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Novel quinazolinone derivatives as 5-HT$_7$ receptor ligands

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Abstract—5-HT$_7$ receptor antagonists generated antidepressant-like effects in animal model and the involvement of the 5-HT$_7$ receptor in other pathophysiological mechanisms such as thermoregulation, learning and memory, and sleep has been highlighted by various studies. As one of our efforts to discover a new type of 5-HT$_7$ receptor antagonists, we here report on the synthesis and binding affinities to the 5-HT$_7$ receptor of the quinazolinone library **1**, which was designed with various substituents (X, Y, R^1, and R^2) on the aromatic rings and different carbon chain length. Total 85 compounds of the quinazolinone library **1** were synthesized and the binding affinities of all the synthesized compounds were obtained by radioligand binding assay for the 5-HT$_7$ receptor. Among the 85 compounds, 24 compounds show very good binding affinities with IC$_{50}$ values below 100 nM. Mainly the compounds with IC$_{50}$ values below 100 nM have *o*-OMe or *o*-OEt as R^2 substituent. The compound with the best binding affinity is **1-68** of which the IC$_{50}$ value is 12 nM. In in vivo animal study, some synthesized compounds really have the antidepressant activity in the forced swimming test in mice.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a major neurotransmitter that plays important roles in physiological and pathophysiological processes such as memory, thermoregulation, sleep, depression, and so on.[1] There are seven types of 5-HT receptors, 5-HT$_1$–5-HT$_7$, which are redivided to 14 subtypes. Among the 14 subtypes of 5-HT receptors, the 5-HT$_7$ receptor is the most recently cloned in 1993.[2] The 5-HT$_7$ receptor is found in the brain, mainly in the hypothalamus, thalamus, hippocampus, and cortex.[3] The 5-HT$_7$ receptor is a G protein-coupled receptor (GPCR) protein and found to stimulate cAMP production and activate the extracellular signal-regulated kinase (ERK) through a mechanism that is dependent on a Ras monomeric GTPase. The stimulation of ERK by the 5-HT$_7$ receptor in hippocampal neurons possibly results in an effect that can be of importance for hippocampal function and mood regulation. Due to the availability of selective antagonists and knockout mice, recent studies suggest that the 5-HT$_7$ receptor is involved in thermoregulation, circadian rhythm, learning and memory, hippocampal signaling, sleep, and endocrine regulation. It is unclear how receptor blockade could lead to an antidepressant effect. However, the direct actions of antidepressants on the 5-HT$_7$ receptor and the reversal of sleep disturbances were observed in depressed patients after the receptor blockade, which leads to suggest that a 5-HT$_7$ receptor antagonist may be sufficient to treat depression.[3]

Up to date, there have not been many 5-HT$_7$ receptor antagonists developed.[4,5] DR-4004 and DR-4485 by Meiji Seika[6] and SB-269970 and SB-656104 by Glaxo-SmithKline[7] are being developed for the treatment of depression and sleep disorders (Fig. 1). Due to the phenolic hydroxyl group, SB-269970 is metabolized by Phase II metabolic mechanism and excreted from the body.[8] To overcome the defect, studies were carried out on replacing the phenolic moiety in SB-269970 with metabolically more stable bioisosteres, resulting in the introduction of SB-656104.[7b]

Keywords: Quinazolinone derivatives; Small molecule library; 5-HT$_7$ receptor; 5-HT$_7$ receptor antagonist.
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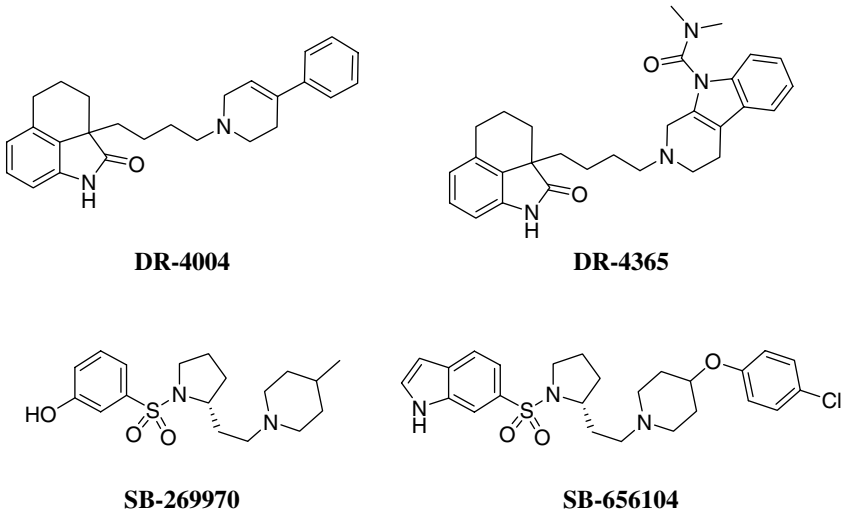


Figure 1. Selective 5-HT_7 receptor antagonists.

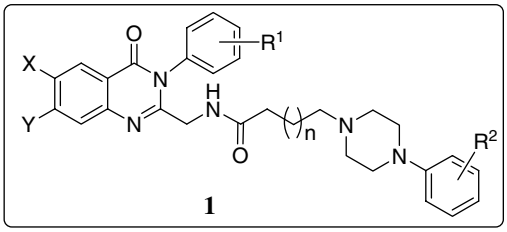
In order to find 5-HT_7 receptor antagonists, our in-house small molecule library, which consists of compounds with a quinazolinone or a sulfonamide as core structure, was assayed against the 5-HT_7 receptor. Among the compounds of the small molecule library, some quinazolinone derivatives were found to show binding affinities to the 5-HT_7 receptor. Based on the structure of the quinazolinone derivatives, a focused small molecule library **1** was designed (Fig. 2). In this paper, we describe the synthesis of the quinazolinone library **1** and the binding affinities to the 5-HT_7 receptor.

