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Synthesis and structure–activity relationship of 1*H*-indole-3-carboxylic acid pyridine-3-ylamides: A novel series of 5-HT_{2C} receptor antagonists

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ABSTRACT

A novel series of 1*H*-indole-3-carboxylic acid pyridine-3-ylamides were synthesized and identified to show high affinity and selectivity for 5-HT_{2C} receptor. Among them, 1*H*-indole-3-carboxylic acid[6-(2-chloro-pyridin-3-yloxy)-pyridin-3-yl]-amide (**15k**) exhibits the highest affinity (IC_{50} = 0.5 nM) with an excellent selectivity (>2000 times) over other serotonin (5-HT_{1A}, 5-HT_{2A}, and 5-HT_6) and dopamine (D_2–D_4) receptors.

The 5-HT_2 receptor family consists of 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} subtypes, classified on the basis of molecular sequence, secondary messenger system, and pharmacological profile.[1–3] All are G-protein coupled receptors (GPCRs) and involve in the processes of activating phospholipase C via Gq-protein.[4] The non-selective 5-HT agonist, *m*-chlorophenyl piperazine (*m*CPP), is known to induce symptoms of compulsive disorder, depression, and anxiety in several animal models.[5,6] As this *m*CPP-induced anxiety seems to be mediated via 5-HT_{2C} receptor, it has been hypothesized that 5-HT_{2C} antagonists might be potential drugs for the treatment of anxiety and other psychiatric disorders.[7,8] Because 5-HT_2 receptor subtypes have approximately 80% amino acid identity in the transmembrane regions, there remains a paucity of selective 5-HT_{2C} receptor antagonists. In recent efforts several selective 5-HT_{2C} antagonists have been developed including **1** (SB-243213, pK_i = 9.0),[7a] **2** (pK_i = 9.2),[6] **3** (pK_i = 8.5)[9], and **4** (pK_i = 8.5) as shown in Figure 1.[10] Researchers at GSK found that bisaryl ether part of **1** (SB-243213) showed high 5-HT_{2C} receptor affinity and selectivity over 5-HT_{2A} receptor in addition to a range of other monoamine receptors, including serotonergic and dopaminergic subtypes.[7,11] Continuous studies showed that H-bond acceptor, the carbonyl oxygen on the vinylic or aromatic lactam moiety of **2**–**4**, plays an important role on the potent and selective 5-HT_{2C} inhibition.[9,10,12] Based on these observations, we designed a novel series of fused heterobicyclic carboxylic acid pyridine-3-ylamides (**12** and **15**) as potential 5-HT_{2C} receptor antagonists. An important structural aspect of them was the incorporation of 6-substituted-pyridine-3-yl moieties as a selectivity inducing part and heterobicyclic carbonyl moieties as H-bond acceptor. It may be expected to have a synergistic influence on their binding activity as well as selectivity for 5-HT_{2C} receptor. Herein, we report the synthesis and biological properties of a series of heterobicyclic carboxylic acid pyridine-3-ylamides.

The preparation of the key intermediates, heterobicyclic carboxylic acids, is outlined in Scheme 1. The substituted indoles **5** were treated with trifluoroacetic anhydride in DMF to yield the indole-3-trifluoroacetates **6**. Indole-3-carboxylic acids **7** were obtained by hydrolyzing **6** with 20% aqueous NaOH.[13] Benzofuran-3- and benzothiophene-3-carboxylic acids **9a** and **9b** were prepared by treatment of the corresponding 3-bromo compounds **8a** and **8b**, respectively, with *n*-butyllithium, followed by addition of excess CO_2. Other bicyclic carboxylic acids and sulfonyl chlorides were prepared by known literature procedures.[14–17] The pyridin-3-yl amines **11** were prepared by treating hydroxyl compounds **10** with 2-chloro-5-nitropyridine with NaH in DMF, followed by reduction of the resulting nitropyridine with $SnCl_2$ in a mixture of EtOH and concentrated HCl. Target sulfonamides **14a** and **14b** were obtained by treatment of heterobicyclic sulfonyl chlorides **13a** and **13b** with the pyridin-3-yl amine **11a** in dichloromethane at room temperature in the presence of triethylamine as a base. Carboxamides **12** and **15** were prepared by coupling the appropriate heterobicyclic carboxylic acids and the pyridin-3-yl amines **11** using DCC or HBTU in DMF as shown in Scheme 2.

