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Evaluation of Isotryptamine Derivatives at 5-HT$_2$ Serotonin Receptors

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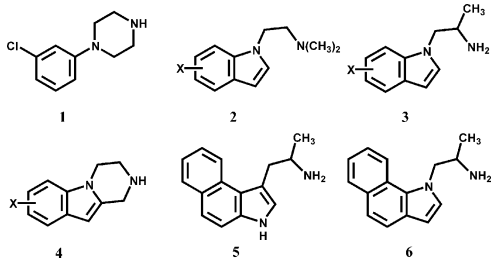
Abstract—On the basis that *meta*-chlorophenylpiperazine (*m*CPP; **1**) is a nonselective 5-HT$_{2C}$ agonist, that benz-fused tryptamines (e.g., **5**) display enhanced 5-HT$_2$ affinity, and that certain isotryptamines **3** reportedly bind with enhanced affinity and selectivity at 5-HT$_{2C}$ receptors, we prepared and examined a series of isotryptamine-related analogues as potentially selective 5-HT$_{2C}$ agonists. None of the compounds displayed selectivity for 5-HT$_{2C}$ versus 5-HT$_{2A}$ receptors. Detailed re-examination of a compound previously reported to display 100-fold 5-HT$_{2C}$ selectivity [i.e., *S*(+)-5,6-difluoro-α-methylisotryptamine] revealed that its selectivity versus 5-HT$_{2A}$ receptors was, at best, only 10-fold.

The identification of 5-HT$_{2C}$-selective serotonergic ligands—particularly 5-HT$_{2C}$-selective agonists—has been an elusive goal. 5-HT$_2$ receptors have been implicated in certain central disorders including depression, schizophrenia, anxiety, and eating disorders.[1–3] However, agents with high affinity for 5-HT$_{2C}$ receptors typically possess high affinity for 5-HT$_{2A}$ receptors and rarely display >10-fold selectivity. Very few 5-HT$_{2C}$-selective agonists are known (e.g., refs 3 and 4). *meta*-Chlorophenylpiperazine (*m*CPP; **1**), although not necessarily selective for 5-HT$_2$ versus certain other populations of 5-HT receptors, is an agent that displays about 10-fold selectivity for 5-HT$_{2C}$ receptors. Because *m*CPP behaves not as a 5-HT$_{2C}$ agonist but as a 5-HT$_{2A}$ antagonist,[5] it might represent an attractive lead for the development of novel agents despite its low selectivity. In fact, others have already reported this strategy.[6]

There exists some remote structural similarity between *m*CPP (**1**) and the tryptamines.[7] Tryptamine derivatives, though, lack selectivity for 5-HT$_{2C}$ versus 5-HT$_{2A}$ receptors.[1,2] We have previously shown that isotryptamines (e.g., **2**) bind at 5-HT$_2$ receptors.[8] Their subpopulation selectivity was not investigated at that time because 5-HT$_{2C}$ receptors had not yet been identified. More recently, Bös et al.[9] reported that certain isotryptamine derivatives (e.g., **3**) display upwards of 100-fold selectivity for 5-HT$_{2C}$ versus 5-HT$_{2A}$ receptors. Furthermore, certain pyrazino[1,2-*a*]indoles (e.g., **4**), which might be generally viewed as conformationally-restricted analogues of *m*CPP with an embedded isotryptamine moiety, have been identified as somewhat selective 5-HT$_{2C}$ partial agonists.[6]



We have previously demonstrated that benz-fusion of tryptamines results in compounds with high affinity, although not necessarily enhanced selectivity, for 5-HT$_{2C}$ receptors (e.g., **5**, 5-HT$_{2C}$ K_i = 9 nM; 5-HT$_{2A}$ K_i = 3 nM).[10] Because it is claimed that certain isotryptamines **3** are 5-HT$_{2C}$-selective, the present study employed the strategy that if ring-fusion of tryptamines results in high-affinity 5-HT$_2$ ligands, and if the isotryptamine nucleus imparts 5-HT$_{2C}$ selectivity, a ring-fused isotryptamine (i.e., **6**) might display enhanced 5-HT$_{2C}$ selectivity. Also, given that pyrazino[1,2-*a*]indoles **4** possess an iso-

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tryptamine moiety and are structurally related to *m*CPP (**1**), we additionally explored several ring-substituted analogues of **4** incorporating substituents that might mimic the lipophilic or electronic effects of a fused ring. In particular, we prepared the 6-, 7-, and 8-chloro and the 6-, 7-, and 8-methoxy derivatives of **4** (e.g., analogues **7**).

