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SAR of psilocybin analogs: Discovery of a selective 5-HT$_{2C}$ agonist

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Abstract—An SAR study of psilocybin and psilocin derivatives reveals that 1-methylpsilocin is a selective agonist at the h5-HT$_{2C}$ receptor. The corresponding phosphate derivative, 1-methylpsilocybin, shows efficacy in an animal model for obsessive–compulsive disorder, as does 4-fluoro-*N*,*N*-dimethyltryptamine. These results suggest a new area for development of novel 5-HT$_{2C}$ agonists with applications for drug discovery.
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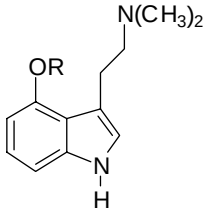
Psilocybin (**1**), a hallucinogenic component of the sacred Mexican mushroom *Psilocybe mexicana*, and its metabolite, psilocin (**2**), are both potent agonists at the 5-HT$_{2a}$ and 5-HT$_{2c}$ receptors (Fig. 1). In recent years, several case reports of the efficacy of psilocybin in the treatment of obsessive–compulsive disorder (OCD) have been published.[1] As a result, an FDA-approved clinical trial for patients suffering from OCD is now underway.[2] The hallucinogenic activity of psilocybin and psilocin is believed to be largely due to activation of 5-HT$_{2A}$ receptors, while the anti-OCD activity is associated with agonist activity at 5-HT$_{2C}$. Thus, it is believed that a selective 5-HT$_{2C}$ agonist would have considerable potential for treatment of OCD and other indications, such as obesity.[3,4]

We recently began an initial structure–activity relationship (SAR) study of some psilocin and psilocybin derivatives with the goal of obtaining selectivity for the 5-HT$_{2C}$ receptor. Only very limited analog work has been reported in the psilocybin area,[5] and to our knowledge, no pharmacological testing of psilocybin or psilocin derivatives has been published since the discovery of 5-HT$_2$ receptor subtypes.[6,7] Although the amino acid sequence of the 5HT$_{2C}$ receptor has been determined, little data are available regarding its three-dimensional structure[3] and therefore, our program has initially relied on an empirical approach.



1: R = P(O)(OH)$_2$
2: R = H

Figure 1.

We examined the influence of structural modification at a number of sites in the psilocybin structure. The compounds prepared (**3–17**)[8] are shown in Figure 2. Known *N*-methylated derivatives **3** and **4** were synthesized following published procedures.[5,9,10] Treatment of 4-benzyloxyindole with oxalyl chloride and then with dimethylamine gave the 3-substituted oxamide. Amide reduction (LAH), N-methylation (NaH, MeI), and hydrogenation (H_2, $Pd(OH)_2$) afforded *N*-methylpsilocin, **3**. Phosphorylation followed by debenzylation gave *N*-methylpsilocybin, **4**. Targets **5**, **6**, **7**,[9] **8**, and **9**[11] were prepared following the same general route (Scheme 1). Analogs **10**,[12] **11**, and **12**[13] were obtained similarly upon treatment of the intermediates formed from 4-benzyloxyindole and oxalyl chloride with the appropriate secondary amines, followed by LAH reduction and debenzylation. The synthesis of **13** followed the reported

