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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1987–1991
Bioorganic & Medicinal Chemistry Letters

Binding of isotryptamines and indenes at h5-HT$_6$ serotonin receptors

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Received 3 February 2005; revised 18 February 2005; accepted 22 February 2005

Abstract—To determine if the indolic nitrogen atom is required for the binding of N_1-benzyltryptamines at h5-HT$_6$ serotonin receptors, several isotryptamines and indene analogs were examined. The affinity of 3-benzyl-N_1-(*N*,*N*-dimethylaminoethyl)indole (**5**, K_i = 32 nM) and 1-benzyl-3-(*N*,*N*-dimethylaminoethyl)indene (**11**, K_i = 3 nM) indicates that the indolic nitrogen atom is not essential for binding.

Serotonin (5-HT) receptors are classified as belonging to one of seven families (5-HT$_1$–5-HT$_7$).[1,2] Human 5-HT$_6$ receptors were identified about a decade ago[3,4] and have been implicated in depression and psychosis, based in large part on their high affinities for many psychiatric medications including antipsychotics and antidepressants.[4–6] Evidence suggests these receptors might also be involved in convulsive disorders, cognition, appetite control, and possibly drug abuse.[5–7] Within the past few years several 5-HT$_6$ antagonists have been reported,[6–8] and among these is one developed in our laboratories: MS-245 (**1**).[9,10] MS-245 (**1**; K_i = 2.1 nM) binds with high affinity at human 5-HT$_6$ (h5-HT$_6$) receptors; it was also shown that a 4'-amino group is tolerated (**2**, K_i = 2.0 nM), an intact tryptamine side chain is not required for binding (**3**, K_i = 12 nM), and that the sulfonyl moiety could be replaced by a methylene group (**4**, K_i = 6.5 nM).[10–12]

In continuing efforts to identify a binding pharmacophore for MS-245-type compounds at h5-HT$_6$ receptors, a question of interest was whether an indolic N$_1$ nitrogen atom is required for high-affinity binding of tryptamine-related compounds. Two strategies were employed to address this issue. First, because isotryptamines have been shown to mimic tryptamines at certain 5-HT receptors,[13,14] an isotryptamine analog of **4** (i.e., **5**) was examined. Second, the indole nitrogen atom of **4** was replaced by an sp^2-hybridized carbon atom to afford indene analog **6**. It was reasoned that the sp^2-hybridized carbon atom to which the 'benzyl' substituent would be attached, both in the isotryptamine and indene series, might mimic the electronic character of the indole nitrogen atom of **4**. Because **3** retains affinity for 5-HT$_6$ receptors, we also targeted abbreviated structures such as **7** for evaluation (see Fig. 1).

Compound **5** was prepared by reaction of 3-benzylindole[15] with *N*,*N*-dimethylaminoethyl chloride in the presence of NaH.

Indene **6** was prepared by reaction of 3-(2-dimethylaminoethyl)indene[17] with benzaldehyde in the presence of ethanolic KOH to yield the *trans* isomer.[18,19]

Reaction of the indenyl anion with benzyl chloride afforded nearly equal mixture of products **9** and **10** (Scheme 1). Treatment of the mixture with NaH and reaction with *N*,*N*-dimethylaminoethyl chloride can, in theory, result in the formation of three products (i.e., **11**–**13**). Such results have been reported for related compounds.[20] The reaction was performed several times. Despite apparent homogeneity upon thin-layer chromatographic analysis,

Keywords: Serotonergic ligands; 5-HT$_6$; Binding requirements.
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doi:10.1016/j.bmcl.2005.02.070