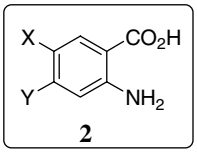
2. Results

2.1. Chemistry

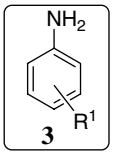
The quinazolinone library **1** is composed of three building blocks: anthranilic acids **2**, anilines **3**, and alkanoic acids **4** possessing various arylpiperazine moieties (Fig. 2). The anthranilic acids **2** contain anthranilic acid **2a**, 4-fluoroanthranilic acid **2b**, and 5-fluoroanthranilic acid **2c**. The anilines **3** have five different substituents as R^1 such as H, *p*-F, *o*-OMe, *m*-OMe, and *p*-OMe. The arylpiperazinylalkanoic acids **4** have 19 different substituents as R^2 which are shown in Figure 2. And the carboxylic acids **4** have different alkyl chains ($n = 0$ or 1) like arylpiperazinylpropanoic acids and arylpiperazinylbutanoic acids.

Anthranilic acids **2** and anilines **3** are commercially available, but no arylpiperazinylalkanoic acids **4** are available. Therefore, arylpiperazinylalkanoic acids **4** were obtained from the commercially available arylpiperazines **5** (Scheme 1). The arylpiperazines have substituents as R^2. The substituents are H, F, Cl, dichloro, trifluoromethyl, methyl, nitro, dimethyl, methoxy, ethoxy, and acetyl. Each arylpiperazine **5** was treated with

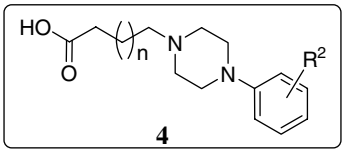




2a X = Y = H
2b X = H, Y = F
2c X = F, Y = H

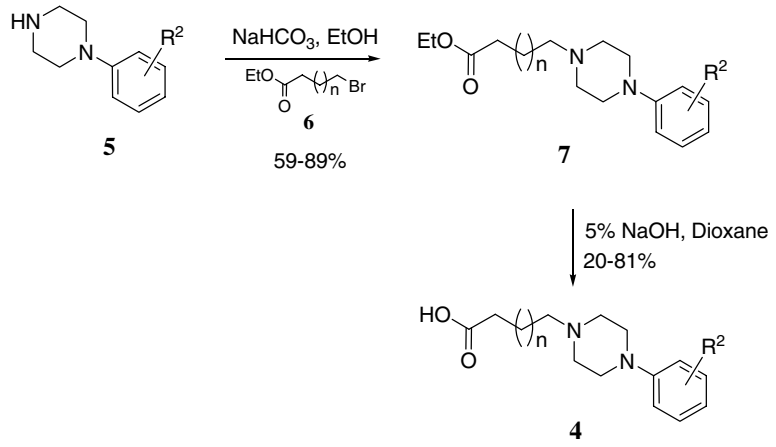


3a R^1 = H
3b R^1 = p-F
3c R^1 = o-OMe
3d R^1 = m-OMe
3e R^1 = p-OMe



4 n = 0 or n = 1
R^2 = H, o-F, p-F, o-Cl, p-Cl, 2,3-Cl_2, m-CF_3, p-Me, p-NO_2, 2,3-Me_2, 2,4-Me_2, 2,5-Me_2, 2,6-Me_2, 3,4-Me_2, o-OMe, m-OMe, p-OMe, o-OEt, p-Ac

Figure 2. Three building blocks **2**, **3**, **4** of the quinazolinone library **1**.



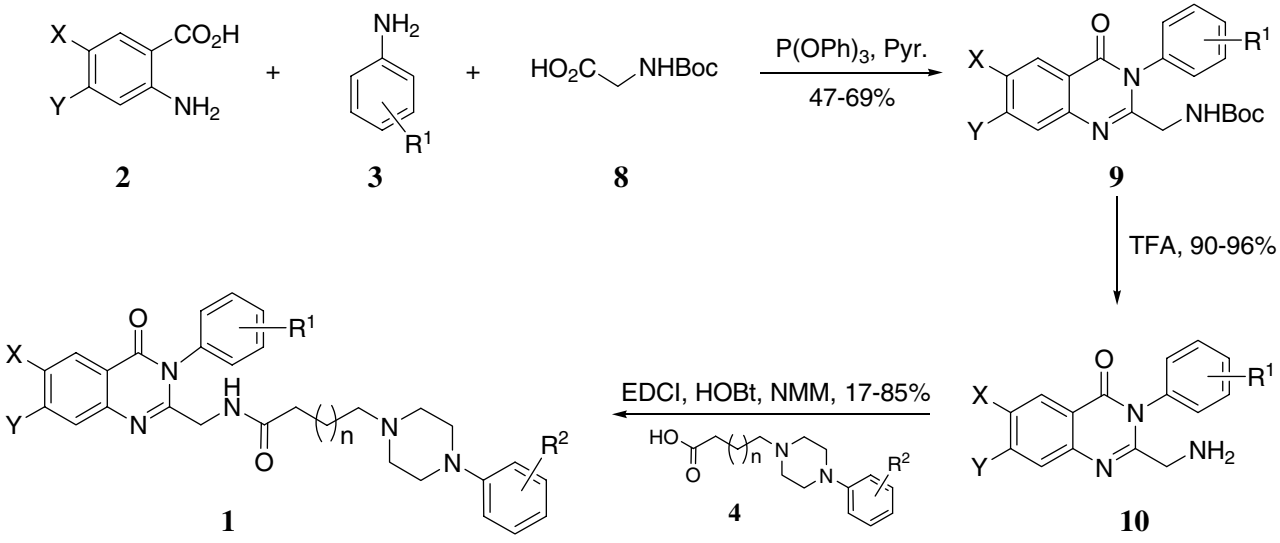
Scheme 1. Synthesis of the arylpiperazinylalkanoic acids **4**.

ethyl 3-bromopropanoate **6** (or ethyl 4-bromobutanoate) and $NaHCO_3$ in EtOH under reflux to give the corresponding compound **7** in 59–89% yield. Compounds **7** underwent hydrolysis under basic conditions to afford the arylpiperazinylalkanoic acids **4** in 20–81% yields.

