-arylsulfonamide moiety. Despite attempts to describe how such agents might bind relative to one another, this issue has yet to be resolved. 5-HT$_6$ receptors are transmembrane-embedded G-protein coupled receptors containing an aspartate moiety in TM helix 3 that, presumably, serves as a ligand–amine binding site.[1] Compounds **1** possess multiple basic amine functions and it has been difficult identifying which is (are) most important for binding.[8] Compound **3** possesses only two basic amines and some investigators view one of the methylamino groups of **1** as mimicking the aryl amine moiety of **3**.[9,10] QSAR studies suggest the aryl amine might be the more important of the two, and one model indicates that the nonaryl amine of **3**-type compounds even contributes negatively to binding.[10] Yet, compounds **2** lack such an amine group. At this time, it is not known how the various amine functions influence affinity.

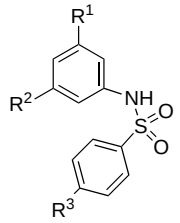
Evidently, one of the pyrimidine nitrogen atoms of **1a** (i.e., **1b**) can be removed without detriment to affinity.[4] One of the methylamino groups α to the pyridine nitrogen atom of **1b** can also be eliminated.[11] However, because this modification results in a symmetrically substituted pyridine, it is not known which of the two secondary amines is the more important. Is the remaining ring nitrogen atom required? It might be argued, because **4** (K_i ca. 600 nM) and **5** (K_i ca. 200 nM)[11] bind with several-fold reduced affinity relative to **1**, that this could be the case. In contrast, neither **2** nor **3** possesses a corresponding 6-membered heteroaryl ring. Furthermore, the high affinity of **6** and **7** (K_i ca. 30–40 nM)[11] indicates that a ring nitrogen atom is not required for binding.



Keywords: 5-HT$_6$ serotonin receptors; Aminoindoles.
* Corresponding author. Tel.: +1 804 828 8487; fax: +1 804 828 7404; e-mail: glennon@hsc.vcu.edu

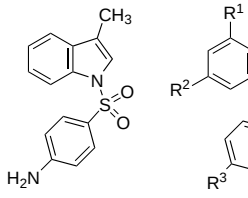
0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.08.059

Hirst et al.[8] have suggested that the 2-methylamino group of **1a** interacts with the TM3 aspartate. Interestingly, although the primary amine of **1** has not been investigated, it has been mentioned (though no data were provided) that its elimination results in loss of affinity,[11] suggesting it could be involved in binding. However, we found that **8** (K_i = 230 nM), which lacks the primary amine, and **9** (K_i = 200 nM), which bears only the primary amine, bind with nearly identical affinity and with an affinity comparable to that of **5**.[12] But, the similar affinity of **2b** (K_i = 0.8 nM)[13] and **2a** argues that the primary amine is not necessary. Yet, it has been demonstrated that **10** (K_i = 12 nM) (i.e., **2b** minus the tryptamine side chain) binds at 5-HT_6 receptors and behaves as an antagonist.[13]



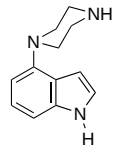
4 R^1=$NHCH_3$, R^2 = H, R^3 = NH_2
5 R^1 = R^2 = $NHCH_3$, R^3 = NH_2
6 R^1 = $NHCH_3$, R^2 = OCH, R^3 = NH_2
7 R^1 = $NHCH_3$, R^2 = CF_3, R^3 = NH_2
8 R^1 = $NHCH_3$, R^2 = R^3 = H
9 R^1 = R^2 = H, R^3 = NH_2

We have suggested that compounds such as **10** might represent conformationally constrained analogs of **1**.[14] Thus, given this structural relationship, it should be possible to probe the importance to binding of the various amine functions. The purpose of this investigation, then, was to examine variously substituted amine analogs **11** to determine which amine moiety is most contributory to binding. In addition, we examined the structurally related aryl amine-bearing analogs **12b** and **c** to determine if the presence of the aliphatic amine moiety detracts from receptor affinity as earlier suggested.

