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Synthesis and biological evaluation of benzoisothiazole derivatives possessing *N,N*-dimethylformimidamide group as 5-HT$_6$ receptor antagonists

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ABSTRACT

A series of novel *N,N*-dimethyl-*N′*-(5-(Ar-sulfonamido) benzo[d]isothiazol-3-yl)formimidamides was designed and synthesized as 5-HT$_6$ ligands. Here *N,N*-dimethyl formimidamides was used as a replacement for an aminoethyl moiety. In vitro functional assays demonstrated compounds **9b** and **9i** significantly inhibited the 5-HT-induced Ca^{2+} increases (**9b**; IC_{50} = 0.36 μM and **9i**; IC_{50} = 0.44 μM), indicating that **9b** and **9i** were potent 5-HT$_6$ receptor antagonists. Compounds **9i** also showed good selectivity on the 5-HT$_6$ over 5-HT$_4$ and 5-HT$_7$ receptors.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT, **1**) is a major neurotransmitter that mediates multiple physiological functions by interacting with 5-HT receptors. These 5-HT receptors are divided into seven families, designated as 5-HT$_1$ through 5-HT$_7$ and further subdivided into 14 subpopulations. The 5-HT$_6$ receptor that is a member of the G-protein subfamily, is positively coupled to adenylate cyclase via the Gs protein. It is one of the most recently identified 5-HT receptors.[1–5] It is mainly localized in the central nervous system (CNS) such as olfactory tubercles, striatum, nucleus accumbens, and hippocampus. There is evidence that its exclusive distribution in the brain is related to cognition, obesity, and certain neuropsychiatric disorders and neurodegenerative diseases, including depression, schizophrenia, and Alzheimer's disease. Its high affinity for typical and atypical antipsychotic agents implies a possible role for 5-HT$_6$ receptor as promising, novel target for CNS-mediated diseases.[6–10]

Up to date, there has been various 5-HT$_6$ receptor antagonists developed. The first antagonist Ro 04-6790 (**2**) was identified in the late 1990s by HTS of compound libraries at Roche.[11,12] And Glennon's group at Merck Sharp and Dohme accomplished pioneering work in the discovery of the antagonists MS-245 (**3**) by synthesizing the of 5-HT$_6$ receptor ligands.[13–15] The first candidate for clinical development, the phenyl-piperazine SB-271046 (**4**) which was developed by GlaxoSmithKline and entered the Phase 1 trials but was discontinued.[16] Several other compounds have entered clinical trials, such as SB-742457 (**5**) and LY-483518 (**6**), for the treatment of cognitive dysfunction associated with Alzheimer's disease, or cognitive impairment associated with schizophrenia (Fig. 1).[17–19]

Despite some of these unsuccessful endeavor, significant efforts have been put forth in order to understand the 5-HT$_6$ receptor antagonists ligand pharmacophore in the past few years.[20] These medicinal chemistry-driven approaches have delivered highly selective lead structures with well-defined functionalities. The structural requirements for the 5-HT$_6$ receptor ligand that are postulated by several research groups are comprised of four key requirements. A model composed of two hydrophobic areas (ARs) connected with hydrogen bond acceptor (HBA), and one proton donor group (PI) was proposed by Holenz.[21,22] The core hydrophobic area is generally an indole, indole-like or a monocyclic/bicyclic aryl motif. The other hydrophobic area's favorite motif include phenyl, naphthyl, benzothiophenyl, imidazo[2,1,-*b*]thiazolyl and *p*-aminophenyl. The hydrogen bond acceptor function, in most cases, a double-hydrogen-bond acceptor, is nearly always represented by a sulfonamide or a sulfonyl motif.[20,21] In addition, the proton donor is an ionizable nitrogen, in majority of cases a tertiary aliphatic amine function such as piperazine, methyl piperidine or *N,N*-dimethyl ethyl group.[20,21]

Based on these pharmacophore models, we designed benzoisothiazole and benzothiazole derivatives having the arylsulfonamides, in which an ionizable nitrogen was introduced as *N,N*-dimethylformimiamide. This is certainly the first report that

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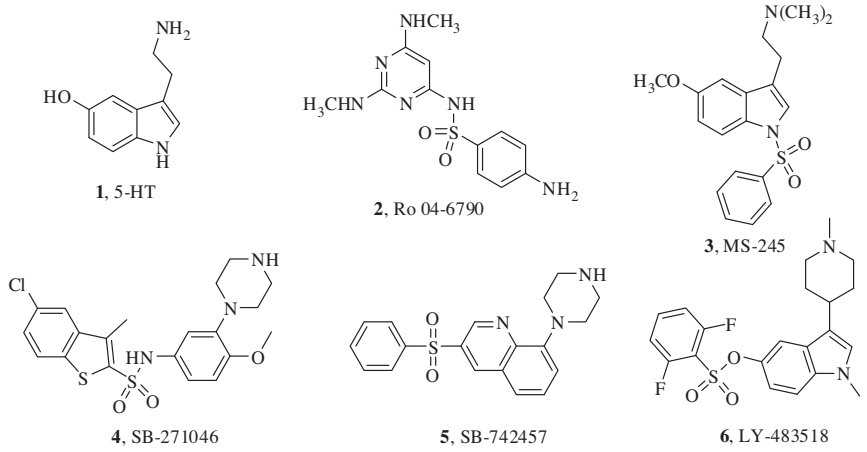


Figure 1. Structures of 5-HT and 5-HT_6 receptor antagonists.