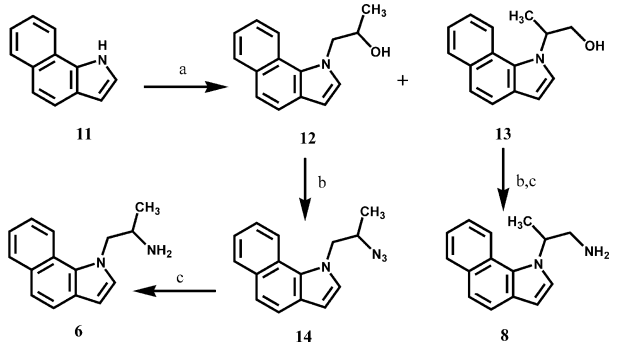
Chemistry

Ethyl benz[*g*]indole-2-carboxylate was prepared by the method of Rydon and Siddappa[11] except that hydrochloric acid was used in place of H_2SO_4 to effect cyclization; the ester was hydrolyzed to the acid, and the resulting acid was decarboxylated by heating at 210 °C to afford benz[*g*]indole (**11**).[10,11]

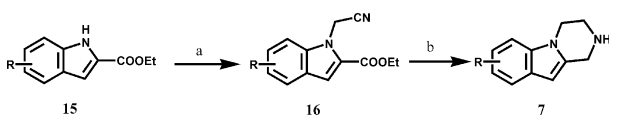
Compound **11** was allowed to react with propylene oxide to yield a 10:1 mixture of **12** and **13** which was separated by column chromatography (10% EtOAc in hexanes; silica gel) (Scheme 1). Reaction of **12** and **13** with diphenylphosphoryl azide (DPPA)/DBU gave the expected azides as oils. Catalytic reduction (10% Pd/C) of the azides afforded the target amines **6** (oxalate salt, mp 208–209 °C from MeOH/Et_2O) and **8** (0.75 oxalate, mp 123–124 °C from MeOH/Et_2O), respectively. Compound **14** was alternatively prepared in >90% yield by conversion of **12** to its mesylate, followed by reaction with sodium azide.

The pyrazino[1,2-*a*]indoles **7** were prepared by the general method of Rajur et al.[12] (Scheme 2). Compound **7h** has been reported by Bös et al.;[6] certain of the other derivatives had also been reported[12] but their melting points were 60–100 °C lower than that found in the present study (see Table 1).

The synthesis of compound **7h** was initiated using the method of Bös et al.;[6] however, once the necessary ethyl



Scheme 1. (a) Propylene oxide, THF, 0 °C→ room temperature, 16 h; (b) DPPA/DBU, toluene, 0 °C→ reflux 16 h; (c) (i) H_2, 10% Pd/C; (ii) oxalic acid.



Scheme 2. (a) (i) NaH; (ii) $ClCH_2CN$, DMF; (b) (i) $LiAlH_4/Et_2O$; (ii) oxalic acid or HCl.

5-chloro-3-methoxyindole-2-carboxylate was obtained, the remainder of the synthesis followed the procedure shown in Scheme 1. Compounds **9**[13] and **2a**–**2c**[10] were prepared by literature methods. 5,6-Difluoroindole (mp 96–97 °C from hexanes), required for the synthesis of **2d** and **10**, was prepared by catalytic reduction of 3,4-difluoro-6-nitrophenylacetonitrile. The indole was elaborated to **2d** by treatment with NaH/HMPA and then 2-chloro-*N*,*N*-dimethylethylamine hydrochloride. Compound **10** was obtained from 5,6-difluoroindole as previously described.[9]

