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Discovery of a Novel 5-HT_3 Antagonist/5-HT_{1A} Agonist 3-Amino-5,6,7,8-tetrahydro-2-{4-[4-(quinolin-2-yl)piperazin-1-yl]butyl}quinazolin-4(3*H*)-one (TZB-30878) as an Orally Bioavailable Agent for Irritable Bowel Syndrome

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We have prepared a series of quinazolinone derivatives linked with piperazinylquinoline for the treatment of irritable bowel syndrome (IBS). Using pharmacophore analysis, we designed and synthesized compounds which bind to both serotonin receptor subtype 1A (5-HT_{1A}) and subtype 3 (5-HT_3). Quinazolinone derivatives with a sulfur atom in the linker showed high affinity in in vitro assays, but low in vivo activity. Focusing on the linker to improve the pharmacokinetic profile, the sulfur atom in the linker was replaced with a methylene group. Further optimization led to the discovery of compound **17m** (TZB-30878) (*J. Pharmacol. Exp. Ther.* **2007**, *322*, 1315−1323, Patent WO2005082887 (A1), 2005), a novel 5-HT_{1A} agonist/5-HT_3 antagonist in the 3-aminoquinazolinone series. In in vivo functional assays, **17m** dose dependently inhibited the Bezold−Jarisch reflex and induced 5-HT_{1A}-mediated behaviors, and in an IBS animal model, **17m** significantly inhibited stress-induced defecation. Pretreatment by WAY-100635 (5-HT_{1A} antagonist) significantly attenuated but did not abolish the inhibitory effects of **17m**. These results suggested that **17m** exerted inhibitory effects via both 5-HT_{1A} agonistic and 5-HT_3 antagonistic activities and that **17m** would be useful as a therapeutic agent for IBS.

Introduction

Irritable bowel syndrome (IBS[a]) is a disease of which the main symptoms are evacuation abnormalities including diarrhea, constipation, or bellyache, and IBS is not caused by an intestinal organic lesion.[1,2] This disease develops as a result of a mutual association of intestinal motion disorder, viscerosensory anaphylaxis, and psychological and social factors.[3,4] Indeed, antimotility and anxiolytic agents have been used for the treatment of this disorder.

Serotonin receptor subtype 3 (5-HT_3) in intestinal tissue plays a role in intestinal contraction, secretion of intestinal juice, peristalsis, and content transport; thus, diarrheal symptoms can be improved by administration of 5-HT_3 antagonists. Alosetron (**3**) and Ondansetron (**4**) are selective 5-HT_3 antagonists[5] useful for diarrhea-predominant IBS patients[6] (Figure 1).

Because psychological and social factors are recognized as one of the causes of IBS, the administration of benzodiazepine antianxiety agents has been investigated for IBS therapy. 8-OH-DPAT (**1**) and Buspirone (**2**) are well-known serotonin receptor subtype 1A (5-HT_{1A}) agonists[7] (Figure 1). **2** is used for the treatment of stress-induced dyspeptic ulcers, and its mechanism of action has been attributed to an antianxiety activity through 5-HT_{1A} agonism.

In a previous paper, we reported the synthesis of a compound which acts as both a 5-HT_{1A} agonist and 5-HT_3 antagonist, with the goal being to find a single compound that acts on both receptors as a treatment for IBS.[8] Compounds in this series showed both 5-HT_{1A} agonist and 5-HT_3 antagonist activity both in vitro and in vivo. From this lead, further structure−activity relationship analysis as well as the design and synthesis of compounds based on our pharmacophore analysis led to the identification of a dual action compound suitable for IBS therapy. In this paper, the design and synthesis of such compounds acting on both 5-HT_{1A} and 5-HT_3 receptors and their in vitro and in vivo activities are presented.

Superposition of Pharmacophores

To design compounds with dual receptor affinity, we analyzed many structures of 5-HT_{1A} agonists and 5-HT_3 antagonists and extracted pharmacophoric elements (Figure 2). For example, from 5-HT_{1A} agonist **2**, we extracted a pharmacophore that includes an aromatic ring as the basic template, a hydrogen bond acceptor, a basic nitrogen a certain constant distance from the aromatic ring or a hydrogen-bond acceptor, and a bulky hydrophobic group linked by a spacer from the basic nitrogen. Similarly, from the 5-HT_3 antagonist Quipazine (**5**),[9,10] we extracted a pharmacophore that includes an aromatic ring as the basic template similar to the 5-HT_{1A}

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[a] Abbreviations: IBS, irritable bowel syndrome; 5-HT_{1A}, serotonin receptor subtype 1A; 5-HT_3, serotonin receptor subtype 3; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; LLR, lower lip retraction; FBP, flat body posture; ΔT, change in rectal temperature; BJ reflex, Bezold−Jarisch reflex.