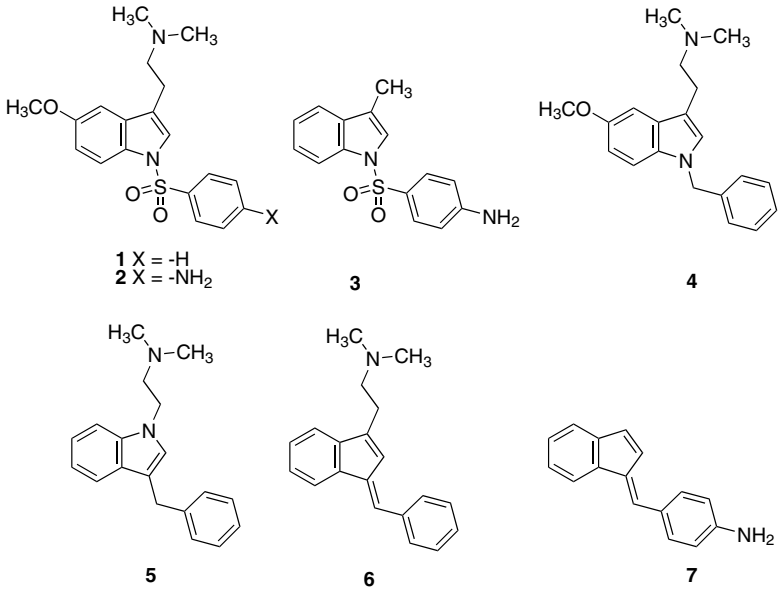
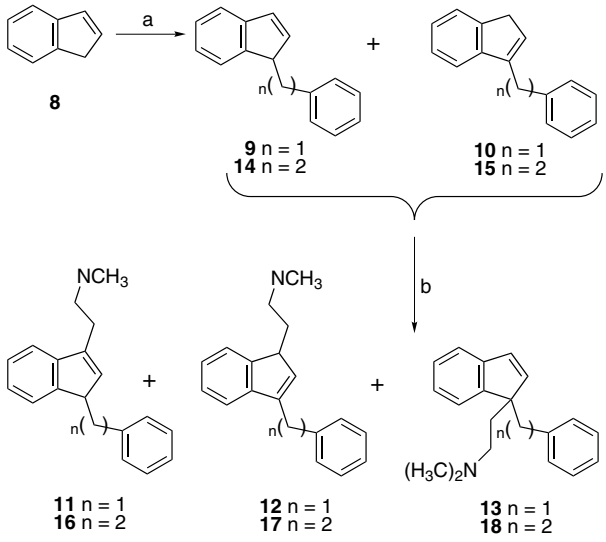


Figure 1. Structures of known (**1**–**4**) and target (**5**–**7**) 5-HT_6 ligands.

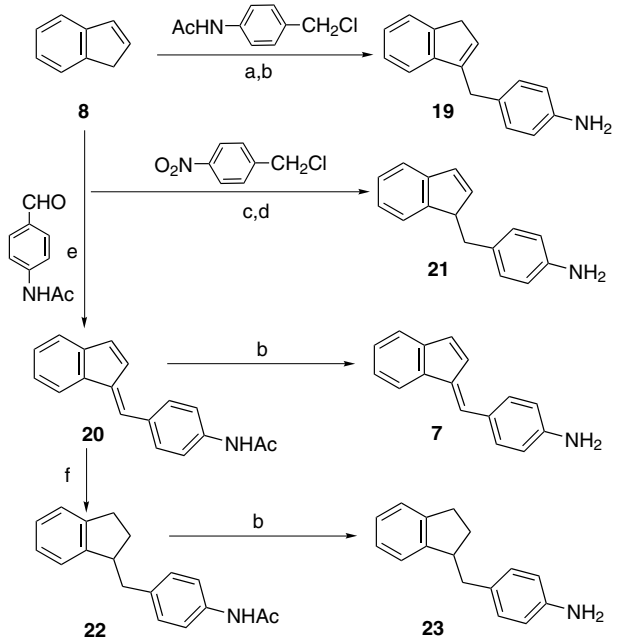


Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, $Ph(CH_2)_nCl$, 0 °C; (b) NaOH, *N*,*N*-dimethylaminoethyl chloride.

the ^1H NMR spectrum indicated that a mixture of products had been formed (predominantly **11** with approximately 3–10% **12** depending upon the particular reaction); **12** could not be completely separated from **11** but the two products could be differentiated by their benzylic proton signals (i.e., a doublet of doublets at δ 2.63 and 3.13 for **11**, and a singlet at δ 3.87 for **12**). In one instance, **13**[21] was isolated in low yield by column chromatography.

