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Discovery of 3-aryl-3-methyl-1*H*-quinoline-2,4-diones as a new class of selective 5-HT$_6$ receptor antagonists

Churl Min Seong,[a,*] Woo Kyu Park,[b] Chul Min Park,[a] Jae Yang Kong[b] and No Sang Park[a]

[a]*Center for Medicinal Chemistry, Drug Discovery Division, Korea Research Institute of Chemical Technology, 100 Chang-dong, Yuseong-gu, Daejeon 305-606, South Korea*
[b]*Center for Drug Discovery Technologies, Drug Discovery Division, Korea Research Institute of Chemical Technology, 100 Chang-dong, Yuseong-gu, Daejeon 305-606, South Korea*

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Abstract—A 5,7-dichloro-3-phenyl-3-methyl-quinoline-2,4-dione (**11a**) has been identified in a random screen as a lead for 5-HT$_6$ antagonist. During the lead optimization process, several analogs were synthesized and their biological activities were investigated. Within this series, several compounds display high binding affinity and selectivity for the 5-HT$_6$ receptor. In particular, 3-(4-hydroxyphenyl)-3-methyl-quinoline-2,4-dione (**12f**) exhibits high affinity (K_i = 12.3 nM) for 5-HT$_6$ receptor with good selectivity over other serotonin and dopamine (D_1–D_4) receptor subtypes. In a functional adenylyl cyclase stimulation assay, this compound exhibited considerable antagonistic activity (IC_{50} = 0.61 μM).

The 5-HT$_6$ receptor is the most recently discovered member of the serotonin receptor family.[1] It was isolated from rat mRNA in 1993, found to consist of 438 amino acids with seven transmembrane domains, and is positively coupled to adenylyl cyclase.[2] Human 5-HT$_6$ receptor was subsequently cloned in 1996 and shares 89% homology with the rat receptors.[3] Expression of the 5-HT$_6$ receptor mRNA is localized exclusively in the central nervous system (CNS).[4] Binding studies have disclosed that certain tricyclic antipsychotic drugs and antidepressants have significant affinity for 5-HT$_6$ receptor.[5] The localization and their affinity for the CNS drugs have sparked efforts to elucidate their role in treatment of CNS disorders, including schizophrenia and depression, and more recent studies implicate possible involvement in cognition and memory dysfunction.[6–8] Most of the early exploration of 5-HT$_6$ pharmacology was largely dependent on the use of nonselective agents. However, this lack of selectivity limited their value for most other pharmacological studies. In more recent efforts several selective 5-HT$_6$ antagonists have been reported including Ro 04-6790 (**1**, K_i = 55 nM), Ro 63-0563 (**2**, K_i = 512 nM),[9] SB-271046 (**3**, K_i = 1 nM),[10] SB-357134 (**4**, K_i = 3 nM),[11] and MS-245 (**5**, K_i = 2.3 nM)[12,13] as shown in Figure 1. Interestingly, although these represented independent discoveries, the compounds were identified by random screening methods and all possess a sulfonamide moiety. In contrast to the above-mentioned sulfonamides or tryptamine derivatives, Hoffmann-LaRoche (**6**, K_i = 1.0 nM)[14] and Pharmacia-Upjohn (**7**, K_i = 1.4 nM)[15] also revealed several aromatic sulfones.

Advent of these selective agents has greatly benefited 5-HT$_6$ studies, and this field of research has experienced resurgence. Despite the high affinity and selectivity of these compounds, a significant problem in preclinical trials was their poor penetration to cross the blood brain barrier. Newer agents are aimed at improving pharmacokinetic and pharmacodynamic properties.

The purpose of our present study was to identify novel, potent antagonists with enhanced selectivity for 5-HT$_6$ receptors that might be useful as potential therapeutics with improved brain-penetrating properties. We began our investigation by exploring the structure–affinity relationships for the known 5-HT$_6$ receptor antagonists and

Keywords: 5-HT$_6$ Receptor antagonist; Schizophrenia; Alzheimer's disease; Quinoline-2,4-dione.
* Corresponding author. Tel.: +82 42 860 7134; fax: +82 42 861 1291; e-mail: cmsung@krict.re.kr