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agonist, a hydrogen bond acceptor (nitrogen) in the aromatic ring, and a basic nitrogen a certain constant distance from the aromatic ring or a hydrogen-bond acceptor.

Because some of these compounds have a common element, an aryl piperazine, in their structure, we superimposed these pharmacophores at the aryl piperazine. We modeled a pharmacophore which could generate compounds with both 5-HT_{1A} and 5-HT_3 receptor binding as follows: (1) an aromatic ring as the basic template, (2) a hydrogen bond acceptor (nitrogen) in the aromatic ring, (3) a basic nitrogen that exists a certain constant distance from the aromatic ring, and (4) a bulky hydrophobic group linked by a spacer from the basic nitrogen. On the basis of this analysis, we designed and synthesized compounds having the features described above which would bind to both 5-HT receptor subtypes.

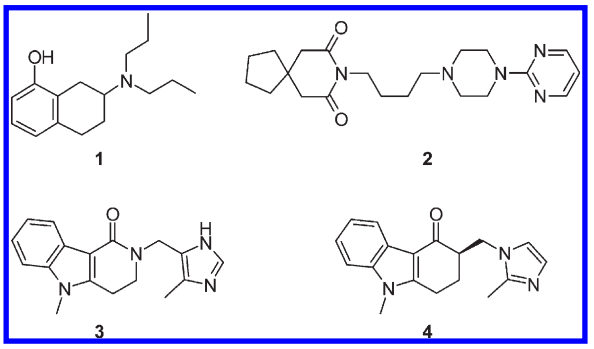


Figure 1. Structure of 5-HT_{1A} agonists and 5-HT_3 antagonists.

Synthesis of Pyrimidinone Derivatives Linked with Aryl Piperazine

Initially, we synthesized pyrimidinone derivatives linked with an aryl piperazine by a thioether (Scheme 1).[11] Starting from the anthranilate analogue (**6**), reaction with thiophosgene gave the isothiocyanate (**7**). Treatment of **7** with hydrazine followed by refluxing in KOH/EtOH provided the potassium salt of the pyrimidinone with an amino group at the 3-position (**9**). To obtain derivatives without an amino group at the 3-position (**11**), the anthranilate analogue was treated with ammonium thiocyanate and benzoyl chloride, followed by heating in KOH/EtOH. Aryl piperazines were synthesized from aryl chloride and piperazine by heating at 140 °C in ethylene glycol.[8] These aryl piperazines were treated with 1-bromo-3-chloropropane to give alkyl chloride derivatives (**13**). The potassium salts (**9** and **11**) were coupled with **13**