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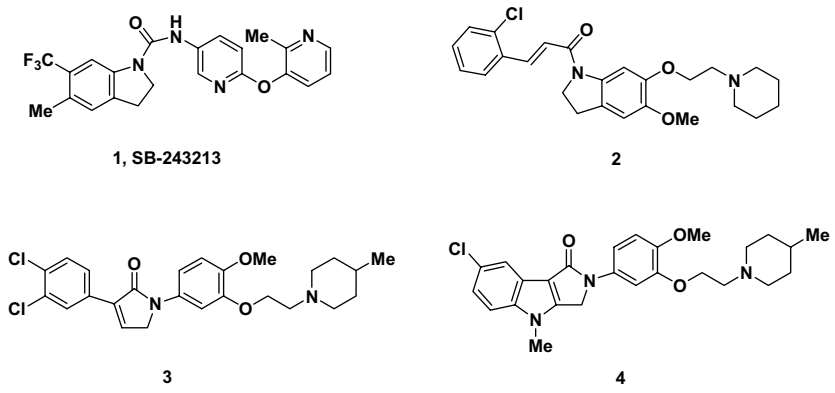
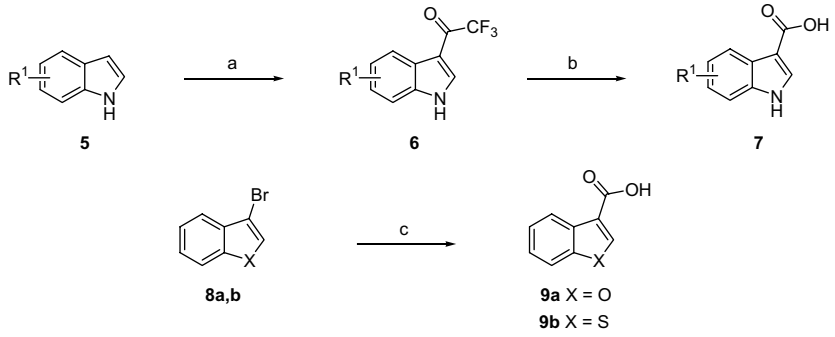
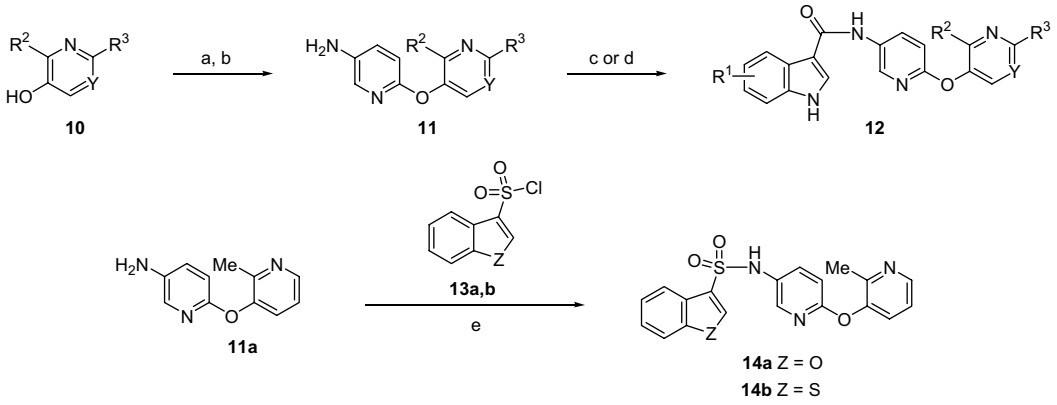


Figure 1. Structures of 5-HT_{2C} receptor antagonists.



Scheme 1. Reagents and conditions: (a) TFAA, DMF, rt; (b) 20% NaOH (aq), 60 °C; (c) *n*-BuLi, CO_2, Et_2O, −78 °C to rt.

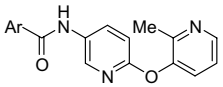


Scheme 2. Reagents and conditions: (a) 2-chloro-5-nitropyridine, NaH, DMF, 0 °C to rt; (b) $SnCl_2$, EtOH/concd HCl, 50 °C; (c) **7**, DCC, DMF, 50 °C; (d) **7**, HBTU, DMF, Et_3N, rt; (e) Et_3N, CH_2Cl_2, rt.

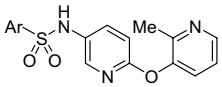
The affinities of the synthesized compounds for the human recombinant 5-HT_{2C} receptor expressed by CHO-K1 cell line were determined using [^{3}H]-mesulergine as a radioligand.[18] The results are summarized in Table 1. Various heterobicyclic carboxamides **12a–e** possess 6-(2-methyl-pyridin-3-yloxy)-pyridin-3-yl subunit as a constant. Indole-2- and 3-carboxamides **12c** and **12d** show a significant affinity. It was expected to replace an indoline subunit of the known antagonist **1** (SB-243213) to the alternative heterobicyclic subunit such as an indole, remaining reasonable receptor binding properties.