Alkylation of the homologous phenylethyl derivative (**14** and **15**) with *N*,*N*-dimethylaminoethyl chloride (Scheme 1) gave three products as identified by TLC and NMR analysis. Only compounds **16** and **18**[21] could be isolated in pure form by column chromatography.

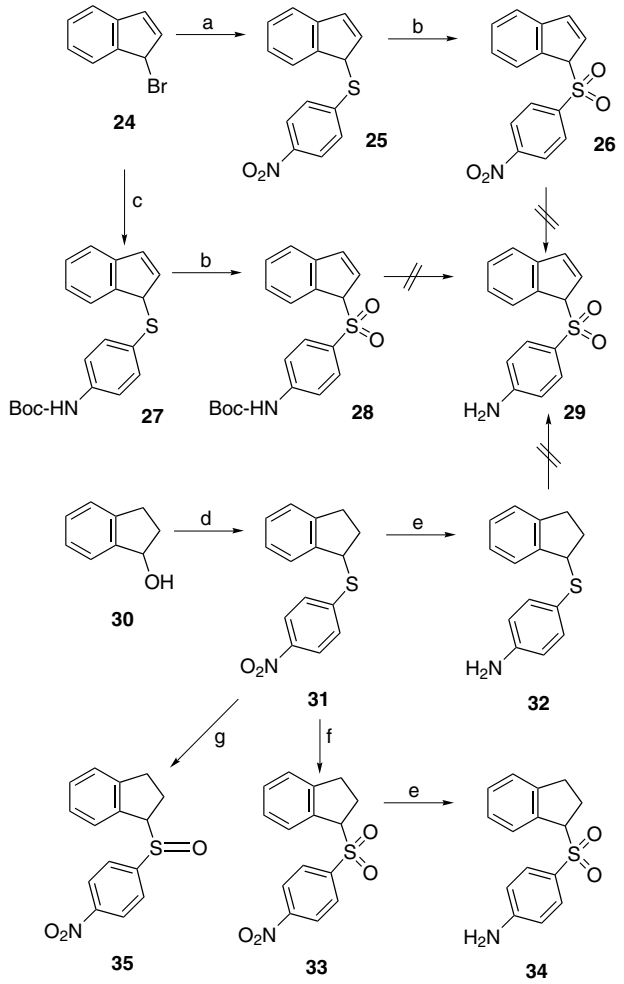
Scheme 2 shows the synthesis of compounds **7**, **19**, **21**, and **23**. Reaction of the indenyl anion with 4-acetamidobenzyl chloride gave compound **19** following hydrolysis of the protecting group. However, when 4-nitrobenzyl chloride was used as the reactant under similar conditions, compound **21** was isolated following reduction of the nitro intermediate; compound **19**, identified as a minor product of the reaction, was removed by recrystallization. Condensation of **8** with 4-acetamidobenzaldehyde afforded **20**; deprotection of **20** led to **7**, whereas reduction of **20** prior to deprotection led to indane **23**.



Scheme 2. Reagents and conditions: (a) *n*-BuLi, hexanes; (b) HCl, absolute EtOH; (c) *n*-BuLi, Et_2O; (d) $SnCl_2$, absolute EtOH; (e) KOH, absolute EtOH; (f) H_2, 5% Pd/C, MeOH.

Attempts were made to prepare the benzenesulfonyl counterpart of **21** (i.e., **29**) to obtain an analog similar in structure to **3**. Compound **26**[22] (Scheme 3) was obtained from **24**,[23] but all efforts to obtain **29** by reduction of the nitro group (under a variety of conditions including catalytic reduction, $SnCl_2$, $Na_2S_2O_4$) were unsuccessful. Although it was possible to obtain the Boc-protected amine **27**, and to oxidize the mercapto group to sulfone **28**,[22] attempts to deprotect **28** under a variety of acidic or basic conditions resulted in nearly immediate decomposition of the crude product that was obtained from the reaction.