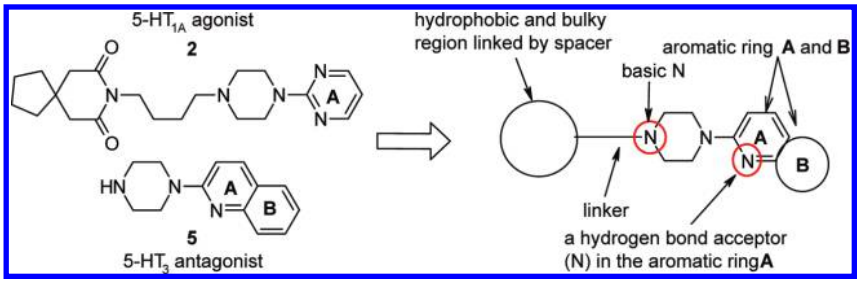
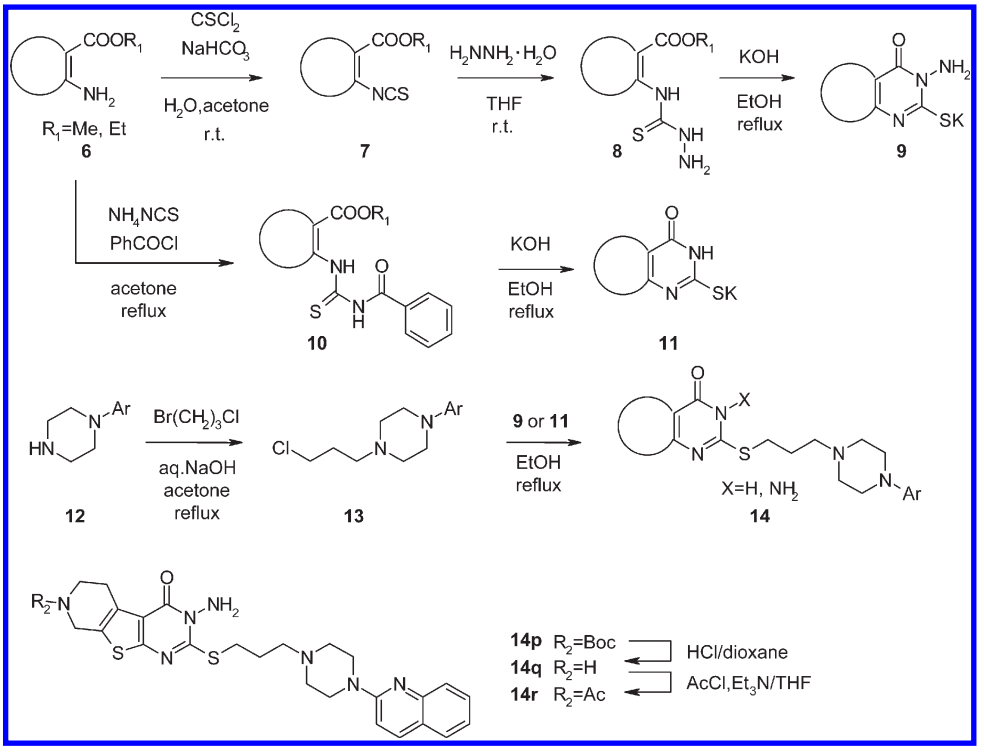
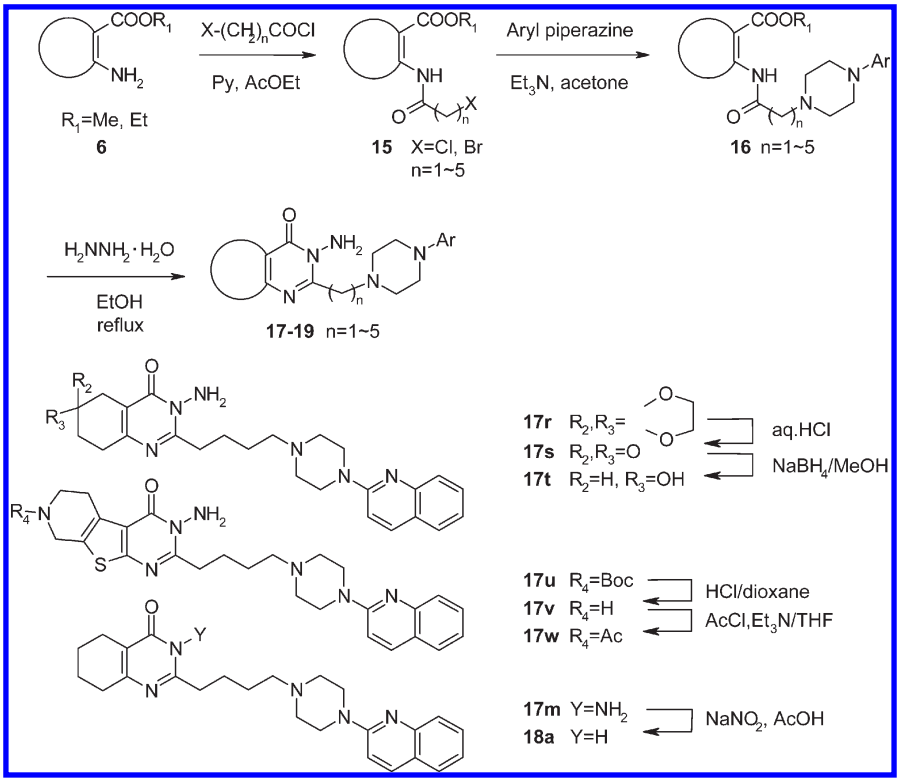


Figure 2. Pharmacophore analyses of compounds that bind to 5-HT_{1A} or 5-HT_3 and development of a hybrid pharmacophore model for compounds that bind to both 5-HT_{1A} and 5-HT_3 receptors.