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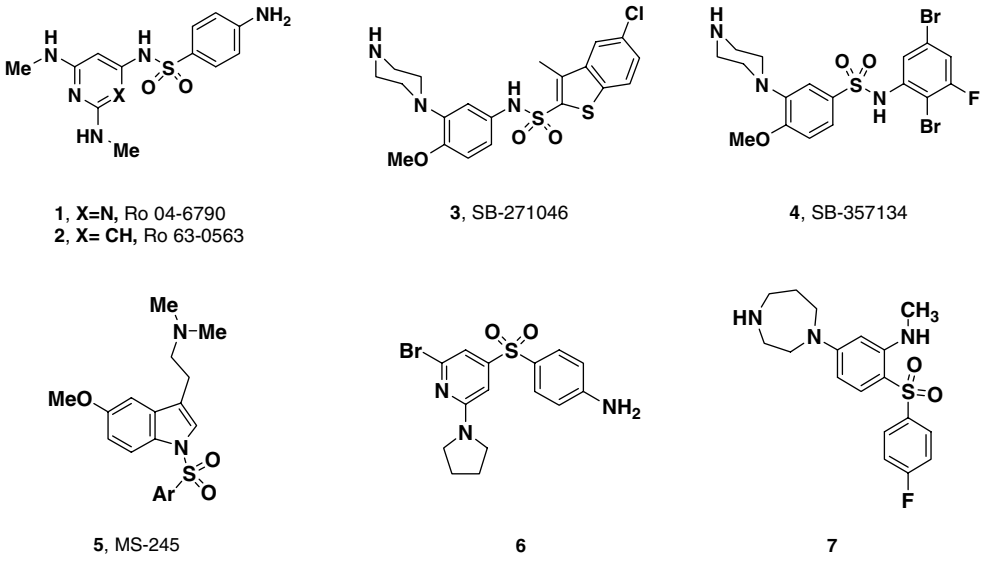
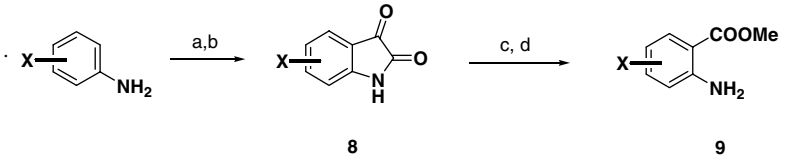
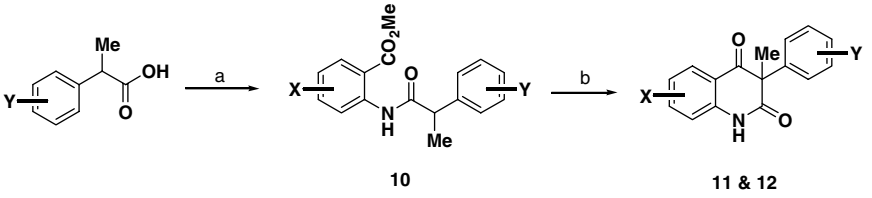


Figure 1. Structures of 5-HT_6 receptor antagonists.

high throughput screening with in-house compounds. We found that (+/−)-5,7-dichloro-3-phenyl-3-methyl-quinoline-2,4-dione (**11a**, K_i = 54.7 nM) exhibits good affinity and significant selectivity for 5-HT_6 receptor. Our studies were then directed toward the structural modification of quinoline-2,4-dione skeleton for the

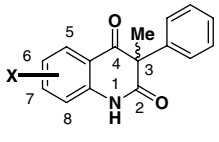


Scheme 1. Reagents and conditions: (a) Chloral hydrate, NH_2OH–HCl, Na_2SO_4, H_2O, reflux; (b) conc. H_2SO_4, 80 °C; (c) H_2O_2/aq.NaOH, rt; (d) CH_2N_2/Et_2O, rt.



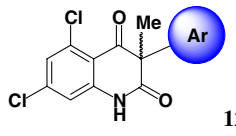
Scheme 2. Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, then **9**, reflux; (b) LiHMDS, THF, −78 °C to reflux.