An alternative approach involved the oxidation of **32**. Compound **31**[22] was obtained from **30**,[24] and successfully reduced to **32**; however, it was not possible to dehydrogenate/oxidize **32** to **29**. Oxidation of **31** afforded **33**,[22] which was reduced to **34**; but, here too, attempts to dehydrogenate **34** to **29** led to decomposition. In one instance, oxidation of **31** with oxone resulted in sulfoxide **35**.[25] In the course of these studies, crude **29** was isolated in several instances as evidenced by ^1H NMR; however, the product quickly decomposed.



Scheme 3. Reagents and conditions: (a) Hunig's base, CH_2Cl_2, THF, 4-nitrobenzenethiol, −60 °C; (b) oxone, H_2O, MeOH, rt; (c) Hunig's base, CH_2Cl_2, THF, *t*-Bu *N*-(4-mercapto-phenyl)carbamate, −60 °C; (d) (i) Tf_2O, CH_2Cl_2, −60 °C; (ii) 4-nitrobenzenethiol; (e) $SnCl_2$, H_2O, EtOH, Δ; (f) *m*CPBA, 0 °C; (g) oxone.

The 4′-amino derivative of **5**, (i.e., **36**; Fig. 2) was obtained by reaction of *N*,*N*-dimethylisotryptamine[14] with 4-(benzotriazol-1-ylmethyl)aniline[16] in methanolic HCl.

Radioligand binding data are provided in Table 1. Isotryptamines **5** (K_i = 32 nM) and **36** (K_i = 50 nM) were found to bind with high affinity, but with lower affinity than N_1-benzyltryptamine **4** (K_i = 6.5 nM). Four explanations are possible for this somewhat lower affinity: (a) compounds **5** and **36** lack a 5-methoxy group, (b) the double bond is in the 'wrong' position, (c) relocation of the ring nitrogen atom is not well tolerated, and/or (d) the tryptamine N_1-nitrogen atom is optimal for binding. The des-methoxy analog of **4** (i.e., **37**, K_i = 6 nM) retains affinity arguing that the methoxy group is not a major contributor to binding. Benzylindene **6** (K_i = 57 nM), which lacks the indole nitrogen atom, binds with an affinity similar to that of **5** suggesting that an N_1 indolic nitrogen atom might not be required. However, with **6** there is an additional complicating factor. Although the presence of the sp^2-hybridized ring carbon atom of **6** might electronically mimic the tryptamine nitrogen atom, it also imposes conformational constraint that could 'lock' the substituent into a conformation that is not particularly favored for binding. Consequently, benzylindene **11** was examined. The enhanced affinity of **11** (K_i = 3.0 nM) indicates that the sp^2-hybridized ring carbon atom is not required for binding. Indeed, racemic **11** binds with an affinity between that of MS-245 (**1**) and N_1-benzyltryptamine **4**. These results suggest that neither the tryptamine N_1-nitrogen atom, nor an sp^2-hybridized carbon atom, is essential at this position for binding to 5-HT_6 receptors. The results with **11** further suggest that the somewhat lower affinity of **5** relative to **4** (or **37**), might be due to the new location of the ring nitrogen atom and/or to the location of the double bond.

Interestingly, although homologation of the benzyl substituent of **37** to a phenylethyl group (i.e., **38**, K_i = 12 nM) only halved affinity, compound **16** (K_i = 100 nM), a homolog of **11**, binds with 30-fold lower affinity than **11**—an observation that cannot be satisfactorily explained at this time, but that might be related to stereochemistry. That is, the benzylic carbon atom of **38** is in the plane of the aromatic ring whereas that of chiral **16** is either behind or in front of the plane; this would place the pendent phenyl substituent in different positions relative to that in **38**. This effect could be more exaggerated with **16** than with **11** due to chain length.