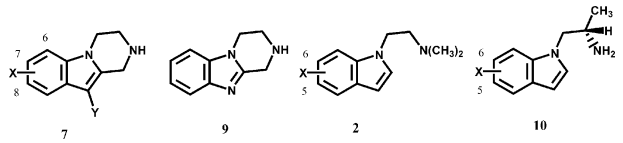
Results and Discussion

Compound **6** was found to bind both at 5-HT_{2A} ($K_i = 38 \pm 9$ nM) and 5-HT_{2C} ($K_i = 42 \pm 4$ nM) receptors. Although 5-HT_{2A} affinity was high, and higher than nearly all of the isotryptamines already reported,[9] selectivity was lacking. Interestingly, however, compound **8**, a positional isomer of **6**, was found to bind with greatly reduced affinity at both populations of receptors (5-HT_{2A} $K_i = 4300 \pm 1130$ nM; 5-HT_{2C} $K_i > 10{,}000$ nM). The low affinity of **8** relative to **6** suggests a possible steric interaction involving the β-methyl group that alters the conformation of the side chain. The individual optical isomers of **6** and **8** should be evaluated in future studies to determine the effect of stereochemistry on binding.

With few exceptions, the pyrazino[1,2-*a*]indoles **7** possessed low affinity at 5-HT_{2A} and 5-HT_{2C} receptors (Table 1). At 5-HT_{2A} receptors, only the 7-chloro derivative **7c** displayed appreciable affinity. Because 5-HT_{2A} agonists tend to show higher affinity for sites labeled by an agonist radioligand (e.g., [^{3}H]DOB) than by an antagonist radioligand (e.g., [^{3}H]ketanserin), 5-HT_{2A} sites were labeled with both radioligands (designated herein as 5-$HT_{2A(D)}$ and 5-$HT_{2A(K)}$, respectively, in Table 1) in order to obtain a more realistic perspective of their affinity. Although somewhat higher affinity was observed in each case (Table 1), the pyrazino[1,2-*a*]-indoles still bind only with modest affinity. The pyrazino[1,2-*a*]indoles also displayed relatively low affinity at 5-HT_{2C} receptors, and showed no selectivity for 5-HT_{2C} relative to 5-HT_{2A} receptors. Compound **7h** has been previously shown by Bös et al.[9] to be a 5-HT_{2C} partial agonist with about 8-fold selectivity for 5-HT_{2C} receptors. They have commented that the presence of the 10-methoxy group contributes to affinity and 5-HT_{2C} selectivity; the present results (comparing **7h** with **7d**) confirm their prior claim that the methoxy group plays a role in binding.[9] Data in Table 1 also show that replacement of the 10-methoxy group by a methyl group (i.e., **7i**) is tolerated, but that replacement of C_{10} with a nitrogen atom (i.e., **9**) decreases affinity.

In an attempt to further determine why the pyrazino[1,2-*a*]indoles bind with low selectivity, we focused on the structurally simpler isotryptamines **2**. Although compounds **2a** and **2c** bind with modest affinity, they lack 5-HT_{2C} selectivity (Table 1). There are several possible explanations for the low affinity and selectivity

Table 1. 5-HT_{2A} and 5-HT_{2C} binding data for compounds examined in this investigation