Table 1. 5-HT_6 receptor binding affinity of 3-methyl-3-phenyl quinoline-2,4-diones, **11a**–**j**[a]

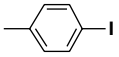
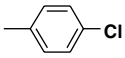
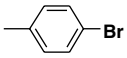
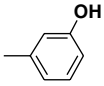
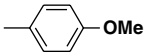
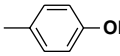
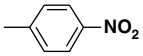
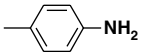
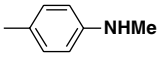
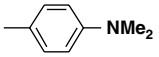
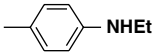
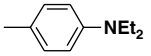
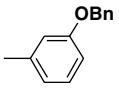
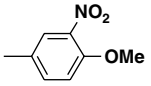
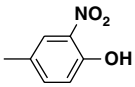
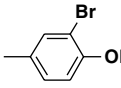
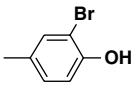
	Compound	Substituents (**X**)	K_i[b] (nM)
	**a**	5,7-Cl_2	54.7 ± 14.4
	b	5,6,7,8-H	>1000
	c	7-Cl	>1000
	d	6,7-Cl_2	>1000
	e	5,7-Br_2	196.9 ± 57
	f	6,8-Br_2	>1000
	g	5-Cl,7-OMe	344.6 ± 46
	h	5-Br,7-OMe	73.2 ± 12
	i	5,7-di-OMe	728 ± 115
	j	5-Cl,7-NMe_2	715 ± 110

[a] Displacement of [^{3}H]-LSD binding to cloned human 5-HT_6 receptors expressed in HEK293 cell line.
[b] All values are means ± SEM of three separate competition experiments.

Table 2. 5-HT_6 receptor binding affinity of 3-aryl-5,7-dichloro-3-methyl quinoline-2,4-diones, **12a–q**[a]



12a~q

Compound	Ar	K_i[b] (nM)
11a		54.7
12a		952
12b		877
12c		304
12d		30.1
12e		147
12f		12.3
12g		>1000
12h		156
12i		257
12j		346
12k		28.3
12l		9.2
12m		82.4
12n		>1000
12o		>1000
12p		>1000
12q		77.5

[a] Displacement of [^{3}H]-LSD binding to cloned human 5-HT_6 receptors expressed in HEK293 cell line.
[b] All values are means of three separate competition experiments.

delineation of optimal substitution patterns. A series of 3-aryl-3-methyl-quinoline-2,4-diones were prepared and their pharmacological profiles for 5-HT_6 receptors were examined. These compounds are structurally different from prototypical antagonists in that they do not contain a sulfonamide or a sulfone functionality, but rather a substituted quinoline-2,4-dione skeleton. Herein, we wish to report the synthesis and biological evaluation of novel analogs of **11a**.

The general synthetic approach to 3,3-disubstituted quinoline-2,4-diones is presented in Schemes 1 and 2. The key intermediates for many of the quinoline-2,4-dione derivatives are substituted anthranilic esters. The anthranilic esters were either purchased or prepared from the corresponding anilines.

The substituted anilines were treated with chloral hydrate and hydroxylamine hydrochloride to produce the *N*-(substituted phenyl)(hydroxylimino)acetamides which were dehydrated in warmed concentrated sulfuric acid to give the isatins **8**.[16] Oxidation with hydrogen peroxide gave the substituted 2-amino-benzoic acid which was converted to the corresponding methyl ester **9** by treatment with ethereal diazomethane at room temperature. The preparation of 3,3-disubstituted-quinoline-2,4-diones was carried out by condensation of the anthranilic methyl ester **9** with the appropriate 2-methyl-2-phenyl propionic acids. The intermediate acid chloride was prepared *in situ* by treatment of the acids with $SOCl_2$ in anhydrous methylene chloride at room temperature.

Coupling of the crude acid chloride with the anthranilic methyl ester **9** was conducted under anhydrous conditions to provide the corresponding amides **10**. Treatment of the amide **10** with LiHMDS [prepared*in situ* using *n*-BuLi in THF at −78 °C] in THF or NaH in DMF at elevated temperatures induced intramolecular cyclization to give the target 3,3-disubstituted quinoline-2,4-diones **11** and **12** in good yields.