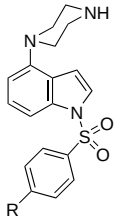


10

11a R^1 =R^2 =$NHCH_3$, R^3 = NH_2
11b R^1 = $NHCH_3$, R^2 = H, R^3 = NH_2
11c R^1 = H, R^2 = $NHCH_3$, R^3 = NH_2
11d R^1 = R^2 = $NHCH_3$, R^3 = H
11e R^1 = R^2 = H, R^3 = NH_2

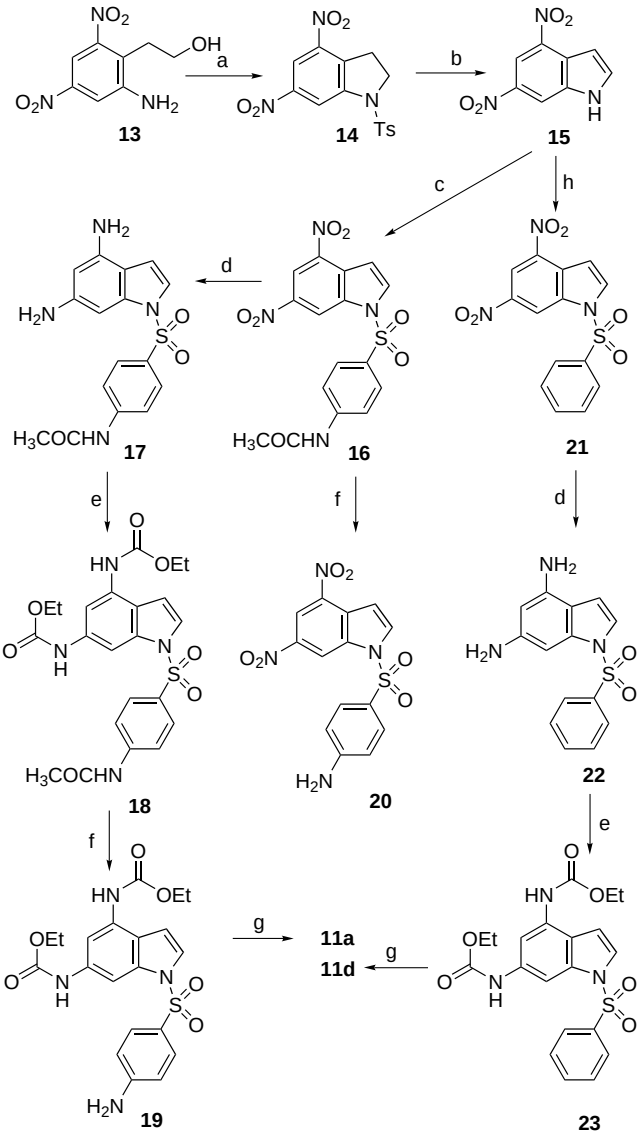


12a



12b R = H
12c R-= NH_2

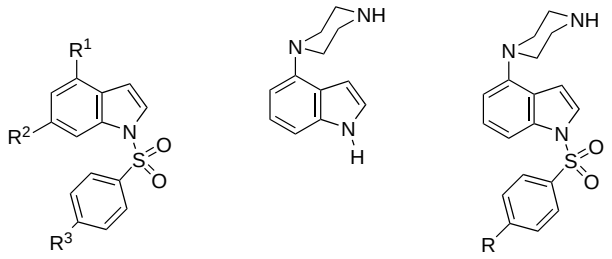
4,6-Dinitroindole (**15**)[15] was prepared and sulfonylated to arylsulfonyl analog **16** (Scheme 1); reduction of the nitro groups, followed by treatment with ethyl chloroformate, afforded the protected indole derivative **18**. Deprotection of **18** followed by reduction of the carbamate groups afforded **11a**. Compound **20** was obtained from **16** by hydrolysis of the amide. Compound **11d** also was obtained from 4,6-dinitroindole (**15**) by sulfonylation with benzenesulfonyl chloride (i.e., **21**) and then by conversion of the nitro groups to their corresponding methylamino groups as described for **11a** (Table 1).



Scheme 1. Reagents and conditions: (a) TsCl, 0 °C/30 min, 50 °C/1 h; (b) KOH, 2-PrOH, Δ; (c) i—NaH, DMF, 80 °C; ii—*N*-acetylsulfanilyl chloride, rt; (d) $SnCl_2{\cdot}2H_2O$, EtOH, Δ; (e) ClCOOEt, DMF, Py; (f) 10% HCl, EtOH, Δ; (g) $LiAlH_4$, THF, Δ; (h) i—NaH, DMF, 80 °C; ii—$PhSO_2Cl$, rt.