N,N-dimethylformimiamide derivative was reported as 5-HT_6 receptor antagonists.

2. Results

2.1. Chemistry

The benzoisothiazole and benzothiazole derivatives which have a *N,N*-dimethylformimidamide group were synthesized using dimethylcarbamoyl chloride (Schemes 1 and 2). Coupling of *N,N*-dialkylformamide with primary amines by a number of coupling agents including P_2O_5, PCl_5, PCl_3, $SOCl_2$, acyl chlorides, and aryl isocyanate has been reported.[22] We evaluated the efficiency of *p*-toluenesulfonyl chloride, dimethylcarbamoyl chloride, diethylcarbamoyl chloride, 4-methylpiperazine-1-carbonyl chloride, and methyl(phenyl)carbamic chloride as a coupling agent. Although not much difference was observed between the reactivity of *p*-toluenesulfonyl chloride, dimethylcarbamoyl chloride, and diethylcarbamoyl chloride for the synthesis of forimidamide, the reaction with dimethylcarbamoyl chloride was slightly more favorable in the reaction rate and product yield. The coupling reaction of 3-amino-5-nitrobenzisothiazole with *N,N*-dimethylformamide and dimethylcarbamoyl chloride afforded the compound **7**. And then the reduction using $SnCl_2$/ultrasonic irradiation gave the amino product.[23] Subsequent reaction to form arylsulfonamide was performed via substitution of sulfonylchloride with amino group. Each and every compound synthesized was characterized by ^1H NMR, ^{13}C NMR and high resolution MS.

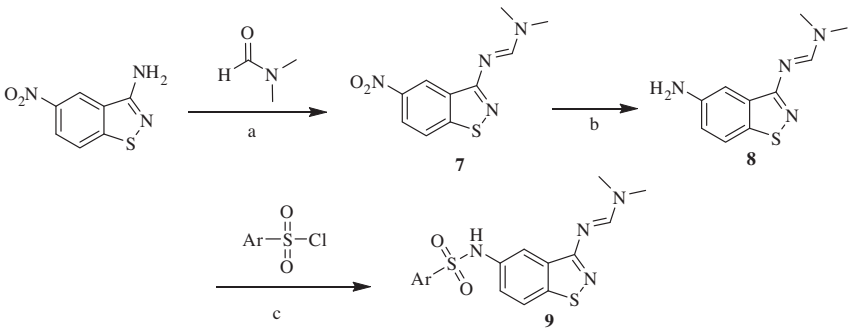
2.2. Biological evaluation

All the synthesized compounds were evaluated in vitro against the human recombinant 5-HT_6 serotonin receptor. The functional efficacy of each compound was evaluated by measuring the 5-HT-induced Ca^{2+} increases using HeLa cell line expressing the cloned human 5-HT_6 receptor (Table 1).[24] The selected compounds were further evaluated for their selectivity on the 5-HT_6 over the 5-HT_4 and 5-HT_7 receptors (Tables 2).

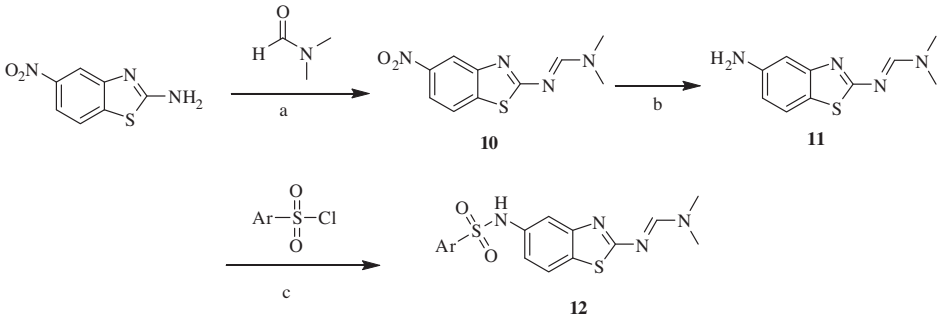
In a study conducted regarding the position of the *N,N*-dimethylformamidine for 5-HT_6 receptor affinity, *N,N*-dimethyl-*N′*-(5-(substituted sulfonamido)benzo[d]isothiazol-3-yl)formimidamide exhibited higher affinity than *N,N*-dimethyl-*N′*-(6-(substituted sulfonamido)benzo[d]thiazol-2-yl)formimidamide (**9h** >**12a** and **9i** >**12b**).