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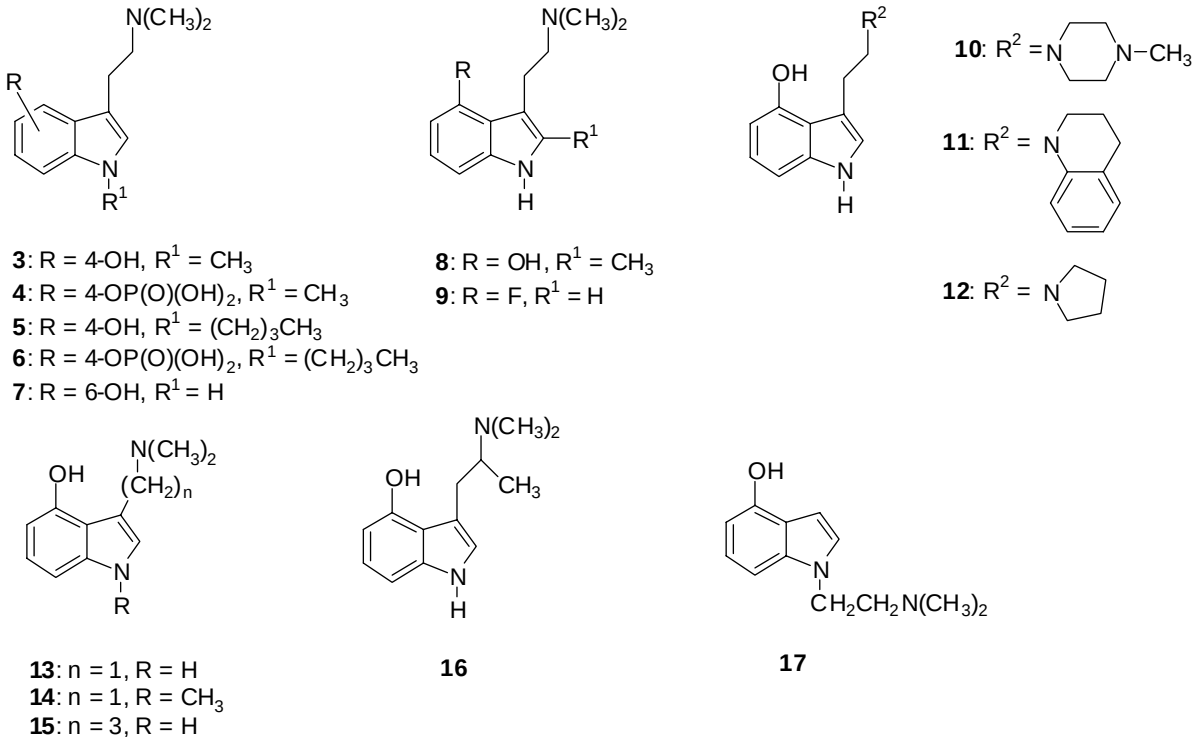
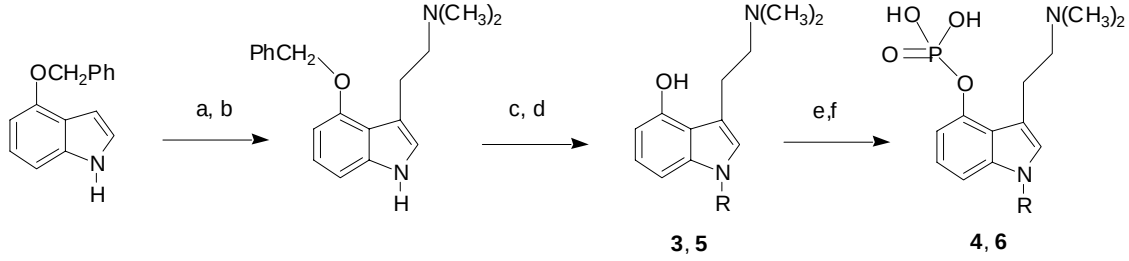
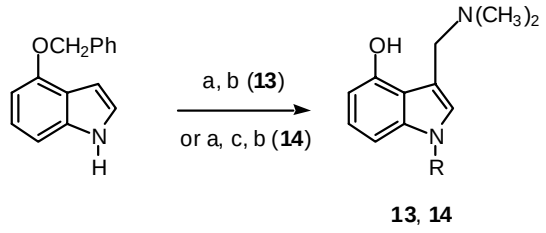


Figure 2.



Scheme 1. Reagents: (a) $(COCl)_2$, $(CH_3)_2NH$ (71%); (b) LAH (73%); (c) NaH, CH_3I (73%), $CH_3CH_2CH_2CH_2I$ (48%); (d) H_2, Pd/C (**3**: 78%, **5**: 67%); (e) LDA $(PhCH_2O)_2P(O)OH$; (f) H_2, Pd/C (**4**: 12%, **6**: 26%).

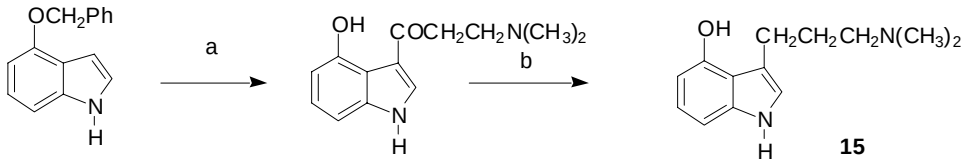
route[14] using a Mannich reaction of formaldehyde and 4-benzyloxyindole to give the intermediate, 4-benzyloxy-3-dimethylaminomethylindole, followed by debenzylation. N-Methylation of 4-benzyloxy-3-dimethylaminomethylindole and debenzylation afforded target **14** (Scheme 2). Compound **15**[9] was obtained from 4-benzyloxyindole upon treatment of the magnesium salt with 3-chloropropionyl chloride, to give an intermediate chloroketone. After reaction with dimethylamine, an intermediate debenzylated ketone was the major product isolated. LAH reduction provided **15** (Scheme 3). A similar sequence using 2-chloropropionyl chloride afforded **16**;[9] however, in this case debenzylation required a separate step (Scheme 4). Alkylation of 4-benzyloxyindole with *N*,*N*-dimethyl-2-chloroethylamine and debenzylation gave **17**.[9]