Compounds **11b** (Scheme 2) and **11c** (Scheme 3) were prepared by a sequence of reactions somewhat similar to those shown for the synthesis of **11a**. Although 4-methylaminoindole (**25**) has been previously described,[16] in the present study it was prepared from commercially available 4-aminoindole (**24**) by acylation with ethyl chloroformate followed by reduction of the carbamate with $LiAlH_4$. Introduction of the arylsulfonyl group followed by deprotection afforded **11b**. The synthesis of **11c** was performed in nearly the reverse manner; that is, 6-nitroindole (**28**)[17] was sulfonylated to arylsulfonyl analog **29**, the nitro group was reduced, and the product converted to the target (Scheme 3).

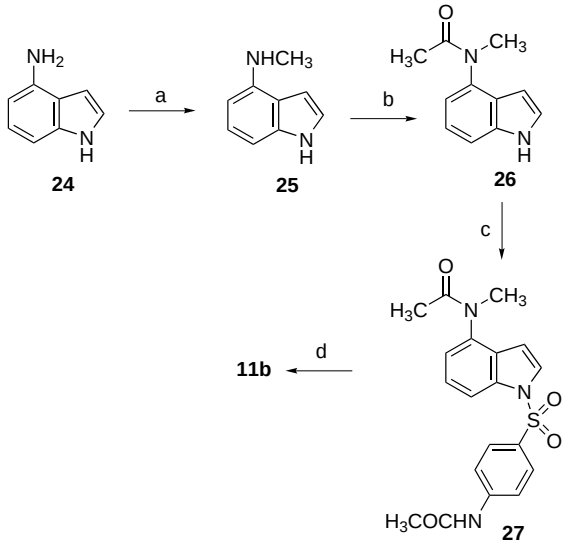
Table 1. Physicochemical and 5-HT_6 receptor binding properties of target benzenesulfonylindoles and piperazinoindole **12a**



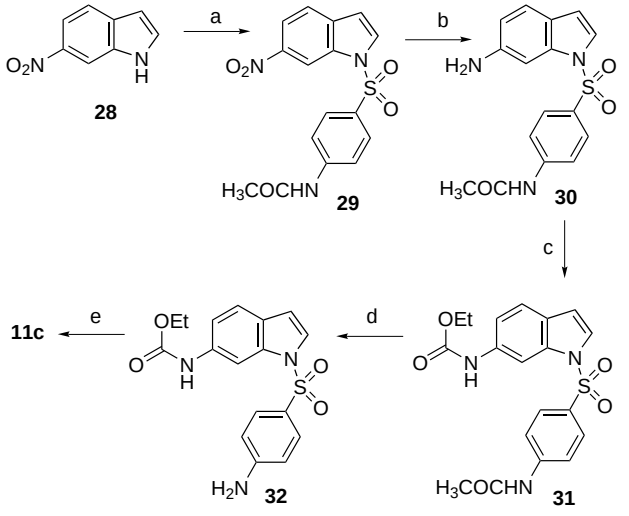
11, **20** **12a** **12b**, **12c**

Compound	R^1	R^2	R^3	R	Recrystallization solvent	Melting point (°C)	Empirical formula[a]	K_i (nM)	±SEM
11a	–NHMe	–NHMe	$–NH_2$	—	$MeOH/Et_2O$	145–146	$C_{16}H_{18}N_4O_2S$ 2.75HCl	1.9	0.4
11b	–NHMe	–H	$–NH_2$	—	$MeOH/Et_2O$	181–182	$C_{15}H_{15}N_3O_2S$ 1.25HCl 0.25H_2O	21	2
11c	–H	–NHMe	$–NH_2$		$MeOH/Et_2O$	103–105	$C_{15}H_{15}N_3O_2S$ 1.25$C_2H_2O_4$	7.0	0.1
11d	–NHMe	–NHMe	–H	—	$MeOH/Et_2O$	149–151	$C_{16}H_{17}N_3O_2S$ 1.75$C_2H_2O_4$	26	2
11e	–H	–H	$–NH_2$		—	88–90	$C_{14}H_{12}N_2O_2S$	10	3
12a[b]	—	—	—	—	MeOH	—	—	2700	500
12b	—	—	—	–H	$MeOH/Et_2O$	291–293	$C_{18}H_{19}N_3O_2S$ HCl	1.0	0.2
12c	—	—	—	$–NH_2$	$MeOH/Et_2O$	>250 (d)	$C_{18}H_{20}N_4O_2S$ HCl 0.5H_2O	0.4	0.1
20	$–NO_2$	$–NO_2$	$–NH_2$	—	$MeOH/CH_2Cl_2$	230–231	$C_{14}H_{10}N_4O_6S$	980	20