The synthesis of the quinazolinone library **1** was started from the formation of quinazolinone moiety (Scheme 2). Quinazolinones **9** were synthesized by an efficient three-component, one-pot reaction from anthranilic acids **2**, anilines **3**, and *N*-Boc-glycine **8**.[9] Anthranilic acid **2** and *N*-Boc-glycine **8** in the presence of the coupling reagent ($P(OPh)_3$) generated the intermediate benzoxazinone derivative, followed by the addition of aniline **3** to give the corresponding quinazolinone **9** in 47–69% yields. The reaction is very sensitive to reaction temperature. If the reaction temperature was over 70 °C or below 60 °C, the yield was low. The optimum yield was obtained between 60 °C and 70 °C. Quinazolinones **9** were deprotected by treating with TFA at room temperature to give the primary amines **10** in 90–96% yields. Primary amines **5** underwent amide coupling with arylpiperazinylalkanoic acids **4** when treated with EDCI, HOBt, and *N*-methylmorpholine (NMM) in methylene chloride at room temperature to afford the corresponding compounds of the quinazolinone library **1** in 17–85% yields. Total 85 compounds were synthesized with three building blocks, each of which has different substituents. All the synthesized 85 compounds were assigned numbers as **1-01**–**1-85**, respectively, as shown in Tables 1–3.

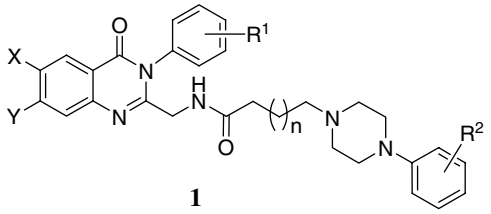
2.2. Binding assay to the 5-HT_7 receptor

All the synthesized compounds of quinazolinone library **1** were biologically evaluated to estimate binding affinities to the human recombinant 5-HT_7 receptor stably expressed by HEK293 cell line through [^{3}H]lysergic acid diethylamide (LSD) binding assay. Among all the biological data, the results of the quinazolinone library **1** where $n = 0$ are shown in Table 1. Compound **1-01** with a phenylpiperazine group shows binding affinity to the 5-HT_7 receptor with an IC_{50} value of 940 nM. Compounds **1-02** and **1-03** with *o*-Cl and *p*-Cl as R^2 show binding affinities with IC_{50} values of 510 nM and



Scheme 2. Synthesis of the quinazolinone library **1**.

Table 1. Binding affinities of the quinazolinone library **1** ($n = 0$) to the 5-HT_7 receptor



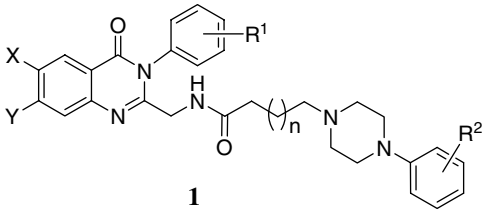
1

Compound	n	X	Y	R^1	R^2	IC_{50} (nM)
1-01	0	H	H	H	H	940
1-02	0	H	H	H	*o*-Cl	510
1-03	0	H	H	H	*p*-Cl	370
1-04	0	H	H	H	*p*-Me	730
1-05	0	H	H	H	2,3-Me_2	95
1-06	0	H	H	H	2,4-Me_2	640
1-07	0	H	H	H	3,4-Me_2	1100
1-08	0	H	H	H	*o*-OMe	80
1-09	0	H	H	H	*m*-OMe	1400
1-10	0	H	H	H	*p*-OMe	710
1-11	0	H	H	H	*o*-OEt	45
1-12	0	H	H	H	*p*-NO_2	>10,000
1-13	0	H	H	H	*p*-Ac	9000
1-14	0	H	H	*p*-F	H	680
1-15	0	H	H	*p*-F	2,4-Me_2	500
1-16	0	H	H	*p*-F	2,6-Me_2	500
1-17	0	H	H	*p*-F	*p*-OMe	2500
1-18	0	H	H	*p*-F	*o*-OEt	1300
1-19	0	H	H	*p*-F	*p*-NO_2	7300
Methiothepin						3.1

370 nM, respectively. Among compounds **1-04**–**1-07** with methyl or dimethyl groups as R^2, compound **1-05** shows very potent binding affinity with an IC_{50} value of 95 nM. Among compounds **1-08**, **1-09**, and **1-10** with *o*-, *m*-, and *p*-OMe as R^2, **1-08** shows very good binding affinity to the 5-HT_7 receptor with an IC_{50} value of 80 nM, while **1-09** shows only marginal binding affinity with an IC_{50} value of 1400 nM. Compound **1-11** with *o*-OEt as R^2 shows the best binding affinity to the 5-HT_7 receptor with an IC_{50} value of 45 nM. In the case of *p*-NO_2 and *p*-Ac as R^2, compounds **1-12** and **1-13** show no binding affinity. Compounds **1-14**–**1-19**, where *p*-fluoro substituent was added to the phenyl ring possessing an R^1 substituent, show moderate to marginal binding affinities with IC_{50} values between 500 nM and 7300 nM.