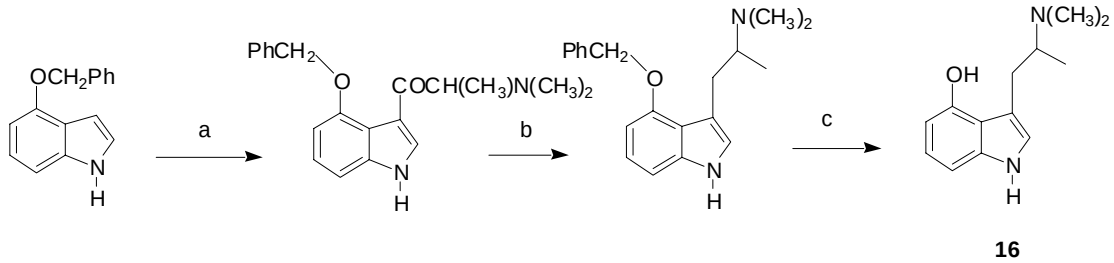
Scheme 2. Reagents: (a) $(CH_3)_2NH$, H_2CO (82%); (b) H_2, Pd/C (**13**: 74%, **14**: 73%); (c) NaH, CH_3I (34%).

The receptor binding of these 15 analogs to the serotonin receptor subtypes 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} was then determined (Table 1).[15] Functional assays of selected compounds were also carried out (Table 2).[16]

1-Methylpsilocin, **3**, displays selective binding at both the INI and VGI isoforms of the h5-HT_{2C} receptor (7.0 and 33 nM, respectively) as compared to the h5-HT_{2A} receptor (900 nM). Functional assays reveal that **3** is an agonist at both receptor subtypes with



Scheme 3. Reagents: (a) MeMgI, $ClCH_2CH_2COCl$, $(CH_3)_2NH$ (46%); (b) LAH (12%).



Scheme 4. Reagents: (a) MeMgI, $CH_3CHClCOCl$, $(CH_3)_2NH$ (16%); (b) LAH (27%); (c) H_2, Pd/C (55%).

Table 1. Receptor binding (K_i) in nM of psilocybin analogs

Compound	r5-HT_{2A}	h5-HT_{2A}	h5-HT_{2B}	r5-HT_{2C}	h5-HT_{2C} INI	h5-HT_{2C} VGI
3	590 ± 80	900 ± 17	38 ± 1.7	48 ± 5.7	7.0 ± 1.6	33 ± 6
4	>10,000	>10,000	5500 ± 3000	>10,000	>10,000	540 ± 320
5	200 ± 16	310 ± 30	5.8 ± 0.5	15.7 ± 0.5	4.4 ± 1.1	14 ± 4
6	>10,000	3000 ± 800	170 ± 40	5700 ± 880	73 ± 21	210 ± 70
7	>10,000	7600 ± 1500	6300 ± 3500	19,000 ± 5600	>10,000	6800 ± 3000
8	1683 ± 486	122 ± 37	ND	1276 ± 177	ND	539 ± 277
9	1008 ± 114	335 ± 66	8.39 ± 0.85	359 ± 125	82 ± 34	84 ± 12
10	6903 ± 1692	9753 ± 4089	116 ± 95	>10,000	103 ± 63	ND
11	8367 ± 1676	ND	5566 ± 3440	1446 ± 312	468 ± 450	ND
12	ND	429 ± 137	423	ND	275 ± 72	1772 ± 1079
13	834 ± 257	923 ± 224	1242 ± 295	49 ± 11	24 ± 0.8	12.6 ± 1.8
14	ND	498 ± 185	1242	ND	87 ± 22	125 ± 74
15	ND	588 ± 219	98 ± 66	ND	1114 ± 41	84 ± 64
16	ND	982 ± 169	745 ± 316	ND	126 ± 19	ND
17	>10,000	ND	ND	9351 ± 5551	2182 ± 848	4200 ± 788

Data represent means ± SD of computer-derived affinities from three or more separate experiments; ND, not determined.