[a] All compounds were homogeneous as determined using thin-layer chromatography; assigned structures are consistent with ^1H NMR spectra, and compounds analyzed within 0.4 for C, H, and N. $C_2H_2O_4$ = oxalate salt.
[b] See Ref. 18.



Scheme 2. Reagents and conditions: (a) i—ClCOOEt, DMF, Py, 0 °C; ii—$LiAlH_4$, THF, Δ; (b) AcCl, toluene, −10 °C; (c) i—NaH, DMF, 80 °C; ii—*N*-acetylsulfanilyl chloride, rt; (d) 10% HCl, EtOH, Δ.



Scheme 3. Reagents and conditions: (a) i—NaH, DMF, 80 °C; ii—*N*-acetylsulfanilyl chloride, rt; (b) $SnCl_2{\cdot}2H_2O$, EtOH, Δ; (c) ClCOOEt, DMF, Py; (d) 10% HCl, EtOH, Δ; (e) $LiAlH_4$, THF, Δ.

Compounds **12b** and **c** (Table 1) were prepared from **12a**[18–20] as previously described in the patent literature.

Compound **11e** (K_i = 10 nM; Table 1),[21] the desmethyl analog of **10**,[13] binds with an affinity comparable to those of **10** (K_i = 12 nM) and **1b**. Introduction of the two methylamino functions found in **1** results in 5-fold enhanced affinity (**11a**; K_i = 1.9 nM), suggesting that the presence of one (or both) secondary amines might make a minor (direct or indirect) contribution to binding. However, elimination of the primary amine (i.e., **11d**; K_i = 26 nM) indicates that its presence is not essential for binding. For comparison, an analog of **11a** was examined where the methylamino groups were replaced with nitro groups; dinitro compound **20** (K_i = 980 nM) binds with >500-fold reduced affinity relative to **11a**.

Which of the two secondary amines of **11a** is the more important? Introduction of the 6-methylamino group (**11c**; K_i = 7.0 nM) has little effect on the affinity of **11e**, whereas introduction of a 4-methylamino group (i.e., **11b**; K_i = 21 nM) only halved affinity. The difference in affinity of the mono-, di-, and tri-amino analogs **11** is quite

small (range ≈ 10-fold), making it nearly impossible to ascribe a major binding role to any single amine function over the others. Comparing **11d** and **e**, however, it would seem unlikely that these compounds are binding at the receptors in a similar fashion (i.e., with superimposable indolic nuclei) if it is assumed they are interacting with a common amine binding site. These findings are not unlike what was found previously with **8** and **9**.

Piperazinoindole **12a** (K_i = 2700 nM; Table 1) lacks significant affinity for 5-HT_6 receptors. Incorporation of the N_1-benzenesulfonyl group results in >2500-fold enhanced affinity and **12b** (K_i = 1 nM) binds with an affinity comparable to that of **3**. Introduction of the primary amine (i.e., **12c**; K_i = 0.4 nM) is tolerated, but the amine is obviously not required for binding. If compound **12c** is viewed as an elaboration of **11b** (K_i = 21 nM), it is also evident that the presence of an intact piperazine ring leads to a >50-fold enhancement in 5-HT_6 receptor affinity. That is, the presence of the alkyl amine group does not detract from receptor affinity when compared with **11b**. Compounds **12b** and **c** were also recently reported by others to bind at human 5-HT_6 receptors with high affinity (K_i = 1 nM).[19]

Fairly apparent from this investigation is that all indole-containing analogs likely do not interact with the receptor with superimposed indolic nuclei, and that multiple amine groups are not a requirement for binding. When multiple amines are present, it remains to be determined which is most critical for interaction with the TM3 aspartate; furthermore, the low affinity of **20** compared with those of **11a** and **e** suggests that the amine groups might additionally have some effect on the electronic character of the indole nucleus. Comparing the compounds that were investigated, the only reasonable conclusion that can be drawn is that multiple modes of binding are possible. As if to underscore this concept, it has recently been shown that tryptamine analogs bearing a arylsulfonamido group at the indole 5-position also bind with nanomolar affinity at 5-HT_6 receptors.[22,23]