To investigate this aspect we prepared two additional scaffolds, benzofuran and benzothiophene, which maintain the indole structural feature. Although benzofuran-3-carboxamide **12a** shows slightly decreased affinity, benzothiophene-3-carboxamide **12b** retains a comparable affinity to that of **12d**. However, the benzoimidazole analog **12e** dramatically loses the binding activity. This suggests that favorable binding modes to 5-HT_{2C} receptor may be accessible to a limited range of heterotricycles. In addition, the alternative use of sulfonamide linkage as a H-bond acceptor in **14**, instead of a carboxamide in **12**, is unfavorable in binding to

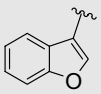
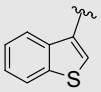
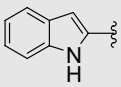
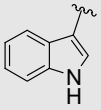
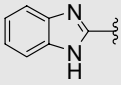
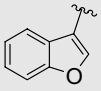
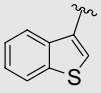
Table 1
5-HT_{2C} receptor binding affinity of bispyridyl amides or sulfonamides[a]



12a–e



14

Compound	Ar	IC_{50}[b] (nM)
12a		167
12b		50
12c		59
12d		67
12e		>10,000
14a		321
14b		572

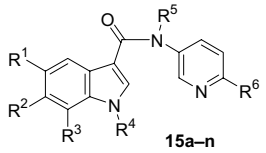
[a] Displacement of [^{3}H]-mesulergine binding to cloned human 5-HT_{2C} receptors expressed in CHO-K1 cell line.
[b] All values are means of two or three separate competition experiments.

the receptor. It implies that the electronic properties of H-bond acceptor may also be of importance.

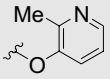
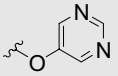
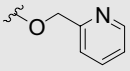
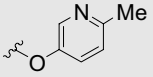
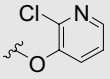
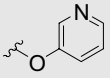
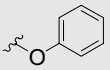
To explore the SAR studies, a wide range of *1H*-indole-3-carboxylic acid pyridine-3-ylamides (**15a**–**n**) as shown in Table 2 were prepared by parallel synthesis. In compounds **15d**, **15f**, and **15g**, the substitution with a methyl group at the positions of R^1, R^2, and R^3 endowed the significantly increased binding affinity (4.5, 1.9, and 8.2 nM, respectively) and a methoxy group at the position of R_1 was also affordable (10.7 nM). However, in compounds **15a**, **15b**, and **15c** the introduction of the *N*-methyl group on either R^4 or R^5 led to a significant loss in 5-HT_{2C} binding affinity, suggesting the presence of a lipophilic pocket in these receptors accommodating an acidic proton such as NH. Alternatively, the presence of *N*-substituent at the indole NH or carboxamide NH may be destructive in binding to the receptor.

The substitution effect on the pyridine-3-ylamide subunit for the receptor binding was investigated in the manner of replacing a 2-methyl-pyridin-3-yloxy group of compound **12d** with various heteroaryl and aryl groups. Both compounds **15k** and **15l**, having a 2-chloro-pyridin-3-yloxy and a pyridine-3-yloxy substituent at R^6, showed a larger increase in receptor affinity, while compound **15n** with non-substituted H at R^6 and compound **15m** with a phenol substituent suffered a dramatic fall.[19] Interestingly, the receptor binding of compound **15j** possessing 6-

Table 2
5-HT_{2C} receptor binding affinity of indole-3-carboxamides, **15a**–**n** [a]



15a–n

Compound	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50}[b] (nM)
12d	H	H	H	H	H		67
15a	H	H	H	Me	H		474
15b	H	H	H	H	Me		9441
15c	H	H	H	Me	Me		6369
15d	Me	H	H	H	H		4.5
15e	MeO	H	H	H	H		10.7
15f	H	Me	H	H	H		1.9
15g	H	H	Me	H	H		8.2
15h	H	H	H	H	H		50
15i	H	H	H	H	H		6
15j	H	H	H	H	H		2253
15k	H	H	H	H	H		0.5
15l	H	H	H	H	H		1.6
15m	H	H	H	H	H		542
15n	H	H	H	H	H	H	>10,000

[a] Displacement of [^{3}H]-mesulergine binding to cloned human 5-HT_{2C} receptors expressed in CHO-K1 cell line.
[b] All values are means of two or three separate competition experiments.

methyl-pyridin-3-yloxy group was detrimental (2253 nM). Substitution with pyridin-2-ylmethoxy or pyrimidin-5-yloxy at R^6 was well tolerated.

Compounds **15d**–**f**, **15k**, and **15l** were examined further for binding affinity toward several serotonergic and dopaminergic receptors (Table 3). 5-HT_{2C} receptor selectivity of these compounds was greater than 200-fold over 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, and dopamine (D_2–D_4) receptor and was higher than that of a reference compound SB-243213.