When eleven substituent groups (phenyl, 4-CH_3 phenyl, 4-$CH(CH_3)_2$ phenyl, 4-F phenyl, 4-Cl phenyl, 4-NO_2 phenyl, 2,6-F_2 phenyl, 1-naphtyl, 2-naphtyl, 8-quinolinyl, and biphenyl), of which the positions are fixed on the sulfonamide group were compared, the 4-CH_3 phenylsulfonamide and 2-naphthanlenesulfonamide significantly inhibited the 5-HT-induced Ca^{2+} increases (**9b**; IC_{50} = 0.36 μM and **9i**; IC_{50} = 0.44 μM) than other derivatives, indicating that compound **9b** and **9i** are potent 5-HT_6 receptor antagonists.

These two active compounds, **9b** and **9i**, were examined further for functional assay toward other serotonergic receptors (Table 2). The compound **9b** displayed higher activity for the serotonin 5-HT_6 receptor than for the serotonin 5-HT_4 receptor, but showed moderate inhibition activity for 5-HT_7 receptor, showing 22 and 69%

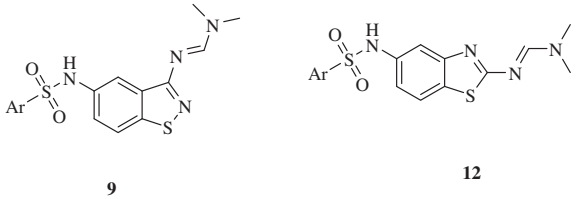


Scheme 1. (a) Dimethylcarbamoyl chloride, *N*-methylmorpholine, DMF, reflux, overnight; (b) $SnCl_2{\cdot}2H_2O$, EtOH, ultrasonic irradiation, RT, 30 min; (c) NaH, DMF, 100 °C, overnight.



Scheme 2. (a) Dimethylcarbamoyl chloride, *N*-methylmorpholine, DMF, reflux, overnight; (b) $SnCl_2{\cdot}2H_2O$, EtOH, ultrasonic irradiation, RT, 30 min; (c) NaH, DMF, 100 °C, overnight.

Table 1
% Inhibition and IC_{50} values of the sulfonamide derivatives **9a–h** and **12a–b** against 5-HT_6 receptor



Compound	Ar	% Inhibition (10 μM)	IC_{50} (μM)
9a	Phenyl	42.6 ± 2.4	
9b	4-Methylphenyl	84.3 ± 3.9	0.36
9c	4-Isopropylphenyl	27.0 ± 1.7	
9d	4-Fluorophenyl	20.2 ± 2.0	
9e	4-Chlorophenyl	51.1 ± 5.1	3.6
9f	4-Nitrophenyl	9.8 ± 5.6	
9g	2,6-Difluorophenyl	10.8 ± 3.1	
9h	1-Naphtyl	43.5 ± 6.7	
9i	2-Naphtyl	99.1 ± 0.3	0.44
9j	8-Quinolinyl	12.1 ± 3.7	
9k	Biphenyl	—	
12a	1-Naphtyl	25.5 ± 5.3	
12b	2-Naphtyl	66.32 ± 6.3	

Table 2
% Inhibition of the selected sulfonamide derivatives against 5-HT receptors

Compound	% Inhibition (10 μM) (n = 3)		
	5-HT_4 receptor	5-HT_6 receptor	5-HT_7 receptor
9b	21.9 ± 1.8	86.5 ± 4.5	68.8 ± 4.8
9e	11.6 ± 1.2	53.9 ± 6.0	40.2 ± 0.0
9i	17.4 ± 1.4	98.8 ± 0.1	28.9 ± 7.8

inhibition, respectively. Compound **9i** delivered low inhibiting activity for the 5-HT_4 and 5-HT_7 receptors showing only 17 and 29% inhibition, respectively.

3. Discussion

The 5-HT_6 receptor is one of the latest subtypes of the mammalian serotonin receptor family. It is highly unusual for a member of serotonin family because its distribution is almost exclusive within the CNS and the related signaling cascades are deeply implicated in the process of information perception, learning and memory formation.[25] The high affinity of a wide range of antipsychotic drugs for the receptor, coupled with its almost exclusive distribution in the brain, prompted much interest into the potential role of the 5-HT_6 as a target for CNS-mediated diseases. Therefore, 5-HT_6 receptors represent an extremely attractive target for the development of novel small molecule therapeutics for the treatment of various neurodegenerative disorders.[9,10]