Initially, these findings are disturbing because they imply that the sulfonamido moiety common to the various agents will not be aligned. However, the present conclusion is not inconsistent with observations that 'reverse sulfonamides'[24] and even sulfones[25,26] bind at 5-HT_6 receptors, and that the benzenesulfonyl group can be effectively replaced with a benzyl group.[3] In other words, although the sulfonamido moiety might contribute to the affinity of some of these ligands, its presence (or specific orientation) may not be essential for binding. The possibility of multiple modes of binding is also disappointing because it makes it rather difficult to utilize or reliably extrapolate the structure–affinity findings from one series to another for purposes of drug design. This might be true even within a given series of agents (e.g., compare **11d** with **e**). Consequently, it will be necessary to exercise caution when conducting QSAR investigations that require alignment of specific structural features until it is known how such agents bind relative to one another.

Finally, the assumption has been made throughout that each of these ligands binds to a common amine site. However, there may be two accessible amine sites in the binding pocket of 5-HT_6 receptors: one in TM3 and another in TM7.[27] The possibility exists, then, that some ligands might utilize one or the other (or both) of these sites. This remains to be further investigated.

Acknowledgment

This work was supported in part by MH 60599.

References and notes

1. Kroeze, W. K.; Kristiansen, K.; Roth, B. L. *Curr. Top. Med. Chem.* **2002**, *2*, 507.
2. Woolley, M. L.; Marsden, C. A.; Fone, K. C. F. *Curr. Drug Top.* **2004**, *3*, 59.
3. Glennon, R. A. *J. Med. Chem.* **2003**, *46*, 2795.
4. Sleight, A. J.; Boess, F. G.; Bos, M.; Levet-Trafit, B.; Riemer, C.; Bourson, A. *Br. J. Pharmacol.* **1998**, *124*, 556.
5. Glennon, R. A.; Lee, M.; Rangisetty, J. B.; Dukat, M.; Roth, B. L.; Savage, J. E.; McBride, A.; Rauser, L.; Hufesien, L.; Lee, D. K. H. *J. Med. Chem.* **2000**, *43*, 1011.
6. Tsai, Y.; Dukat, M.; Slassi, A.; MacLean, N.; Demchyshyn, L.; Savage, J. E.; Roth, B. L.; Hufesein, S.; Lee, M.; Glennon, R. A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2295.
7. Bromidge, S. M.; Brown, A. M.; Clarke, S. E.; Dodgson, K.; Gager, T.; Grassam, H. L.; Jeffrey, P. M.; Joiner, G. F.; King, F. D.; Middlemiss, D. N.; Moss, S. F.; Newman, H.; Riley, G.; Routledge, C.; Wyman, P. *J. Med. Chem.* **1999**, *42*, 202.
8. Hirst, W. D.; Abrahamsen, B.; Blaney, F. E.; Calver, A. R.; Aloj, L.; Price, G. W.; Medhurst, A. D. *Mol. Pharmacol.* **2003**, *64*, 1295.
9. Russell, M. G.; Baker, R. J.; Barden, L.; Beer, M. S.; Bristow, L.; Broughton, H. B.; Knowles, M.; McAllister, G.; Patel, S.; Castro, J. L. *J. Med. Chem.* **2001**, *44*, 3881.
10. Wu, Y. J.; He, H.; Hu, S.; Huang, Y.; Scola, P. M.; Grant-Young, K.; Bertekap, R. L.; Wu, D.; Gao, Q.; Li, Y.; Klakouski, C.; Westphal, R. S. *J. Med. Chem.* **2003**, *46*, 4834.
11. Bös, M.; Sleight, A. J.; Godel, T.; Martin, J. R.; Riemer, C.; Stadler, H. *Eur. J. Med. Chem.* **2001**, *36*, 165.
12. Pullagurla, M. R. PhD Thesis, Virginia Commonwealth University, December 2003.
13. Pullagurla, M.; Setola, V.; Roth, B. L.; Glennon, R. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3355.
14. Pullagurla, M.; Dukat, M.; Roth, B. L.; Setola, V.; Glennon, R. A. *Med. Chem. Res.*, in press.
15. Samet, A. V.; Zakharov, E. P.; Semenov, V. V.; Buchanan, A. C.; Gakh, A. A. *Synth. Commun.* **2001**, *31*, 1441.
16. Quick, J.; Saha, B. *Tetrahedron Lett.* **1994**, *35*, 8553.
17. Caixach, J.; Capell, R.; Galvez, C.; Gonzalez, A.; Roca, N. *J. Heterocycl. Chem.* **1979**, *16*, 1631.
18. Compound **12a** (commercially available from Bridge Organics Co., Vicksburg, MI, as its dihydrochloride salt) was purified by recrystallization from aqueous MeOH. Compound **12b** has been variously described in the patent literature as a low-melting hydrochloride salt (mp 60 °C),[19] and as a dihydrochloride hydrate[20] for which no mp or other physicochemical data were provided. Compound **12c**, although also cited in the patent literature,[19] was not characterized. In the present investigation,