Table 2. Functional assay (EC_{50}) in nM of psilocybin and analogs

Compound	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
1	3475 ± 2904 (31 ± 8%)	74 (24%)	506 ± 164 (51 ± 3%)
2	24 ± 2 (43 ± 17%)	58 (45%)	30 ± 18 (51 ± 37%)
3	633 ± 1.14 (31 ± 2.9%)	Inverse agonist	12 ± 1.5 (45 ± 5.5%)
5	32 ± 29.7 (0.12 ± 0.065%)	Inverse agonist	595 ± 42.5 (83 ± 12.9%)
6	Antagonist	Antagonist	Antagonist
9	949 ± 1.04 (49 ± 2%)	1180 ± 316 (38 ± 1.82%)	99 ± 168 (93 ± 49%)
13	Antagonist	Antagonist	Antagonist

Data represent mean EC_{50} values for activation of phosphoinositide hydrolysis in cells expressing human 5-HT_{2A}, 5-HT_{2B} or 5-$HT_{2C\text{-}INI}$ receptors, relative to serotonin at 100%. When SD is given $N \geqslant 3$.

considerable selectivity (EC_{50} at 5-HT_{2C} = 12 nM, EC_{50} at 5-HT_{2A} = 633 nM). Evaluation of the affinity of **3** for the 5-HT_{2B} receptor was also carried out, as agonist activity at 5-HT_{2B} is strongly associated with heart valve toxicity.[17,20] It was gratifying to find that although high affinity for the 5-HT_{2B} receptor was found (38 nM), a functional assay revealed that **3** is an inverse agonist at this receptor subtype. The observed selectivity of compound **3** for the 5-HT_{2C} receptor proved to be remarkably sensitive to structural variation. An increase in the size of the 1-substituent of **3** to *n*-butyl (**5**) afforded a compound that was a much weaker agonist at the 5-HT_{2C} receptor in the functional assay used.[21] Modification of the 4-hydroxy group (**7**), the 2-position (**8**), and the diethylamino substituent (**10**, **11**, and **12**) all resulted in much less potent binding at the 5-HT_{2C} receptor and less selectivity over the 5-HT_{2A} receptor. However, the 4-fluoro analog **9** showed modest

functional activity as an agonist at the 5-HT_{2C} receptor with some selectivity over the 5-HT_{2A} and 5-HT_{2B} receptors (about 10-fold each). A shortening in the length of the linker at the 3-position of the indole ring to a methylene group (**13**) gave an antagonist at the 5-HT_{2A} and 5-HT_{2C} receptors, while **14** (*N*-methyl, methylene linker), **15** (propylene linker), and **16** (α-methyl) all showed reduced affinity and selectivity for the 5-HT_{2C} receptor. Finally, **17** (side chain moved to the 1-position) was also quite inactive in binding at the 5-HT_{2C} receptor. The psilocybin analogs (**4**, **6**) gave very low binding as compared to their psilocin analogs (**3**, **5**). However, metabolic dephosphorylation of psilocybin to psilocin is known to readily occur in vivo[22] and thus, these psilocybin derivatives could act as prodrugs for their psilocin analogs. In fact, **4** shows improved in vivo activity as compared to **3** (vide infra, Fig. 3).

We next examined selected analogs in a mouse model for OCD. Serotonin produces an itching sensation when applied to the human skin and has been suggested to be involved in pruritic diseases. Further research demonstrated that an ip injection of 5-HT into the rostral back of the mouse elicits scratching with the hind paws, which is itch-associated rather than a pain response.[23] The 5-HT action is at least partly mediated by 5-HT_2 receptors in the skin, as shown by blocking with specific antagonists.[24] Psilocin and its analogs' effect on itch-associated scratching in the mice was thought to indicate their action on 5-HT receptors, and a study was devised as an animal model for OCD.[25] Selected results are shown in Figure 3. Psilocybin strongly inhibits scratching in this model, and more so than psilocin; however, psilocin shows much greater functional activity at the 5-HT_{2C} receptor. This difference may be due to active transport of psilocybin across the blood–brain barrier prior to dephosphorylation. *N*-Methyl derivatives **3** and **4** show comparable activity, but only at higher concentrations. Compound **4**, like psilocybin, dramatically inhibits scratching. *N*-Butylpsilocin, **5**, however, is nearly inactive, in contrast to **3**. Thus, positive effects seen in this mouse model are consistent with 5-HT_{2C} agonist activity. Finally, 4-fluoro-*N*,*N*-dimethyltryptamine, **9**, also exhibits strong activity, which is comparable to that of psilocybin and compound **4**, despite displaying only modest agonist activity at the 5-HT_{2C} receptor (99 nM).[26] One possible explanation for these results is that relatively lipophilic **9** may more readily penetrate the blood–brain barrier, and thus affording the observed in vivo activity.