After obtaining the binding affinities of quinazolinone derivatives **1-01**–**1-19**, we synthesized compounds with a longer carbon chain such as $n = 1$ and compounds with fluorine substitution (X = F or Y = F) to determine how the carbon chain length and the electronegative fluorine affect the binding affinity to the 5-HT_7 receptor. Due to the high electronegativity and the small size, the fluorine substituent is widely used in medicinal chemistry.[10] Total 51 compounds from **1-20** to **1-70** were synthesized and biologically evaluated to obtain binding affinities to the 5-HT_7 receptor. The results are shown in Table 2. Compound **1-20** with no substituent in aromatic rings shows binding affinity with an IC_{50} value

Table 2. Binding affinities of the quinazolinone library **1** ($n = 1$, $R^1 = H$) to the 5-HT_7 receptor



1

Compound	n	X	Y	R^1	R^2	IC_{50} (nM)
1-20	1	H	H	H	H	650
1-21	1	H	H	H	*o*-F	400
1-22	1	H	H	H	*p*-F	2400
1-23	1	H	H	H	*o*-Cl	130
1-24	1	H	H	H	*m*-Cl	110
1-25	1	H	H	H	*p*-Cl	450
1-26	1	H	H	H	3,4-Cl_2	160
1-27	1	H	H	H	2,3-Me_2	460
1-28	1	H	H	H	2,4-Me_2	690
1-29	1	H	H	H	2,5-Me_2	420
1-30	1	H	H	H	3,4-Me_2	200
1-31	1	H	H	H	*o*-OMe	21
1-32	1	H	H	H	*m*-OMe	>10,000
1-33	1	H	H	H	*p*-OMe	2000
1-34	1	H	H	H	*o*-OEt	26
1-35	1	H	H	H	*m*-CF_3	350
1-36	1	H	H	H	*p*-Ac	>10,000
1-37	1	H	F	H	H	930
1-38	1	H	F	H	*o*-F	770
1-39	1	H	F	H	*p*-F	770
1-40	1	H	F	H	*o*-Cl	52
1-41	1	H	F	H	*m*-Cl	190
1-42	1	H	F	H	*p*-Cl	2200
1-43	1	H	F	H	3,4-Cl_2	450
1-44	1	H	F	H	2,3-Me_2	75
1-45	1	H	F	H	2,4-Me_2	940
1-46	1	H	F	H	2,5-Me_2	1500
1-47	1	H	F	H	3,4-Me_2	590
1-48	1	H	F	H	*o*-OMe	85
1-49	1	H	F	H	*m*-OMe	>10,000
1-50	1	H	F	H	*p*-OMe	1100
1-51	1	H	F	H	*o*-OEt	19
1-52	1	H	F	H	*m*-CF_3	92
1-53	1	H	F	H	*p*-Ac	680
1-54	1	F	H	H	H	470
1-55	1	F	H	H	*o*-F	1000
1-56	1	F	H	H	*p*-F	420
1-57	1	F	H	H	*o*-Cl	200
1-58	1	F	H	H	*m*-Cl	130
1-59	1	F	H	H	*p*-Cl	1200
1-60	1	F	H	H	3,4-Cl_2	270
1-61	1	F	H	H	2,3-Me_2	55
1-62	1	F	H	H	2,4-Me_2	620
1-63	1	F	H	H	2,5-Me_2	200
1-64	1	F	H	H	3,4-Me_2	260
1-65	1	F	H	H	*o*-OMe	120
1-66	1	F	H	H	*m*-OMe	920
1-67	1	F	H	H	*p*-OMe	350
1-68	1	F	H	H	*o*-OEt	12
1-69	1	F	H	H	*m*-CF_3	74
1-70	1	F	H	H	*p*-Ac	590
Methiothepin						3.1

Table 3. Binding affinities of the quinazolinone library **7** ($n = 1$, R^1 = OMe) to the 5-HT_7 receptor

Compound	*n*	X	Y	R^1	R^2	IC_{50} (nM)
1-71	1	H	H	*o*-OMe	*o*-OMe	210
1-72	1	H	H	*o*-OMe	*o*-OEt	29
1-73	1	H	F	*o*-OMe	*o*-OEt	110
1-74	1	F	H	*o*-OMe	*o*-OMe	79
1-75	1	F	H	*o*-OMe	*o*-OEt	48
1-76	1	H	H	*m*-OMe	*o*-OMe	98
1-77	1	H	H	*m*-OMe	*o*-OEt	55
1-78	1	H	F	*m*-OMe	*o*-OEt	91
1-79	1	F	H	*m*-OMe	*o*-OMe	230
1-80	1	F	H	*m*-OMe	*o*-OEt	16
1-81	1	H	H	*p*-OMe	*o*-OMe	97
1-82	1	H	H	*p*-OMe	*o*-OEt	29
1-83	1	H	F	*p*-OMe	*o*-OEt	49
1-84	1	F	H	*p*-OMe	*o*-OMe	190
1-85	1	F	H	*p*-OMe	*o*-OEt	16
Methiothepin						3.1

of 650 nM. In the case of halogen substituents such as compounds **1-21**–**1-26**, compounds **1-23**–**1-26** with cholorine substituents show better binding affinities to the 5-HT_7 receptor than compounds **1-21** and **1-22** with fluorine substituents. Compound **1-24** with *m*-Cl as R^2 shows good binding affinity with an IC_{50} value of 110 nM. Compounds **1-26**–**1-30** with dimethyl substituents show fairly good binding affinities with IC_{50} values between 200 nM and 690 nM. Among compounds **1-31**–**1-34** with alkoxy substituents, the binding affinities are diverse according to the position of substituents. Compound **1-32** with *m*-OMe as R^2 shows no significant binding affinity up to 10 μM, while compounds **1-31** and **1-34** with *o*-OMe and *o*-OEt show best binding affinities to the 5-HT_7 receptor with IC_{50} values of 21 nM and 26 nM, respectively. Compound **1-33** with *p*-OMe shows only marginal binding affinity. Compound **1-35** with *m*-CF_3 shows moderate binding affinity with an IC_{50} value of 350 nM and compound **1-36** with *p*-Ac shows no significant binding affinity up to 10 μM.