The affinities of the synthesized compounds for the human recombinant 5-HT_6 receptor expressed by HEK293 cell line were determined using [^{3}H]-lysergic acid diethylamide (LSD).[17] The results are summarized in Table 1. All compounds were tested as the racemates. The 3-methyl-3-phenyl-quinoline-2,4-dione (**11a**) as a hit compound in a random screen demonstrated reasonably high binding affinity (K_i = 54.7 nM) to 5-HT_6 receptor. This implies that the sulfonamide backbone separated from a basic amine by an aryl group in well-

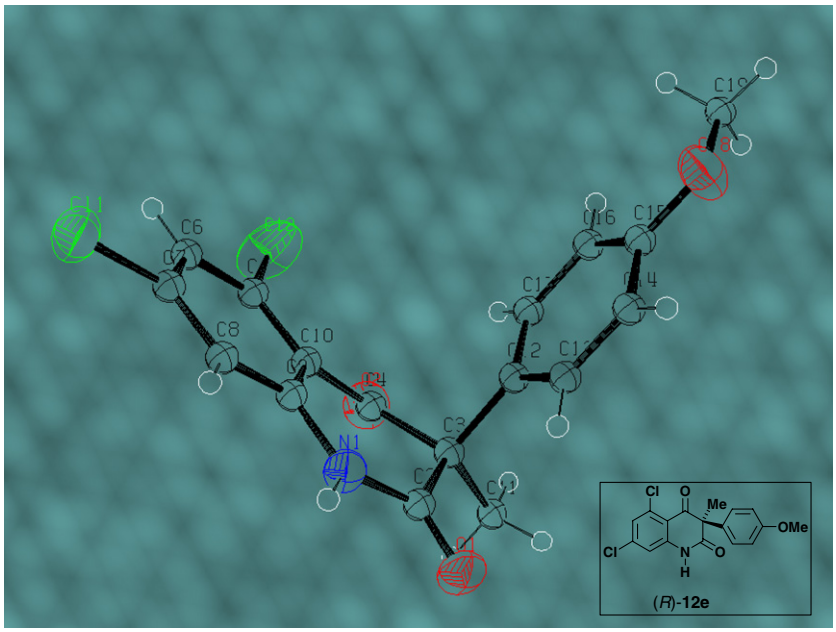


Figure 2. ORTEP representation of (*R*)-form isomer of **12e**.

known antagonists such as SB-271046 or Ro 04-6790 is not an essential requirement for binding to the 5-HT_6 receptor and the substituted quinoline-2,4-diones could be the alternative structure for new potent 5-HT_6 receptor ligand.

Additional analogs of compound **11a** provided consistent results to further support the hypothesis. The 5,7-disubstituted analogs (**11e** and **11h**) showed moderate binding activities to 5-HT_6 receptor, whereas the unsubstituted analog **11b** did not exhibit any significant binding activity. Within this series, none of the analogs showed better binding affinity compared to hit compound **11a**.

Next, we investigated the substituent effects on the phenyl ring at the C3 position of quinolone nucleus with remaining the 5,7-dichloro-substitution as shown in Table 2.

A wide range of substituted 3-aryl-3-methyl-quinoline-2,4-diones were prepared by parallel synthesis. In particular, compounds **12d**, **12f**, **12k**, and **12l** containing a hydroxyl, ethylamino or diethylamino group gave high binding affinities. In contrast, the binding activities of compounds **12g**, **12n** and **12o** containing the electron withdrawing NO_2 substituent were detrimental. Introduction of halogen atom on C2-phenyl ring in compounds **12a**, **12b**, and **12c** was unfavorable in 5-HT_6 receptor binding. To elucidate the optimal configuration in binding for 5-HT_6 receptor, each optically pure enantiomer of 3-methyl-3-(4-methoxyphenyl-quinoline-2,4-dione **12e** was separated by a chiral chromatography using Pirkle-type stationary phase.[18] The absolute configuration of each isomer was determined by X-ray analysis, as shown in Figure 2. (*S*)-isomer (*S*)-**12e** (K_i = 293 nM) showed higher binding affinity than (*R*)-isomer (*R*)-**12e** (K_i = 893 nM) and its racemate **12e** (K_i = 563 nM). This implies (*S*)-configuration on C3 in the quinolone nucleus is preferable in binding for 5-HT_6 receptor.

Compounds **11a**, **12d**, **12f**, and **12m** were examined further for binding activity toward several serotonergic and dopaminergic receptors (Table 3). The 5-HT_6 receptor selectivity of compounds **11a** and **12f** was greater than 200-fold over 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_7, and dopamine (D_1–D_4) receptors and was comparable to that of a reference compound SB-271046. Meanwhile, compounds **12m** produced moderate binding affinity for the 5-HT_{1A} and D_1 receptors showing 0.17 and 0.12 μM of K_i values, respectively.