According to the literature, the pharmacophore model of 5-HT_6 receptor antagonist shows four key pharmacophore elements: A positive ionizable atom (PI), an aromatic ring (AR), a hydrogen bond acceptor group (HBA), and a hydrophobic site (HYD).[20] On the basis of these structural requirements, we have designed a series of new compounds (**9a–9j**) that contain novel –N=C–N(CH_3)$_2$ system as PI. It has also been expected that a benzisothiazole ring, a –SO_2 group, and benzene or napthalene or quinoline served as AR, HBA, or HYD, respectively. Hence all the newly designed derivatives could represent a new class of 5-HT_6 receptor antagonists, contain a benzisothiazole scaffold in the central core. The –N=C–N(CH_3)$_2$ system, as well as piperazine, methyl piperazine, piperidine or *N,N*-dimethylethyl, resemble the –C–C–NH_2 system of serotonine. Many compounds containing piperazine, methyl piperazine, piperidine, aminoethyl or *N,N*-dimethylethyl, have been reported as 5-HT_6 antagonists, but none of *N,N*-dimethylformimiamide derivative was reported as 5-HT_6 receptor antagonists yet. Also the introduction of a nitrogen made the synthesis of target compounds much easier than aminoethyl moiety containing congeners.

In addition, the benzothiazole analogs (**12a** and **12b**) showed lower activities than benzoisothiazoles (**9h** and **9i**). The results could be interpreted that two different orientations of the AR,

HBA, and HYD moieties relative to the receptor-anchoring PI group in these two scaffolds relative to others might cause the different affinity to receptor. In the compounds **9a–9b** the $-N{=}C-N(CH_3)_2$ (PI) and HBA are attached to the position 3 and 5 of the benzisothiazole ring, while in compounds **12a–12b** they are attached to the position of 2 and 5 of benaothiazole ring.

In terms of SAR (structural–activity relationship) for HYD, compounds having 4-methyl phenyl, and 2-napthyl ring (IC_{50} = 0.36 and 0.44 μM, respectively) at the position number 5 of the benzisothiazole ring attached with the $-SO_2-NH-$ system showed good activity with 5-HT_6 receptor. For substituted phenyl groups, fluoro-, difluoro-, or nitro substitution resulted in the decreased activity. Only chloro substituted compound **9e** showed moderate activity with IC_{50} values of 3.6 μM. The larger substituent, for example **9c** (isopropyl group), on the phenyl ring also reduced the activity. In the previous QSAR study of bioactivities of 1-(azacyclyl)-3-arylsulfonyl-1H-pyrrolo[2,3-b] pyridines as 5-HT_6 receptor ligands, it has been known that the topological descriptors, derived from hydrogen suppressed molecular graphs representing connections between atoms, and molecular/group size, representing possible steric interactions with the receptor, are the important properties to be considered for the design of new analogs of the titled compounds.[26]

Indeed, 2-naphthyl and 1-naphthyl groups are commonly introduced in 5-HT receptor antagonists design.[27] 2-Naphthalenesulfonamides were previously described in the literature as multireceptor ligands with high affinity for 5-HT receptors.[28,29] The replacement of the 2-naphthyl ring with a nitrogen containing aromatic heterocycle, 8-quinolinyl, decreased the 5-HT_6 receptor antagonistic activity. Compound **9i** showed high inhibition activity toward 5-HT_6 receptor and good selectivity over the other related serotonergic receptor subtypes. It produced low inhibiting activity for the 5-HT_4 and 5-HT_7 receptors showing 17 and 29% inhibition, respectively, while 98.8% inhibition on 5-HT_6 at 10 μM. Compounds **9b** and **9e** showed moderate activity over 5-HT_7 receptor.

4. Conclusion

Novel *N,N*-dimethyl-*N′*-(5-(Ar-sulfonamido) benzo[d]isothiazol-3-yl)formimidamides were designed and synthesized as 5-HT_6 ligands using *N,N*-dimethyl formimidamides as replacement for an aminoethyl moiety. Compounds **9b** and **9i** significantly inhibited the 5-HT-induced Ca^{2+} increases (**9b**; IC_{50} = 0.36 μM and **9i**; IC_{50} = 0.44 μM, respectively), indicating that these compounds are potent 5-HT_6 receptor antagonists.

5. Experimental

5.1. Materials and methods

All the melting points of the synthesized compounds were taken in Pyrex capillaries using electrothermal digital melting point apparatus (Buchi) and were not corrected. ^{1}H NMR spectra were recorded on a 400 MHz Varian FT-NMR using tetramethylsilane as an internal standard. Mass spectra data were obtained on a Jeol JMS 700 high resolution mass spectrometer at the Korea Basic Science Institute (Daegu). Most of the reagents were purchased from Aldrich Chemical Company and Merck Company.

5.2. General procedure for the preparation of *N, N*-dimethyl-*N′*-(5-nitrobenzo[d]isothiazol-3-yl)formimidamide and *N,N*-dimethyl-*N′*-(6-nitrobenzo[d]thiazol-2-yl)formimidamide (7 and 10)

To a solution of 3-amino-5-nitrobenzoisothiazole or 2-amino-5-nitrobenzothiazole (0.4 g, 2.04 mmol) to be used in the coupling in DMF (0.3 M) and *N*-methylmorpholine (0.14 mL, 5.1 mmol), a solution of dimthylcarbamoyl chloride (1.88 mL, 2.04 mmol) in DMF (0.05 M) was added. The reaction was heated to 100 °C with stirring overnight. The resulting mixture was allowed to cool at room temperature and poured into water and extracted with EtOAc. The combined organic layers were washed with aqueous HCl (1 N), saturated aqueous $NaHCO_3$ and brine solution and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo and the crude product was purified by column chromatography using hexane: ethyl acetate (5:5) as eluent.