We have also synthesized compounds **1-37**–**1-53** with 7-fluorine substituents (Y = F) in the quinazolinone ring moiety and compounds **1-54**–**1-70** with 6-fluorine substituents (X = F). Compounds **1-37** and **1-54** with hydrogens as R^2 show moderate binding affinities with IC_{50} values of 930 nM and 470 nM, respectively. Among compounds **1-38**–**1-43** and **1-55**–**1-60** with halogen substituents, compound **1-40** with *o*-Cl and Y = F shows the best binding affinity to the 5-HT_7 receptor with an IC_{50} value of 52 nM. On the other hand, compounds **1-44**–**1-47** and **1-61**–**1-64** with dimethyl substituents show very good to moderate binding affinities with IC_{50} values between 55 nM and 1500 nM. The best compounds are **1-44** and **1-61** with 2,3-dimethyl substituents with IC_{50} values of 75 nM and 55 nM, respectively. In the case of the substituents such as alkoxy groups (compounds **1-48**–**1-51** and **1-65**–**1-68**), compounds **1-48**, **1-51**, **1-65**, and **1-68** with *o*-alkoxy as R^2 show very good binding affinities with IC_{50} values of 85 nM, 19 nM, 120 nM, and 12 nM, respectively. Compound **1-68** shows the best binding affinity to the 5-HT_7 receptor among all the synthesized compounds. Compounds **1-52** and **1-69** with *m*-CF_3 as R^2 show also very good binding affinities with IC_{50} values of 92 nM and 74 nM, respectively. The binding affinities of compounds **1-53** and **1-70** with *p*-Ac are much improved compared with compounds **1-13** and **1-36**.

According to the results of Tables 1 and 2, the compounds with *o*-OMe or *o*-OEt as R^2 show very good binding affinities to the 5-HT_7 receptor. We decided to synthesize compounds **1-71**–**1-85** where R^2 is fixed with *o*-OMe or *o*-OEt and R^1 is replaced with *o*-, *m*-, and *p*-OMe instead of hydrogen. The results are shown in Table 3. Among 15 compounds, 11 compounds show very good binding affinities with IC_{50} values below 100 nM. The other 4 compounds (**1-71**, **1-73**, **1-79**, and **1-84**) show also fairly good binding affinities to the 5-HT_7 receptor with IC_{50} values of 210 nM, 110 nM, 230 nM, and 190 nM, respectively. Among 15 compounds in Table 3, the best compounds are **1-80** and **1-85** with the same IC_{50} values of 16 nM.

2.3. Selectivities to other neurotransmitter receptors and antidepressant effects

Compounds with various binding affinities as well as different R^1 and R^2 groups, such as **1-68**, **1-85**, **1-83**, **1-61**, **1-44**, **1-52**, **1-65**, and **1-27** in order of decreasing binding affinities, were selected to test how selectively those synthesized compounds would bind to the 5-HT_7 receptor and whether they would have antidepressant effects in animal model, that is, the forced swimming test in mice.[11] The binding affinities of the selected 8 compounds were evaluated against the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and D_2 receptors which are also target receptors for antidepressant effects and/or antipsychotic effects.[12] The results are shown in Table 4. Most of the compounds bind more selectively to the 5-HT_7 receptor than other receptors tested such as 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and D_2 receptors.

The antidepressant effects of the selected 8 compounds were tested. The compounds were orally injected at a dose of 100 mg/kg to mice in the force swimming test and the total duration of immobility of the mice was monitored. After administration of compounds **1-68**, **1-85**, **1-83**, **1-52**, **1-65**, and **1-27**, the total duration of immobility was not reduced and was same as control. After administration of compounds **1-44** and **1-61**, the total duration of immobility was reduced to 75.9% and 94.4%, respectively (Fig. 3). In the case of Prozac® as positive control, the duration of immobility was reduced to 59.5%.

3. Discussion

The binding affinities of all the 85 synthesized compounds were obtained by radioligand [^{3}H]lysergic acid diethylamide (LSD) binding assay for the 5-HT_7 receptor. Among the 85 compounds, 24 compounds show very good binding affinities with IC_{50} values below 100 nM. Mainly the compounds with IC_{50} values below 100 nM have *o*-OMe or *o*-OEt as R^2 substituent. Except

Table 4. Selectivities to 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and D_2 receptors

Compound	5-HT_{1A}		5-HT_{2A}		5-HT_{2C}		D_2		5-HT_7
	IC_{50} (nM)	SI[a]	IC_{50} (nM)	SI[a]	IC_{50} (nM)	SI[a]	IC_{50} (nM)	SI[a]	IC_{50} (nM)
1-27	500	1.1	1100	2.4	460	1.0	6900	15	460
1-44	400	5.3	1700	23	3400	45	1500	20	75
1-52	140	1.5	620	6.7	1300	14	6600	72	92
1-61	220	4.0	420	7.6	80	1.5	4000	73	55
1-65	960	8.0	>10,000	>83	220	1.8	5300	44	120
1-68	500	42	>10,000	>830	1600	130	1000	83	12
1-83	370	7.6	2100	43	3800	78	1500	31	49
1-85	120	7.5	7900	490	>10,000	>630	140	8.8	16

[a] SI, selectivity index = the binding affinity to the target receptor (in IC_{50})/the binding affinity to the 5-HT_7 (in IC_{50}).