7 9 2 10

	X	Y	Mp (°C)[a]	K_i, (nM) (±SEM)						5-HT_{2A} selectivity[c]
				5-$HT_{2A(K)}$[b]		5-$HT_{2A(D)}$[b]		5-HT_{2C}		
7a	H	H	233[d]	1040	(30)	635	(75)	4780	(390)	7.5
7b	6-Cl	H	223–225	630	(40)	330	(40)	520	(40)	1.6
7c	7-Cl	H	221–222	58	(18)	80	(10)	560	(100)	7.0
7d	8-Cl	H	230[e]	1670	(190)	320	(50)	1460	(250)	4.6
7e	6-OMe	H	225–227	1000	(60)	300	(20)	1990	(160)	6.6
7f	7-OMe	H	204–206	5190	(800)	1300	(120)	5160	(420)	4.0
7g	8-OMe	H	238[f]	5830	(720)	930	(20)	9930	(200)	10.7
7h	8-Cl	OMe	—	130	(10)	60	(15)	330	(50)	5.5
7i	8-Cl	Me	—	1250	(250)	—[g]		450	(20)	—
9	—	—	—	> 10,000		> 10,000		> 10,000		—
2a	H	—	—	600	(80)	650	(170)	720	(80)	1.1
2b	5-OMe	—	—	> 10,000		> 10,000		> 10,000		
2c	6-OMe	—	—	1040	(100)	—[g]		490	(100)	—
2d	5-F,6-F	—	206–207[h]	640	(90)	230	(60)	300	(20)	1.3
10	5-F,6-F	—	—	320	(50)	15	(2)	85	(15)	5.7

[a]All new compounds (i.e., those for which melting points are provided), and **7a**, **7d**, and **7g** were submitted for microanalysis and determined values for C, H, and N were within 0.4% of theory. Analogues **7** were recrystallized from aqueous EtOH. All compounds were isolated as their oxalate salts except for **7h** (HCl), **9** (di HCl), and **10** (fumarate).
[b]5-$HT_{2A(K)}$ and 5-$HT_{2A(D)}$ are 5-HT_{2A} sites labeled by [^{3}H]ketanserin and [^{3}H]DOB, respectively.
[c]Selectivity = 5-HT_{2C} K_i value/5-$HT_{2A(D)}$ K_i value.
[d]Lit.[12] mp 130 °C; also reported as HCl salt.[7]
[e]Lit.[12] mp 162 °C.
[f]Present compound is $C_{11}H_{11}ClN_2.0.75C_2H_2O_4$; lit.[12] mp 180 °C for oxalate salt.
[g]Binding data not obtained.
[h]Compound recrystallized from MeOH.

observed with these derivatives. One of the highest affinity and most 5-HT_{2C}-selective agents reported by Bös et al.[9] is the 5,6-difluoro analogue of **3**; perhaps the fluoro groups are important contributors to selectivity. We examined the 5,6-difluoro derivative of **2a** (i.e., **2d**), but **2d** failed to display substantially improved affinity or selectivity over **2a**. Another explanation for low affinity and lack of selectivity is that compound **3** is an α-*methyl*isotryptamine whereas compounds **2** are *N,N-dimethyl*isotryptamines. Hence, we prepared compound **10**. Compound **10** has been reported to bind at 5-HT_{2A} sites with reduced affinity (K_i ca. 100 nM) relative to 5-HT_{2C} sites (K_i ca. 1 nM) resulting in a compound with 100-fold selectivity.[9] We found that compound **10** binds with somewhat higher affinity at 5-HT_{2A} sites ($K_i = 15$ nM), and with lower affinity at 5-HT_{2C} sites ($K_i = 85$ nM) than previously reported.

Not only did the compounds in the present study lack selectivity for 5-HT_{2C} receptors, compound **10**, which was previously reported to be selective,[9] also failed to exhibit the expected selectivity. Hence, the results cannot be solely attributed to structure; a remaining explanation for the apparent disparity might be the assay conditions. The earlier study used human 5-HT_{2A} and 5-HT_{2C} receptors whereas the present investigation used rat 5-HT_{2A} receptors and human 5-HT_{2C} receptors. The earlier study also used [^{3}H]DOB to label 5-HT_{2A} receptors and [^{3}H]5-HT to label 5-HT_{2C} receptors, whereas, although [^{3}H]DOB was used herein to label 5-HT_{2A} receptors, [^{3}H]mesulergine was employed to label 5-HT_{2C} receptors. Compound **10** was re-examined using various assay conditions (Table 2). The results show that compound **10** binds with similar affinity at rat and human 5-HT_{2A} receptors when an agonist radioligand is employed, and that there is 20-fold increase in affinity when [^{3}H]DOB is used in place of [^{3}H]ketanserin. Compound **10** binds at human 5-HT_{2C} receptors with about 20-fold higher affinity when agonist radioligand is employed in place of [^{3}H]mesulergine. Nevertheless, the selectivity of compound **10** for h5-HT_{2C} receptors over h5-HT_{2A} receptors is, at best, only about 10-fold using