5.2.1. *N,N*-dimethyl-*N′*-(5-nitrobenzo[d]isothiazol-3-yl)formimidamide (7)

Red solid (81%), mp 147–148 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.73 (d, J = 2.4 Hz, 1H), 8.34 (s, 1H), 8.07 (dd, J = 9.7 Hz, 2.4 Hz, 1H), 7.51 (d, J = 9.6 Hz, 1H), 3.35 (s, 3H), 3.30 (s, 3H).

5.2.2. *N,N*-dimethyl-*N′*-(6-nitrobenzo[d]thiazol-2-yl)formimidamide (10)

Yellow solid (63%), mp 176–178 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.73 (d, J = 2.4 Hz, 1H), 8.65 (s, 1H), 8.21 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 3.33 (s, 3H), 3.17 (s, 1H).

5.3. General procedure for the preparation of *N, N′-(5-aminobenzo[d]isothiazol-3-yl)-N,N*-dimethylformimidamide and *N′-(6-aminobenzo[d]thiazol-2-yl)-N,N*-dimethylformimidamide (8 and 11)

To a solution of (E)-*N,N*-dimethyl-N-(5-nitrobenzo[d]isothiazol-3-yl)formamidine (1 mmol) in ethanol (5 mL), was added $SnCl_2{\cdot}2H_2O$ (10 mmol). The reaction mixture was exposed to ultrasonic irradiation for 30 minutes at 25 °C until the reaction was complete as indicated by TLC analysis. The solvent was removed under reduced pressure, and the crude residue was partitioned between ethyl acetate and 2 M KOH. The aqueous layer was extracted with further portions of ethyl acetate (3 × 25 mL), and the combined organic extracts were washed with brine (2 × 25 mL) and water (3 × 50 mL), dried ($NaSO_4$), and concentrated under reduced pressure. The crude residue was subjected to silica-gel column chromatography, using hexane and ethyl acetate as eluent (3:7).

5.3.1. *N,N′-(5-aminobenzo[d]isothiazol-3-yl)-N,N*-dimethylformimidamide (8)

Brown solid (35%), mp 100–102 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.97 (s, 1H), 7.23 (d, J = 9.6 Hz, 1H), 6.97 (dd, J = 9.2 Hz, 2.4 Hz, 1H), 7.74 (d, J = 2.4 Hz, 1H), 4.54 (s, 2H), 3.18 (s, 3H), 3.12 (s, 3H). HR-EI Calcd for $C_{10}H_{13}N_4S$ (M^++H): 221.0866, found: 221.0853.

5.3.2. *N′-(6-aminobenzo[d]thiazol-2-yl)-N,N*-dimethylformimidamide (11)

Brown solid (31%), mp 103–104 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.36 (s, 1H), 7.31 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 2.4 Hz, 1H), 6.70 (dd, J = 8.4 Hz, 2.2 Hz, 1H), 3.19 (s, 3H), 3.04 (s, 3H). HR-EI Calcd for $C_{10}H_{13}N_4S$ (M^++H): 221.0866, found: 221.0857.

5.4. General procedure for the preparation of *N,N*-dimethyl-*N′*-(5-(Ar-sulfonamido) benzo[d]isothiazol-3-yl)formimidamide and *N,N*-Dimethyl-*N′*-(6-(Ar-sulfonamido)benzo[d] thiazol-2-yl)-formimidamide (9a–h and 12a–b)

For the further conversion to sulfonamides, sodium hydride (1 mmol) was added to a suspension of 2-(4-methylpiperazin-1-yl)benzo[d]thiazol-6-amine (**8**) or *N′*-(6-aminobenzo[d]thiazol-2-yl)-*N,N*-dimethylformimid- amide (**11**) (0.5 mmol) in DMF (5 mL).

After stirring at 60 °C for 30 min under nitrogen, Ar-sulfonylchloride (1 mmol) in DMF (5 mL) was added. The reaction mixture was stirred at 100 °C for 16 h. After cooling, the mixture was extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4, and the solvent was removed in vacuo. The crude product was purified by column chromatography using hexane:ethyl acetate (7:3) as eluent.