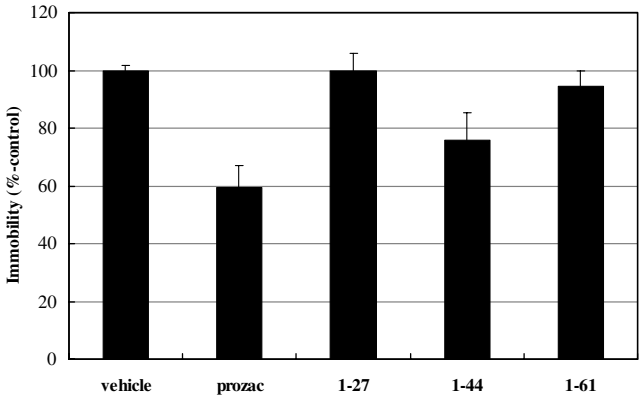


Figure 3. Antidepressant effects of Prozac® and synthesized compounds **1-27**, **1-44**, and **1-61** on immobility in forced swimming test in mice. Prozac® and the tested compounds (100 mg/kg) were injected orally (po) 60 min before the testing, and the total duration of immobility was recorded during the last 5 min of the 6-min testing period. Values are means ± SEM.

these substituents, 2,3-dimethyl, *o*-Cl, and *m*-CF_3 substituents have a favorable effect on the binding affinity to the 5-HT_7 receptor. Compounds **1-05**, **1-40**, **1-44**, **1-52**, **1-61**, and **1-69** with 2,3-dimethyl, *o*-Cl or *m*-CF_3 substituent as R^2 show binding affinities with IC_{50} values between 52 nM and 95 nM. Generally, substituents at the *para* position as R^2 have an unfavorable effect on the binding affinity. The compounds with chlorines at the *para* position show the least binding affinities among the compounds with chlorines at *ortho*, *meta*, and *para* positions. The compounds with other substituents such as *p*-NO_2 and *p*-Ac show almost no binding affinity. In the case of OMe substituents, the compounds with *meta* positions at the aromatic ring show no binding affinity. When the carbon length was changed from $n = 0$ to $n = 1$, the compounds with longer carbon chains show better binding affinities than those with shorter carbon chains. Compounds **1-08** and **1-11** with $n = 0$ show binding affinities with IC_{50} values of 80 nM and 45 nM, while compounds **1-31** and **1-34** with $n = 1$ with IC_{50} values of 21 nM and 26 nM. And other compounds with $n = 1$ also have better IC_{50} values. When fluorine substitution is added to the quinazolinone ring like X = F or Y = F, the overall binding affinities are equivalent or better than the binding affinities of the compounds without substitution in the quinazolinone ring (Table 2). There is apparent improvement in the binding affinities of the fluorinated compounds **1-44**, **1-52**, **1-61**, and **1-69** where R^2 is 2,3-dimethyl group and *m*-CF_3. Compounds **1-27** and **1-35** without fluorine substituent have IC_{50} values of 460 nM and 350 nM, and the fluorinated compounds **1-44**, **1-52**, **1-61**, and **1-69** have IC_{50} values of 75 nM, 92 nM, 55 nM, and 74 nM. And also, we have tried to figure out an effect of substitution in the aromatic ring containing R^1 group. We fixed R^2 as *o*-OMe or *o*-OEt and gave substitution of methoxy groups as R^1 at *ortho*, *meta*, and *para* positions. From the results of the binding assay, there is no effect of the aromatic position on the binding affinity to the 5-HT_7 receptor. All the compounds with methoxy groups as R^1 show good binding affinities to the 5-HT_7 receptor (Table 3).

Compounds **1-27**, **1-44**, **1-52**, **1-61**, **1-65**, **1-68**, **1-83**, and **1-85**, which show good binding affinities, were selected to test selectivity to other neurotransmitter receptors and the in vivo efficacy in the animal model. Most of the compounds bind more selectively to the 5-HT_7 receptor than other receptors tested such as 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and D_2 receptors. Compound **1-68** with the best binding affinity to the 5-HT_7 receptor binds 42 to >830 times more selectively to the 5-HT_7 receptor than other receptors such as 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and D_2 receptors (Table 4). The other compounds also bind quite selectively to the 5-HT_7 receptors. The eight selected compounds were tested to detect antidepressant effects in the forced swimming test in mice. The forced swimming test is one of the most widely used preclinical tests for detecting antidepressant activity. It has been well established that the reduction of immobility time in the forced swimming test depends mainly on the enhancement of serotonin and catecholamine.[13] Among the 8 compounds, two compounds **1-44** and **1-61** show the antidepressant effects. These two compounds will be further evaluated for the pharmacokinetic profile and metabolic stability.

4. Conclusion

We have described the synthesis of the quinazolinone library **1** and their binding affinities to the 5-HT_7 receptor. Total 85 compounds were synthesized and among those, 24 compounds show very good binding affinities with IC_{50} values below 100 nM. The structure–activity relationship study on the quinazolinone library **1** indi-

cated that among the various substituents as R^2, the compounds with *ortho* methoxy and the *ortho* ethoxy groups show the best binding affinities to the 5-HT_7 receptor. We found that the quinazolinone derivatives **1-44** and **1-61** show antidepressant effects in the forced swimming test as well as selective binding affinities to the 5-HT_7 receptor. The two compounds will be further evaluated for the pharmacokinetic profile and metabolic stability. In addition, a small molecule library with more various substituents will be designed, synthesized, and biologically evaluated against the 5-HT_7 receptor.

5. Experimental

All the commercially available reagents were obtained from Aldrich and Fluka, and generally used without further purification. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker Advance 400 (or 300) spectrometer. Nuclear magnetic resonance spectra were acquired at 400 (or 300) MHz for ^{1}H, and 100 MHz for ^{13}C NMR. HR-MS spectra were obtained on a JMS-700 mass spectrometer (Jeol). Analytical thin layer chromatographies (TLC) were carried out on precoated silica gel plates (Merck Kieselgel 60F254, layer thickness 0.25 mm). Flash column chromatographies were conducted with silica gel grade 230–400 mesh (Merck Kieselgel 60 Art 9385).