Potent and selective 5-HT_{2C} agonists may have application in other therapeutic areas besides OCD, including appetite suppression,[7] Alzheimer's disease,[27] and epilepsy.[28] Further studies are underway to more fully develop this class of compounds.[29]

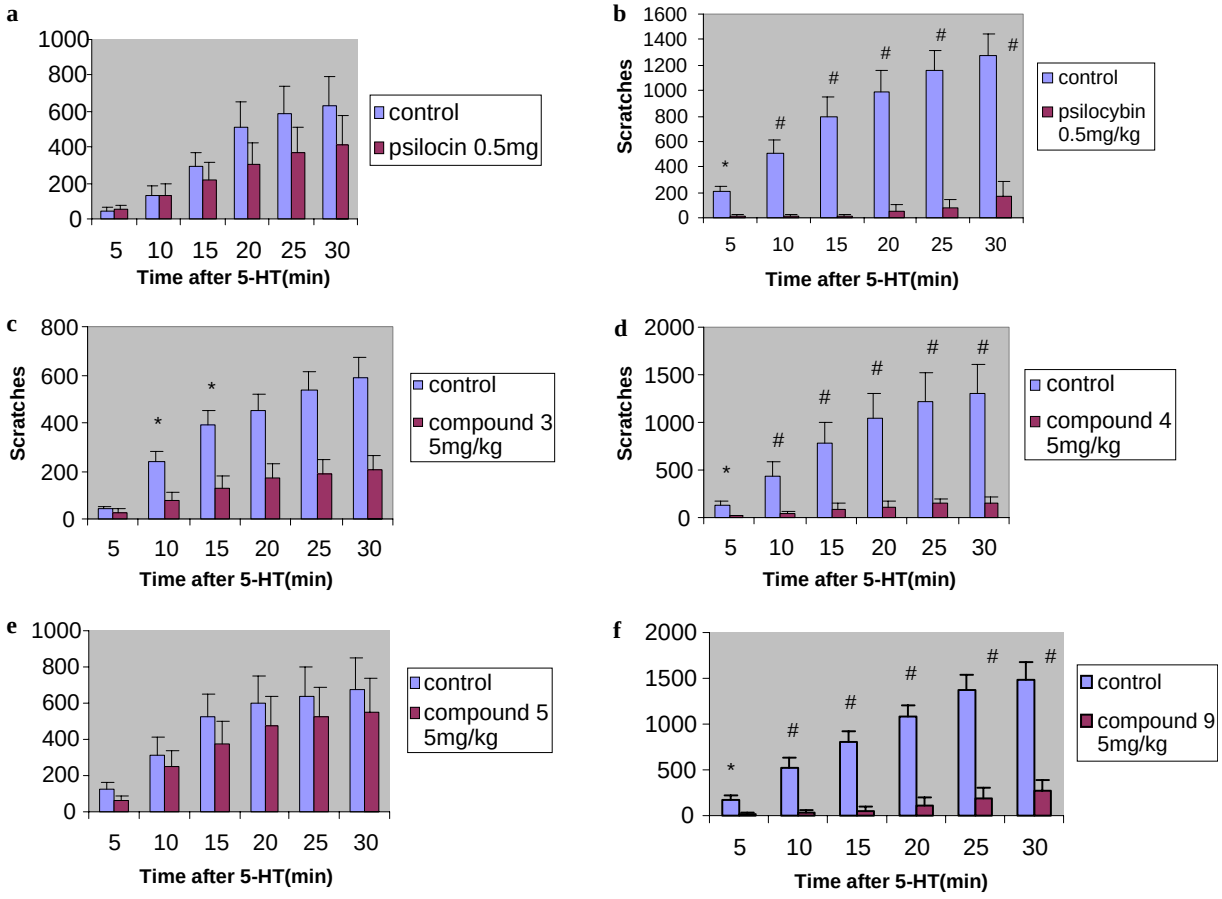


Figure 3. Inhibition of serotonin-induced scratching by psilocybin and analogs (a) $N = 7$, (b) $N = 5$, (c) $N = 12$, (d) $N = 8$, (e) $N = 5$, (f) $N = 5$; $^{\#}P < 0.01$, $^{*}P < 0.05$.

Acknowledgments

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