5.4.1. *N,N*-dimethyl-*N′*-(5-(phenylsulfonamido)benzo[d]isothiazol-3-yl)formimidamide (9a)

Yellow solid (19%), mp 189–190 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.06 (s, 1H), 7.80 (d, J = 8.0 Hz, 2H), 7.61–7.57 (m, 1H), 7.54–7.48 (m, 3H), 7.31 (d, J = 9.6 Hz, 1H), 7.22 (dd, J = 9.6 Hz, 2.2 Hz, 1H), 3.23 (s, 3H), 3.16 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 175.48, 159.23 (2C, aromatic), 156.62 (1C, N=C–N), 139.51, 132.83, 130.08, 129.20, 126.65, 125.81, 125.22, 121.63, 111.24 (11C, aromatic), 34.51 (2C, N–C). HR-EI Calcd for $C_{16}H_{17}N_4O_2S_2$ (M^++H): 361.0971, found: 361.0975.

5.4.2. *N,N*-dimethyl-*N′*-(5-(4-methylphenylsulfonamido)benzo-[d]isothiazol-3-yl)formimidamide (9b)

Yellow solid (17%), mp 180–182 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.06 (s, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.52–7.48 (m, 2H), 7.31 (dd, J = 8.8 Hz, 2.2 Hz, 2H), 7.23 (d, J = 9.2 Hz, 1H), 3.23 (s, 3H), 3.16 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 159.80 (1C, aromatic), 155.48 (1C, aromatic), 153.94 (1C, N=C–N), 143.34, 136.42, 131.74, 129.64, 126.86, 125.63, 121.89, 120.93, 108.34 (11C, aromatic), 20.93 (1C, methyl). HR-EI Calcd for $C_{17}H_{19}N_4O_2S_2$ (M^++H): 375.0949, found: 375.0947.

5.4.3. *N,N*-dimethyl-*N′*-(5-(4-isopropylphenylsulfonamido)-benzo[d]isothiazol-3-yl)formimidamide (9c)

Yellow solid (41%), mp 159–161 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.05 (s, 1H), 7.72 (d, J = 8.4 Hz, 2H), 7.49 (d, J = 2.4 Hz, 1H), 7.38 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 9.2 Hz, 1H), 7.23 (dd, J = 9.6 Hz, 2.2 Hz, 1H), 3.22 (s, 3H), 3.15 (s, 3H), 2.98–2.91 (m, 1H), 1.20 (d, J = 6.8 Hz, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 175.37, 159.22 (2C, aromatic), 156.60 (1C, N=C–N), 153.57, 137.16, 130.21, 127.13, 126.82, 125.74, 125.25, 121.64, 110.81 (11C, aromatic), 34.50, 33.28 (2H, N–C), 23.33 (3C, isopropyl). HR-EI Calcd for $C_{19}H_{23}N_4O_2S_2$ (M^++H): 403.1262, found: 403.1265.

5.4.4. *N,N*-dimethyl-*N′*-(5-(4-fluorophenylsulfonamido)benzo-[d]isothiazol-3-yl)formimidamide (9d)

Brown solid (23%), mp 172–174 °C: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (s, 1H), 7.77–7.74 (m, 2H), 7.41 (d, J = 8.0 Hz, 2H), 7.11–7.07 (m, 2H), 7.00 (dd, J = 9.2 Hz, 2.8 Hz, 1H), 6.47 (s, 1H), 3.17 (s, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 175.64, 165.51, 163.00, 162.30, 159.29 (5C, aromatic), 156.58 (1C, N=C–N), 135.88, 129.86, 125.96, 121.71, 116.53, 111.70 (6C, aromatic), 35.77, 34.52 (2H, N-C). HR-EI Calcd for $C_{16}H_{16}FN_4O_2S_2$ (M^++H): 379.0699, found: 379.0696.

5.4.5. *N,N*-dimethyl-*N′*-(5-(4-chlorophenylsulfonamido)benzo-[d]isothiazol-3-yl)formimidamide (9e)

Yellow solid (35%), mp 200–201 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.07 (s, 1H), 7.78 (d, J = 8.8 Hz, 2H), 7.56 (d, J = 8.8 Hz, 2H), 7.47 (d, J = 1.6 Hz, 1H), 7.33 (d, J = 9.6 Hz, 1H), 7.20 (dd, J = 9.2 Hz, 2.4 Hz, 1H), 3.24 (s, 3H), 3.16 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 175.73, 159.28 (2C, aromatic), 156.71 (1C, N=C–N), 138.32, 137.72, 129.63, 129.41, 128.62, 125.93, 125.21, 121.76, 111.86 (11C, aromatic), 34.52 (2H, N–C). HR-EI Calcd for $C_{16}H_{16}ClN_4O_2S_2$ (M^++H): 395.0403, found: 395.0400.

5.4.6. *N,N*-dimethyl-*N′*-(5-(4-nitrophenylsulfonamido)benzo-[d]isothiazol-3-yl)formimidamide (9f)

Brown solid (11%), mp 213–215 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.38 (d, J = 6.8 Hz, 2H), 8.08 (s, 1H), 8.04 (d, J = 6.8 Hz, 2H), 7.50 (s, 1H), 7.33 (d, J = 9.6 Hz, 1H), 7.20 (dd, J = 9.6 Hz, 2.4 Hz, 1H), 3.23 (s, 3H), 3.15 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 175.92, 162.28, 159.79, 159.30 (4C, aromatic), 156.75 (1C, N=C–N), 149.78, 144.97, 129.25, 128.29, 125.20, 125.20, 124.62, 121.89, 112.25, 109.84 (9C, aromatic), 35.77, 34.53 (2H, N–C). HR-EI Calcd for $C_{16}H_{16}N_5O_4S_2$ (M^++H): 406.0644, found: 406.0647.