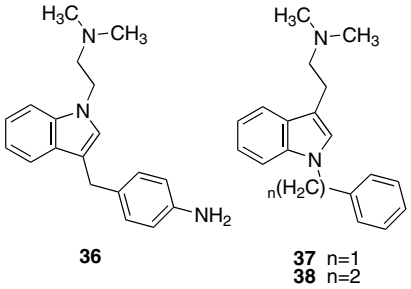


Figure 2. Structures of arylalkyl analogs **36**–**38**.

Table 1. Physicochemical properties and h5-HT_6 serotonin receptor affinities of the compounds investigated

	Melting point (°C)	Recrystallization solvent	Empirical formula[a]	h5-HT_6 K_i, nM (±SEM)[b]	
5	182–184	CH_2Cl_2/Et_2O	$C_{19}H_{22}N_2 \cdot HCl$[c]	32	(±5)
6	211	2-PrOH	$C_{20}H_{21}N \cdot HCl$	57	(±14)
7	202–205	$EtOH/Et_2O$	$C_{16}H_{15}N \cdot HCl$[c]	640	(±100)
11[d]	167–168	MeOH	$C_{20}H_{23}N \cdot C_2H_2O_4$	3	(±1)
16	138–139	Abs EtOH	$C_{21}H_{25}N \cdot C_2H_2O_4$[e]	100	(±10)
19	220–222	$EtOH/Et_2O$	$C_{16}H_{15}N \cdot HCl$	11,800	(±2570)
21	190–192	$EtOH/Et_2O$	$C_{16}H_{13}N \cdot HCl$	4470	(±620)
23	189–190	$EtOH/Et_2O$	$C_{16}H_{17}N \cdot HCl$	6100	(±1300)
32	195–196	$EtOH/Et_2O$	$C_{15}H_{15}NS \cdot HCl$	4200	(±600)
34	210–213	$EtOH/Et_2O$	$C_{15}H_{15}NO_2S \cdot HCl$	740	(±60)
36	164–166	CH_2Cl_2/Et_2O	$C_{19}H_{23}N_3 \cdot 2HCl$[f]	50	(±4)
37	155–156	MeOH	$C_{19}H_{22}N_2 \cdot C_2H_2O_4$	6	(±2)
38	144–146	MeOH	$C_{20}H_{24}N_2 \cdot C_2H_2O_4$[e]	12	(±3)
39[g]	169–170	—	$C_{15}H_{14}N_2 \cdot HCl$[c]	2530	(±200)

[a] Compounds analyzed within 0.4% of theory for C, H, and N. $C_2H_2O_4$ = oxalate salt.
[b] The radioligand binding assay was performed in triplicate as previously reported.[28]
[c] Crystallized with 0.25 mol H_2O.
[d] Homogeneous by thin-layer chromatography, but contains approximately 3% of **12** as identified by ^1H NMR.
[e] Crystallized with 0.5 mol H_2O.
[f] Crystallized with 1.0 mol H_2O.
[g] Prepared according to a literature procedure[29] and converted to HCl salt; the salt was washed with anhydrous Et_2O.

An intact tryptamine moiety is not required for the binding of **2**; that is, the abbreviated tryptamine **3** (K_i = 12 nM) binds with only 6-fold reduced affinity. The abbreviated isotryptamine **39** (Fig. 3) (K_i = 2530 nM), however, binds with 50-fold lower affinity than isotryptamine **36** (K_i = 50 nM). Indene **7** (K_i = 640 nM) also binds with low affinity; again, this might be partly attributable to conformational constraint imposed by the exocyclic double bond. But, its reduced counterpart 1-(4-aminobenzyl)indene (**21**, K_i = 4470 nM) binds with even lower affinity. These results indicate that in the abbreviated series the indolic nitrogen atom or the benzenesulfonyl group of **3** might contribute to binding. The low affinity of 3-(4-aminobenzyl)indene (**19**, K_i = 11,800) provides evidence that the location of ring unsaturation can also influence affinity.