compounds **12b** and **c** were prepared as described[20] but were obtained as high-melting salts. Both compounds were characterized by spectroscopic and elemental analysis; compound **12b** was obtained as its monohydrochloride salt, and **c** as its monohydrochloride hemihydrate.

19. Kelly, M. G.; Cole, D. C. U.S. Patent US 2004/0192749, 2004.
20. Caldirola, P.; Johansson, G.; Nilsson, B. World Patent WO0232864, 2002.
21. The *h*5-HT_6 radioligand binding assay was performed as previously described.[28] In brief, *h*5-HT_6 cDNA was transiently expressed in HEK-293 cells using Fugene6 according to the manufacturer's recommendations. Twenty-four hours after transfection, the medium was replaced; 24 h later, medium containing dialyzed serum (to remove 5-HT) was added. At 75 h after transfection, cells were harvested by scraping and centrifugation. Cells were then washed by centrifugation and resuspension once in phosphate-buffered saline (pH = 7.40; PBS) and then frozen as tight pellets at −80 °C until use. Binding assays were performed at room temperature for 90 min in binding buffer (50 mM Tris–Cl, 10 mM $MgCl_2$, and 0.1 mM EDTA, pH = 7.40) with [^{3}H]LSD (1 nM final concentration) using 10 μM clozapine for non-specific binding. Various concentrations of unlabeled test agent were used for K_i determinations, with K_i values calculated using the program LIGAND. Specific binding represented 80–90% of total binding. K_i values are the result of triplicate determinations.
22. Holenz, J.; Merce, R.; Diaz, J. L.; Guitart, X.; Codony, X.; Dordal, A.; Romero, G.; Torrens, A.; Mas, J.; Andaluz, B.; Hernandez, S.; Monroy, X.; Sanchez, E.; Hernandez, E.; Perez, R.; Cubi, R.; Sanfeliu, O.; Buschmann, H. *J. Med. Chem.* **2005**, *48*, 1781.
23. Cole, D. C.; Lennox, W. J.; Lombardi, S.; Ellingboe, J. W.; Bernotas, R. C.; Tawa, G. J.; Mazandarani, H.; Smith, D. L.; Zhang, G.; Coupet, J.; Schechter, L. E. *J. Med. Chem.* **2005**, *48*, 353.
24. Bromidge, S. M.; Clarke, S. E.; Gager, T.; Griffith, K.; Jeffrey, P.; Jennings, A. J.; Joiner, G. F.; King, F. D.; Lovell, P. J.; Moss, S. F.; Newman, H.; Riley, G.; Rogers, D.; Routledge, C.; Serafinowska, H.; Smith, D. R. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 55.
25. Riemer, C.; Borroni, E.; Levet-Trafit, B.; Martin, J. R.; Poli, S.; Porter, R. H. P.; Bös, M. *J. Med. Chem.* **2003**, *46*, 1273.
26. Lee, M.; Rangisetty, J. B.; Pullagurla, M. R.; Dukat, M.; Setola, V.; Roth, B. L.; Glennon, R. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1707.
27. Pullagurla, M. R.; Westkaemper, R. B.; Glennon, R. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4569.
28. Kohen, R.; Metcalf, M. A.; Khan, N.; Druck, T.; Huebner, K.; Lachowicz, J. E.; Meltzer, H. Y.; Sibley, D. R.; Roth, B. L.; Hamblin, M. W. *J. Neurochem.* **1996**, *66*, 47.