Table 2. Binding of compound **10** at 5-HT_{2A} and 5-HT_{2C} receptors using different assay conditions

Receptor	Species	Radioligand	K_i, nM (SEM)[a]
5-HT_{2A}	Rat	[^{3}H]Ketanserin	320 (±50)
	Rat	[^{3}H]DOB	15 (±2)
	Human	[^{3}H]DOB	39 (±4)
	Human	[^{3}H]5-HT	12 (±1)
5-HT_{2C}	Human	[^{3}H]Mesulergine	85 (±15)
	Human	[^{3}H]DOB	3.9 (±0.8)
	Human	[^{3}H]5-HT	3.8 (±0.5)

[a]K_i values, obtained at least in triplicate, are followed by SEM. Details of the assay procedures are provided.[14]

assay conditions that match the literature conditions as closely as possible. The h5-HT_{2A} binding results were confirmed using a second agonist radioligand ([^{3}H]5-HT), as were the h5-HT_{2C} results [using ([^{3}H]DOB). In fact, under the latter conditions, compound **10** displayed only 3-fold selectivity for 5-HT_{2C} receptors.

In conclusion, none of the examined pyrazinoindoles nor isotryptamines displayed substantial selectivity for 5-HT_{2C} versus 5-HT_{2A} receptors. As reported by Bös et al.,[6] the presence of a 10-methoxy substituent on the pyrazinoindole nucleus might be necessary for enhanced 5-HT_{2C} affinity. In contrast, we were unable to confirm the selective nature of compound **10**. As a consequence, caution is advised when interpreting results of pharmacological studies using **10** as a selective 5-HT_{2C} agonist.

Acknowledgements

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14. **Cell culture and transfection (for human $5HT_{2A}$ assays):** COS-7 cells were grown in DMEM (Life Technologies) with 10% fetal bovine serum (Life Technologies) in 5% CO_2 at 37 C and subcultured 1:12 twice a week. Twenty-four hours before transfection, cells were seeded at 80% confluence in 100-mm dishes. Cells were transfected with human 5-HT_{2A} DNA by Lipofectamine (Life Technologies). This was accomplished by combining 20 μL Lipofectamine with 5 μg of plasmid per dish. Transfections were performed in serum-free DMEM for 4.5 h at 37 °C. **Radioligand binding:** Thirty-six hours after transfection, membranes were prepared from COS-7 cells (for human 5-HT_{2A}) by scraping in 50 mM Tris–HCl, 0.5 mM EDTA, 5 mM $MgCl_2$, pH 7.4 (tissue buffer) and centrifugation at 10,000*g* for 30 min. Membranes were re-suspended in tissue buffer, homogenized and centrifuged again. For human 5-HT_{2C}, NIH3T3 cells stably transfected with human 5-HT_{2C} INI receptors were grown to confluence and prepared in the same manner as the COS-7 cells. After re-suspension in assay buffer (tissue buffer + 0.1% ascorbate), 0.5-mL membrane aliquots were added to each assay tube containing either 2 nM [^{3}H]DOB or 0.4 nM [^{3}H]ketanserin for 5-HT_{2A} or 2 nM [^{3}H]5-HT or 1 nM [^{3}H]mesulergine for 5-HT_{2C} and varying concentrations of competing drug in a final volume of 1 mL mianserin (10 μM) was used to define non-specific binding. Samples were incubated at room temperature for 30 min (5-HT_{2A}) or at 37 °C for 30 min (5-HT_{2C}), filtered through glass fiber filters (presoaked in 0.3% polyethyleneimine) on a Brandel cell harvester, and counted in Ecoscint cocktail in a liquid scintillation counter (Beckman, Berkeley, CA, USA) at 40% efficiency. **Data analyses:** Data analyses were performed using Prism software (GraphPad, San Diego, CA, USA). The Cheng-Prusoff equation was used to calculate K_i values from the IC_{50} values.