5.4.7. *N,N*-dimethyl-*N′*-(5-(2,6-difluorophenylsulfonamido)-benzo[d]isothiazol-3-yl)formimidamide (9g)

Yellow solid (12%), mp 217–218 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.07 (s, 1H), 7.71–7.63 (m, 1H), 7.61 (d, J = 2.4 Hz, 1H), 7.36 (d, J = 9.2 Hz, 1H), 7.30 (dd, J = 9.2 Hz, 2.0 Hz, 1H), 7.16 (t, J = 10.0 Hz, 2H), 3.24 (s, 3H), 3.17 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 175.55, 160.29, 159.12, 157.74 (4C, aromatic), 156.64 (1C, N=C–N), 136.12, 136.02, 135.90, 129.31, 125.21, 125.06, 121.85, 113.56, 113.34, 109.94 (9C, aromatic), 34.45 (2H, N–C). HR-EI Calcd for $C_{16}H_{15}F_2N_4O_2S_2$ (M^++H): 397.0605, found: 397.0602.

5.4.8. *N,N*-dimethyl-*N′*-(5-(naphthalene-1-sulfonamido)benzo-[d]isothiazol-3-yl)formimidamide (9h)

Brown solid (11%), mp 165–167 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.87 (d, J = 8.8 Hz, 1H), 8.23 (dd, J = 7.2 Hz, 1.2 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 8.05 (d, J = 8.8 Hz, 1H), 7.99 (s, 1H), 7.71 (t, J = 7.6 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.55 (t, J = 8.0 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H), 7.20 (d, J = 9.6 Hz, 1H), 7.08 (dd, J = 9.2 Hz, 2.4 Hz, 1H), 3.21 (s, 3H), 3.12 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 175.41, 159.18 (2C, aromatic), 156.58 (1C, N=C–N), 136.63, 134.21, 131.52, 129.96, 129.41, 129.17, 128.92, 127.94, 127.81, 127.65, 125.86, 125.20, 122.11, 121.63, 111.21 (15C, aromatic), 34.47 (2C, N–C). HR-EI Calcd for $C_{20}H_{19}N_4O_2S_2$ (M^++H): 411.0949, found: 411.0951.

5.4.9. *N,N*-dimethyl-*N′*-(5-(naphthalene-2-sulfonamido)benzo-[d]isothiazol-3-yl)formimidamide (9i)

Yellow oil (25%): ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.40 (s, 1H), 8.04 (d, J = 8.0 Hz, 2H), 8.00 (s, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.83 (dd, J = 8.8 Hz, 1.6 Hz, 1H), 7.69–7.60 (m, 2H), 7.47 (d, J = 2.0 Hz, 1H), 7.29 (d, J = 9.2 Hz, 1H), 7.24 (dd, J = 9.2 Hz, 2.0 Hz, 1H), 3.20 (s, 3H), 3.11 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 162.31, 159.79 (2C, aromatic), 155.45(1C, N=C–N), 136.26, 134.27, 131.58, 131.48, 129.41, 129.15, 128.96, 128.21, 127.83, 127.71, 125.70, 122.10, 121.89, 120.90, 108.63 (15C, aromatic), 35.76 (2C, N–C). HR-EI Calcd for $C_{20}H_{19}N_4O_2S_2$ (M^++H): 411.1127, found: 411.1130.

5.4.10. *N,N*-dimethyl-*N′*-(5-(quinoline-8-sulfonamido)benzo-[d]isothiazol-3-yl)formimidamide (9j)

Yellow solid (68%), mp 270–272 °C: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.17 (dd, J = 4.4 Hz, 1.9 Hz, 1H), 8.53 (dd, J = 8.4 Hz, 1.7 Hz, 1H), 8.31 (dd, J = 7.3 Hz, 1.4 Hz, 1H), 8.26 (dd, J = 7.3 Hz, 1.4 Hz, 1H), 8.04 (s, 1H), 7.74 (dd, J = 8.3 Hz, 4.2 Hz, 1H), 7.68 (dd, J = 8.4 Hz, 7.6 Hz, 1H), 7.17 (dd, J = 6.1 Hz, 0.7 Hz, 1H), 7.15 (s, 1H), 7.04 (dd, J = 9.6 Hz, 2.0 Hz, 1H), 3.12 (s, 3H), 3.04 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 175.83, 159.83 (2C, aromatic), 157.16 (1C, N=C–N), 152.17, 143.46, 137.77, 135.97, 134.89, 132.69, 130.79, 129.01, 126.84, 126.30, 125.80, 123.35, 121.88, 111.63, (14C, aromatic), 35.13 (2C, N–C). HR-EI Calcd for $C_{19}H_{18}N_5O_2S_2$ (M^++H): 412.1080, found: 412.0904.