To examine the possible contribution of the benzenesulfonyl group, attempts were made to prepare benzenesulfonyl indene **29**. Repeated trials employing several different methods were unsuccessful; although crude product was obtained in several instances, it quickly decomposed. The indane counterpart of **29** (i.e., **34**, K_i = 740 nM), however, was stable. The nearly 10-fold higher affinity of **34** relative to **23** argues that in the abbreviated series, the benzenesulfonyl moiety might contribute to binding.

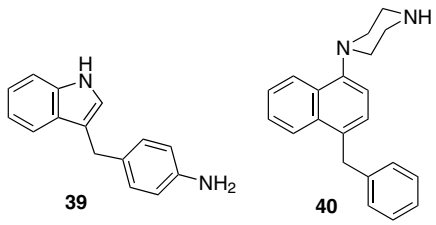


Figure 3. Structures of 3-(4-aminobenzyl)indole (**39**) and naphthylpiperazine **40**.

Human 5-HT_6 receptors possess an aspartate moiety in the third transmembrane helix (TM3).[26] It is likely that analogs possessing the *N*,*N*-dimethylaminoethyl side chain (e.g., **4**–**6**, **11**, **37**) bind in a common manner interacting with this aspartate. For these compounds, the presence of the ring nitrogen atom does not seem to be required for binding. This conclusion is consistent with recent findings on 1-(1-naphthyl)piperazine (1-NP) derivatives that appear to behave as tryptamine-mimics at 5-HT_6 receptors.[27] For example, 4-benzyl-1-NP (**40**; Fig. 3) (K_i = 38 nM), which lacks an indolic nitrogen atom (and an indolic nucleus) binds at 5-HT_6 receptors with an affinity comparable to that of **5**.

In contrast, those compounds lacking the basic amine side chain (i.e., the abbreviated analogs **7**, **19**, **21**, **23**, **32**, **34**) must bind in an altogether different orientation if they interact with the same aspartate residue; that is, with the latter compounds it must be the anilino amine function that interacts with the aspartate. Given the high affinity of **3**, the presence of the indolic nitrogen atom, the presence and location of ring unsaturation, and/or the nature of the substituent (i.e., benzyl vs benzenesulfonyl) seem to play a greater role in binding for the latter compounds. The influence of each of these factors will require further investigation.

Acknowledgements

The present work was supported in part by MH60599, and studies on the abbreviated indene analogs were funded by BTG International.

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21. Compounds **13**: (mp 164–165 °C; $C_{20}H_{23}N{\cdot}C_2H_2O_4$) and **18** (mp 129–130 °C; $C_{21}H_{25}N{\cdot}1.3C_2H_2O_4$) were isolated as their oxalate salts following recrystallization from absolute EtOH. Both analyzed within 0.4% of theory for C, H, and N.
22. The following intermediates were prepared during these studies: **25** mp 120–122 °C, $C_{15}H_{11}NO_2S$; **26** mp 135–136 °C, $C_{15}H_{11}NO_4S$; **28** mp 164–166 °C, $C_{20}H_{21}NO_4S$; **31** mp 91–93 °C, $C_{15}H_{13}NO_2S$; **33** mp 135–137 °C, $C_{15}H_{13}O_4S$, and analyzed within 0.4% of theory for C, H, and N.
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28. The h5-HT_6 radioligand binding assay was performed as previously described.[3] In brief, h5-HT_6 cDNA was transiently expressed in HEK-293 cells using Fugene6 according to the manufacturer's recommendations; 24 h after transfection the medium was replaced, and 24 h later, medium containing dialyzed serum (to remove 5-HT) was added. At 72 h after transfection, cells were harvested by scraping and centrifugation. Cells were then washed by centrifugation and resuspension in phosphate-buffered saline (pH = 7.40; PBS) and 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–HCl, 10 mM $MgCl_2$, 0.1 mM EDTA, pH = 7.40) with [^{3}H]LSD (1 nM final concentration) using 10 μM clozapine for non-specific binding. Concentrations of unlabeled test agent (1 to 10,000 nM) were used for K_i determinations with K_i values calculated using the program GraphPad Prizm (V4.0). Specific binding represented 80–90% of total binding. K_i values are the result of triplicate determinations.
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