The functional efficacy of compound **12f** was evaluated by measuring 5-HT-stimulated cAMP accumulation using HeLa cell line expressing the cloned human 5-HT_6 receptor.[19] In this study, 5-HT-stimulated cAMP accumulation was significantly inhibited by compound **12f** (IC_{50}: 615 nM), indicating that compound **12f** is a selective and potent 5-HT_6 receptor antagonist. Nonselective antagonist methiothepin was also found to be an antagonist showing 127 nM of IC_{50} value in the present experiments.

In summary, 5,7-dichloro-3-methyl-3-phenyl-quinoline-2,4-dione (**11a**) was isolated in high-throughput screening and represents a suitable template for the further development of serotonin 5-HT_6 receptor ligands. The incorporation of appropriate substituents (e.g., **12d**, **12f**, **12k**, and **12l**) resulted in compounds with improved affinity for the receptor. These compounds are noteworthy for their structural uniqueness and selectivity as 5-HT_6 receptor. Additional studies with such compounds are now in progress.

Table 3. 5-HT_6 Receptor selectivity profiles over serotonin (5-HT_{1A}–5-HT_7) and dopamine (D_1–D_4) receptor subtype[a]

Compounds	K_i[b] (μM)								
	5-HT_6	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	5-HT_7	D_1	D_2	D_3	D_4
11a	0.054	>10	>10	>10	>10	>10	>10	>10	>10
12f	0.012	>10	>10	>10	>10	>10	>10	>10	>10
12m	0.082	0.17	0.72	>10	0.60	0.12	>10	>10	>10
12d	0.030	>10	>10	>10	>10	1.81	>10	>10	>10
SB-271046	0.0005	0.34	1.74	0.53	0.26	0.12	0.46	1.33	0.13

[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 & 5-HT_7), SCH-23390 (D_1), and spiperone (D_2–D_4).
[b] K_i values are means of two or three separate competition experiments.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.045.

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17. (a) Drummond, A. H.; Bucher, F.; Levitan, I. B. *Brain Res.* **1980**, *184*, 163; (b) Drummond, A. H.; Bucher, F.; Levitan, I. B. *J. Biol. Chem.* **1980**, *255*, 6679; (c) Membranes from stable HEK-293 cell line expressing the human recombinant 5-HT_6 serotonin receptor (Perkin-Elmer Life and Analytical Sciences, Boston, USA) were used. For the binding assay, aliquots of receptor membranes, 1.6 nM [^{3}H]LSD, and appropriate concentrations of test compounds were added to 0.25 mL of 50 mM Tris–HCl (pH 7.4) buffer containing 10 mM $MgCl_2$ and 0.5 mM EDTA. Incubations were carried out for 60 min at 37 °C, and these were terminated by rapid filtration using an Innotech cell harvester (Innotech Biosystems, Switzerland) through Whatman GF/C glass fiber filter presoaked in 0.3% polyethylenimine. 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). Nonspecific binding was determined in the presence of 0.5 μM methiothepin. 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 nonlinear regression analysis (GraphPad Prism Program, San Diego, USA) to yield inhibition constant (K_i) values.
18. (a) Hyun, M. H.; Min, C. S. *Chirality* **1998**, *10*, 592; (b) Tambute, A.; Siret, L.; Caude, M.; Begos, A.; Rosset, R. *Chirality* **1991**, *3*, 427; (c) Chiral chromatography on silica gel (5 μm) grafted with a chiral stationary phase, (*S*)-*N*-(1-(3,5-dimethyl-phenylcarbamoyl)-2-{4-[3-(methoxy-dimethyl-silanyl)-propoxy]-phenyl}-ethyl)-3,5-dinitro-benzamide; Flow rate: 1.0 mL/min, Eluent: *n*-hexane-EtOAc(20:1), Detector UV 254 nm; (*R*)-**12e**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.52 (s, 3H, CH_3), 3.67 (s, 3H, CO_2CH_3), 6.89

(d, J = 8.9 Hz, 2H, ArH), 6.99–7.08 (m, 3H, ArH), 7.23 (d, J = 1.9 Hz, 1H, ArH), 11.25 (s, 1H, NH); mp. 208–210 °C; MS(EI) *m/e* 349[M^+], 162, 134; HRMS *m/e* calcd for $C_{17}H_{13}NO_3Cl_2$ 349.0272, found 349.0278; Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 650024. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.com.ac.uk].

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