5.1. Ethyl 4-(4-(2-methoxyphenyl)piperazin-1-yl) butanoate (7)

Ethyl 4-bromobutanoate (1.01 g, 5.20 mmol) was added to the solution of 1-(2-methoxyphenyl)piperazine (**5**) (1.0 g, 5.20 mmol) in ethanol (25 mL) at room temperature and the resulting solution was refluxed under nitrogen atmosphere for 6 h. After the completion of reaction, the mixture was cooled to room temperature. Sat. $NaHCO_3$ (50 mL) was added to the reaction mixture and the resulting solution was extracted with CH_2Cl_2 (3× 50 mL). The combined organic solution was dried over $MgSO_4$ and concentrated. The residue was purified with 5% MeOH in CH_2Cl_2 by silica gel column chromatography to give the product (1.40 g) in 88% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.01–6.84 (m, 4H), 4.15 (q, J = 7.2 Hz, 2H), 3.87 (s, 3H), 3.10 (br s, 4H), 2.66 (br s, 4H), 2.50–2.35 (m, 4H), 1.87 (quintet, J = 7.3 Hz, 2H), 1.78 (t, J = 7.1 Hz, 3H).

5.2. 4-(4-(2-Methoxyphenyl)piperazin-1-yl)butanoic acid (4)

5% NaOH (8.5 mL) was added to the solution of ethyl 4-(4-(2-methoxyphenyl)piperazin-1-yl)butanoate (**7**) (1.35 g, 4.41 mmol) in dioxane (3 mL) at room temperature. The resulting solution was stirred at room temperature for 2 h. After removing solvent under reduced pressure, the residue was dissolved in minimum amount of water and acidified with 6 N HCl solution until pH 6 or 5. The precipitate was filtered and dried to give the product (241 mg) in 20% yield: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.04–6.90 (m, 4H), 3.79 (s, 3H), 3.63–3.40 (m, 4H), 3.14–3.09 (m, 6H), 2.35 (t, J = 7.3 Hz, 2H), 1.99–1.92 (m, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 177.49, 155.94, 141.90, 128.70, 124.98, 122.97, 116.39, 59.61, 54.69, 51.05, 34.90, 22.77.

5.3. *tert*-Butyl (4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl) methylcarbamate (9)

Triphenyl phosphite (4.8 mL, 18.2 mmol) was added dropwise to the solution of anthranilic acid **2** (1.00 g, 7.29 mmol) and *N*-Boc-glycine **8** (1.28 g, 7.29 mmol) in anhydrous pyridine (4 mL) under nitrogen atmosphere. The resulting mixture was stirred at 70 °C under nitrogen atmosphere. After 2 h, aniline **3** (798 mL, 8.95 mmol) was added to the reaction mixture. The resulting mixture was stirred at 70 °C under nitrogen atmosphere for 4 h. After the reaction completed, the reaction mixture was concentrated and diluted with EtOAc (100 mL). The solution was washed with satd $NaHCO_3$ (2× 50 mL) and dried over $MgSO_4$. After concentration, the residue was purified with a mixture of EtOAc, ether, and hexane (1:1:3) by silica gel column chromatography to give the product (1.19 g) in 47% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.30 (d, J = 7.9 Hz, 1H), 7.81 (m, 2H), 7.52 (m, 4H), 7.29 (m, 2H), 6.05 (br s, 1H), 3.98 (d, J = 3.9 Hz, 2H), 1.47 (s, 9H).

5.4. 2-(Aminomethyl)-3-phenylquinazolin-4(3*H*)-one (10)

Trifluoroacetic acid (2.5 mL) was added dropwise to a solution of *tert*-butyl (4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl) methylcarbamate **9** (550 mg, 1.56 mmol) in CH_2Cl_2 (6 mL) at 0 °C and the resulting mixture was stirred at room temperature for 1 h. After completion of reaction, the reaction mixture was diluted with EtOAc (50 mL) and the solution was washed with sat $NaHCO_3$ (3× 25 mL) and dried over $MgSO_4$. After concentration, the product (377 mg) was obtained in 96% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.31 (d, 7.6 Hz, 1H), 7.80–7.75 (m, 2H), 7.57–7.49 (m, 4H), 7.26–7.24 (m, 2H), 3.51 (s, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 162.37, 156.59, 147.40, 136.19, 134.86, 130.31, 129.81, 128.43, 127.40, 127.31, 127.16, 121.18, 45.17.

5.5. 4-(4-(2,3-Dimethylphenyl)piperazin-1-yl)-*N*-((4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)methyl)butanamide (1-27)

EDCI (106.8 mg, 0.557 mmol) and HOBT (64.0 mg, 0.474 mmol) were added to a solution of 4-(4-(2,3-dimethylphenyl)piperazin-1-yl) butanoic acid **4** (124 mg, 0.446 mmol) in anhydrous CH_2Cl_2 (3 mL) at room temperature. The resulting mixture was stirred at room temperature for 2 h. A solution of 2-(aminomethyl)-3-phenylquinazolin-4(3*H*)-one **10** (70 mg, 0.28 mmol) in CH_2Cl_2 (1 mL) and NMM (52 mL, 0.47 mmol) were added successively to the reaction mixture at room temperature. The resulting mixture was stirred for additional 2 h. After reaction was completed, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with aqueous $NaHCO_3$, water, and brine, successively. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified with 10%

methanol in EtOAc (or 7% methanol in CH_2Cl_2) by silica gel column chromatography to give the product (107 mg) in 75% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.31 (d, J = 6.6 Hz, 1H), 7.80–7.75 (m, 2H), 7.58–7.52 (m, 4H), 7.33 (br s, 1H), 7.28–7.25 (m, 2H), 7.07 (t, J = 7.1 Hz, 1H), 6.91–6.80 (m, 2H), 4.07 (d, J = 4.3 Hz, 1H), 2.91 (t, J = 4.7 Hz, 4H), 2.64 (br s, 4H), 2.52 (t, J = 7.0 Hz, 2H), 2.41 (t, J = 7.3 Hz, 2H), 2.27 (s, 3H), 2.21 (s, 3H), 1.95–1.90 (m, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 173.06, 162.17, 152.30, 151.76, 146.87, 138.26, 135.57, 135.05, 131.52, 130.64, 130.27, 128.34, 127.58, 127.25, 126.08, 125.24, 121.38, 116.81, 57.84, 53.88, 52.37, 42.44, 34.57, 22.76, 20.90, 14.22; IR (KBr) 3282, 3062, 2939, 2814, 1681, 1638, 1609, 1590, 1473, 1261, 1012, 774, 695 cm^{-1}; HR-MS (TOF, ES+, M+H) Calcd for $C_{31}H_{36}N_5O_2$: 510.2869. Found: 510.2867.