The functional efficacy of compound **15k** was evaluated by measuring 5-HT-stimulated binding of [^{35}S]GTPγS using CHO cells expressing the cloned human 5-HT_{2C} receptors.[20] In this study, compound **15k** was able to block 5-HT-stimulated binding of [^{35}S]GTPγS in a dose-dependent manner with IC_{50} value of 0.59 μM. It was assumed that compound **15k** is a selective and potent 5-HT_{2C} receptor antagonist.

In summary, we report the synthesis and biological profiles of a series of heterobicyclic carboxylic acid pyridine-3-ylamides. Most of compounds, particularly **15f** and **15k**, have been identified to

Table 3
5-HT_{2C} receptor selectivity profiles over serotonin (5-HT_{1A}–5-HT_7) and dopamine (D_2–D_4) receptor subtypes[a]

Compound	IC_{50}[b] (nM)							
	5-HT_{2C}	5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7	D_2	D_3	D_4
SB-243213	0.7	>1000	32.4	667	>1000	>1000	>1000	>1000
15d	4.5	>10,000	>1000	>10,000	>10,000	>10,000	>10,000	>10,000
15e	10.7	>10,000	>10000	>10,000	>10,000	>10,000	>10,000	>10,000
15f	1.9	>10,000	>10000	>10,000	>10,000	>10,000	>10,000	>10,000
15k	0.5	>10,000	>1000	>10,000	>10,000	>10,000	>10,000	>10,000
15l	1.6	>10,000	>1000	>10,000	>10,000	>10,000	>10,000	>10,000

[a] Receptors were all cloned human receptors expressed in CHO, HEK293 or SF9 cells, and [^{3}H] radioligands were as follows: 8-OH-DPAT (5-HT_{1A}), ketanserin (5-HT_{2A}), mesulergine (5-HT_{2C}), LSD (5-HT_6, and 5-HT_7), and spiperone (D_2–D_4).
[b] IC_{50} values are means of two or three separate competitive experiments.

possess the excellent 5-HT_{2C} affinity and the selectivity over the 5-HT_{2A} receptor and other related serotonergic and dopaminergic receptor subtypes. Additional studies with such compounds are now in progress.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.064.

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18. (a) Pazos, A.; Hoyer, D.; Palacios, J. M. *Eur. J. Pharmacol.* **1985**, *106*, 539; (b) Park, W. K.; Jeong, D.; Cho, H.; Lee, S. J.; Cha, M. Y.; Pae, A. N.; Choi, K. I.; Koh, H. Y.; Kong, J. Y. *Pharmacol. Biochem. Behav.* **2005**, *82*, 361; (c) Frozen membranes from stable CHO-K1 cell line expressing the human recombinant 5-HT_{2C} receptor was used. For the binding assay, [^{3}H]-mesulergine (1 nM), receptor membrane and test compounds were added into 50 mM Tris–HCl (pH 7.7) buffer containing 0.1% ascorbic acid and 10 μM pargyline. The incubations were performed for 30 min at 37 °C, and these were terminated by rapid filtration through Whatman GF/C glass fiber filter presoaked in 1% BSA. The filter was covered with MeltiLex, sealed in a sample bag followed by drying in the microwave oven, and counted by Microbeta Plus (Wallac, Finland). Non-specific binding was determined using 0.5 μM mianserin. Competition binding studies were carried out with 7–8 varied concentrations of the test compounds run in duplicate tubes, and isotherms from three assays were calculated by computerized non-linear regression analysis (GraphPad Prism Program, San Diego, USA) to yield IC_{50} (nM) values.
19. The compound **15n** was described to exhibit inhibitory activity against 5-HT_3 receptor in JP 03161470.
20. (a) Adlersberg, M.; Arango, V.; Mann, J. J.; Underwood, M. D.; Liu, K.; Kassir, S. A.; Ruggiero, D. A.; Tamir, H. J. *Neurosci. Res.* **2000**, *61*, 674; (b) Cussac, D.; Newman-Tancredi, A.; Duqueyroix, D.; Pasteur, V.; Millan, M. J. *Mol. Pharmacol.* **2002**, *62*, 578; (c) Receptor-linked G-protein inhibition by antagonists at h5-HT_{2C} receptors was determined by measuring the [^{35}S]GTPγS binding. The assay mixture consists of 20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA. Incubation was started by addition of membrane suspension and test compounds. Following 45 min incubation at 30 °C, bound [^{35}S]GTPγS levels were determined by liquid scintillation counting. A compound showing ⩾50% inhibitory effects on 5-HT-stimulated [^{35}S]GTPγS was classified as an antagonist.