5.4.11. *N′*-(5-(Biphenyl-4-ylsulfonamido)benzo[d]isothiazol-3-yl)-*N*,*N*-dimethylformimidamide (9k)

Brown solid (15%), mp 104–106 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.04 (s, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 10.4 Hz, 2H), 7.72–7.66 (m, 2H), 7.51–7.45 (m, 3H), 7.43–7.39 (m, 1H), 7.33 (dd, J = 9.4 Hz, 0.8 Hz 1H), 7.27 (dd, J = 9.4 Hz, 2.2 Hz, 1H), 3.20 (s, 3H), 3.12 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 162.29 (1C, aromatic), 156.63 (1C, N=C–N), 150.87, 141.50, 138.23, 129.06, 127.34, 127.01, 126.85, 124.20, 121.68, 116.6, 111.21, 101.80 (18C, aromatic), 35.75, 30.74 (2C, N–C). HR-EI Calcd for $C_{22}H_{21}N_4O_2S_2$ (M^++H): 437.1106, found: 437.1102.

5.4.12. *N*,*N*-dimethyl-*N′*-(6-(naphthalene-1-sulfonamido)benzo-[d]thiazol-2-yl)formimidamide (12a)

Pale brown solid (14%), mp 225–227 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.83 (d, J = 8.4 Hz, 1H), 8.40 (s, 1H), 8.20 (dd, J = 7.2 Hz, 1.2 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.71–7.62 (m, 2H), 7.54 (t, J = 8.0 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 6.99 (dd, J = 8.4 Hz, 2.4 Hz, 1H), 3.21 (s, 3H), 3.05 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.46, 160.35, 159.52 (3C, aromatic), 157.09 (1C, N=C–N), 148.43, 134.31, 133.70, 132.24, 129.81, 129.04, 128.04, 127.47, 126.93, 124.44, 124.33, 119.87, 118.98, 112.92 (14C, aromatic), 34.69 (2C, N–C). HR-EI Calcd for $C_{20}H_{19}N_4O_2S_2$ (M^++H): 410.0871, found: 410.0869.

5.4.13. *N*,*N*-dimethyl-*N′*-(6-(naphthalene-2-sulfonamido)benzo-[d]thiazol-2-yl)formimidamide (12b)

Pale brown solid (16%), mp 205–207 °C: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.42 (s, 1H), 8.36 (d, J = 2.0 Hz, 1H), 8.03 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.0 Hz, 1H), 7.77 (dd, J = 8.8 Hz, 2.0 Hz, 1H), 7.69–7.60 (m, 3H), 7.39 (d, J = 8.4 Hz, 1H), 7.13 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 3.22 (s, 3H), 3.06 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.65 (1C, aromatic), 157.03 (1C, N=C–N), 149.08, 136.50, 134.16, 133.14, 132.29, 131.51, 129.32, 129.17, 128.87, 127.87, 127.78, 127.61, 122.07, 119.97, 119.88, 113.97 (16C, aromatic), 34.64 (2C, N–C). HR-EI Calcd for $C_{20}H_{19}N_4O_2S_2$ (M^++H): 411.0949, found: 411.0948.

5.5. Functional assays

5.5.1. Cell culture and transfection

HeLa and HEK293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. For 5-HT_6R activity, HEK293 or HeLa cell lines stably expressing the human 5-HT_6R, were used. For 5-HT_4R or 5-HT_7R activity, human 5-HT_4R or 5-HT_7R receptor gene was transiently expressed in HEK293 cells and the receptor activity assayed using the FDSS6000 system.

5.5.2. Assay of 5-HT receptors using the FDSS6000 system

We measured 5-HT-induced Ca^{2+} increases using a promiscuous $G\alpha_{15}$ protein that facilitates coupling of $G\alpha_S$-coupled receptors to phospholipase C and consequent intracellular Ca^{2+} release, which is subsequently detected using an FDSS6000 96-well fluorescence plate reader as previously reported.[24] Briefly, HeLa or HEK293 cells were loaded with the Ca^{2+} indicator dye Fluo-4-AM (5 μM) and 0.001% Pluronic F-127 (Molecular Probes, Eugene, OR) and incubated in a HEPES-buffered solution (150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, 2 mM $CaCl_2$) for 1 h at 37 °C. Then, the cells were washed three times with a HEPES-buffered solution and maintained with a volume of 80 μL/well in 96-well plates. For antagonist experiments, cells were pre-incubated with compounds for 15 min before the addition of an agonist. The fluorescence intensity (F), and the initial fluorescence intensity (F_0) were measured at 480 nm. All data were collected and analyzed using the FDSS6000 system and related software (Hamamatsu Photonics, Japan).

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