5.6. 4-(4-(2,3-Dimethylphenyl)piperazin-1-yl)-*N*-((7-fluoro-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)methyl)butanamide (1-44)

The procedure was same as that of synthesis of the compound **1-27**. 4-(4-(2,3-Dimethylphenyl)piperazin-1-yl)butanoic acid **4** (115 mg, 0.416 mmol) and 2-(aminomethyl)-7-fluoro-3-phenylquinazolin-4(3*H*)-one (70 mg, 0.26 mmol) were used and the product was obtained in 69% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.33 (dd, J = 8.7, 6.0 Hz, 1H), 7.63–7.57 (m, 3H), 7.45–7.38 (m, 2H), 7.32–7.22 (m, 3H), 7.11 (t, J = 7.8 Hz, 1H), 6.96–6.92 (m, 2H), 4.10 (d, J = 4.5 Hz, 2H), 2.96 (t, J = 4.5 Hz, 4H), 2.70 (br s, 4H), 2.58 (t, J = 6.9 Hz, 2H),2.45 (t, J = 7.5 Hz, 2H), 2.31 (s, 3H), 2.25 (s, 3H), 2.01–1.94 (m, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 173.07, 166.98 (d, J = 255 Hz), 161.41, 153.92, 151.68 (d, J = 12.9 Hz), 138.27, 135.38, 131.50, 130.68, 130.43, 130.37, 130.29, 128.29, 126.13, 125.32, 118.11, 116.81, 116.25 (d, J = 23.2 Hz), 112.77 (d, J = 22.1 Hz), 57.85, 53.88, 52.33, 42.52, 34.55, 22.69, 20.93, 14.23; IR (KBr) 3282, 3062, 2942, 2812, 1683, 1642, 1607, 1590, 1576, 1546, 1485, 1279, 781, 696 cm^{-1}; HR-MS (TOF, ES+, M+H) Calcd for $C_{31}H_{35}FN_5O_2$: 528.2775. Found: 528.2776.

5.7. 4-(4-(2,3-Dimethylphenyl)piperazin-1-yl)-*N*-((6-fluoro-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)methyl)butanamide (1-61)

The procedure was same as that of synthesis of the compound **1-27**. 4-(4-(2,3-Dimethylphenyl)piperazin-1-yl)butanoic acid **4** (115 mg, 0.416 mmol) and 2-(aminomethyl)-6-fluoro-3-phenylquinazolin-4 (3*H*)-one (70 mg, 0.26 mmol) were used and the product was obtained in 71% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.95 (dd, J = 8.4, 2.7 Hz, 1H), 7.78 (dd, J = 9.0, 4.8 Hz, 1H), 7.63–7.51 (m, 4H), 7.38 (br s, 1H), 7.33–7.29 (m, 2H), 7.11 (t, J = 7.5 Hz, 1H), 6.96–6.91 (m, 2H), 4.10 (d, J = 3.9 Hz, 2H), 2.96 (t, J = 4.5 Hz, 1H), 2.70 (br s, 4H), 2.76 (t, J = 6.9 Hz, 2H), 2.45 (t, J = 6.9 Hz, 2H), 2.31 (s, 3H), 2.25 (s, 3H), 2.01–1.94 (m, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 173.05, 161.50, 161.46, 161.33 (d, J = 249 Hz), 151.82, 151.70, 143.63, 138.29, 135.43, 131.50, 130.69, 130.37, 129.73 (d, J = 8.0 Hz), 128.28, 126.10, 125.31, 123.52 (d, J = 24.2 Hz), 122.69 (d, J = 8.5 Hz), 116.79, 112.47 (d, J = 23.6 Hz), 57.83, 53.86, 52.32, 42.40, 34.54, 22.69, 34.54, 22.69, 20.93, 14.25; IR (KBr) 3312, 3064, 2939, 2813, 1681, 1649, 1611, 1591, 1545, 1485, 1237, 1013, 754, 696 cm^{-1}; HR-MS (TOF, ES+, M+H) Calcd for $C_{31}H_{35}FN_5O_2$: 528.2775. Found: 528.2778.

5.8. [^{3}H]LSD binding assay to serotonin 5-HT$_7$ receptor

Membranes from stable CHO cell line expressing the human recombinant 5-HT$_7$ serotonin receptor (Perkin-Elmer Life and Analytical Sciences, Boston, USA) were used. For 5-HT$_7$ receptor binding assay, cell membrane, 3 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. The mixture was incubated for 90 min at 27 °C, and the reaction was terminated by rapid filtration through Whatman GF/C glass fiber filter presoaked in 0.3% polyethyleneimine. 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 values (IC_{50}).

5.9. Forced swimming test in mice

The forced swimming test was performed according to the methods described by Porsolt et al.[14] Each mouse was placed in a 25-cm glass cylinder (10 cm diameter) containing 15 cm of water maintained at 23 ± 1 °C, and was forced to swim for 10 min. Twenty-four hours later, the mouse was replaced into the cylinder and the total duration of immobility was recorded during the last 5 min of the 6-min testing period. Mice are judged immobile when they float in an upright position and make only small movements to keep their head above water. Drugs (50 or 100 mg/kg) were suspended in 3% Tween 80 solution and administered 30 min (ip) or 60 min (po) before the testing.

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