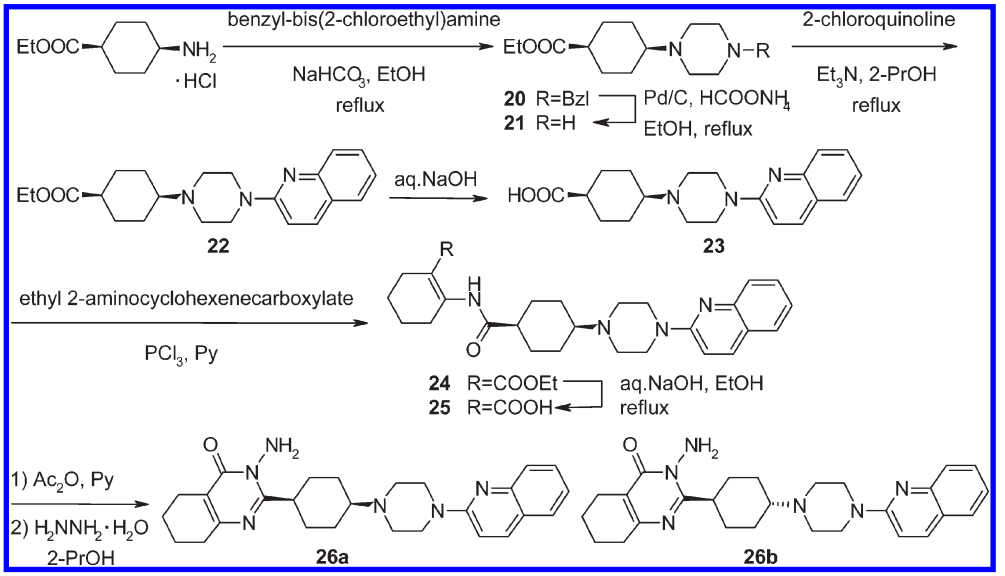
Scheme 1. Synthesis of Pyrimidinone Derivatives Linked with Aryl Piperazine by a Thioether



Scheme 2. Synthesis of Pyrimidinone Derivatives Linked with Aryl Piperazine by Methylene Groups



Scheme 3. Synthesis of Pyrimidinone Derivatives Linked with Aryl Piperazine by a Rigid Linker

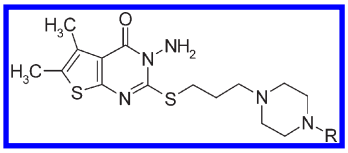


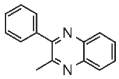
by heating to give the target compounds (**14**). Tetrahydropyridine derivative **14r** was synthesized from **14p** by deprotection of the Boc group and acetylation.

To improve in vivo activities, we synthesized the pyrimidinone derivatives linked with an aryl piperazine by a methylene spacer (Scheme 2). Treatment of the anthranilate analogue (**6**) with a haloalkyl acid chloride gave the alkyl halide derivative (**15**). The alkyl halide intermediates were reacted with aryl piperazine followed by heating with hydrazine to give the 3-aminopyrimidinone derivatives (**17**−**19**), which had a methylene spacer. Protected ketal derivative (**17r**) was deprotected and reduced to give the 6-hydroxytetrahydroquinazolinone (**17t**). The *N*-acetylated compound (**17w**) was synthesized from the *N*-Boc derivative analogous to the synthesis of **14r**. Deamination of **17m** was conducted with $NaNO_2$ in acetic acid to give **18a**.

Synthesis of compounds with the conformationally restricted linker was as follows (Scheme 3). Ethyl *cis*-4-aminocyclohexanecarboxylate, which was prepared from *cis*-4-aminocyclohexanecarboxylic acid, was treated with *N*-benzyl-bis(2-chloroethyl)amine to prepare the piperazine derivative (**20**). After the deprotection, the piperazine derivative (**21**) was treated with 2-chloroquinoline to give the *N*-substituted **5** derivative (**22**). Hydrolyzing the ester to the carboxylic acid (**23**) and coupling with a anthranilate analogue by PCl_3 in pyridine gave compound **24**. After the hydrolysis of **24**, heterocyclization of **25** with hydrazine gave the rigid *cis*-form linker compound (**26a**)

Table 1. Preliminary Analysis of Binding Inhibition Activity for Lead Compound Aryl Piperazine Substitution



compd no.	R	5-HT$_{1A}$ IC$_{50}$ (nM)	5-HT$_3$ IC$_{50}$ (nM)
14a		4.2	> 100
14b		36.4	48.4
14c		4.3	50.5
14d		6.1	> 100
14e		66.6	87.6
14f		> 100	> 100
14g		55.4	> 100

and a small amount of the rigid *trans*-form linker compound **26b** to which **26a** was isomerized.

Preliminary Studies to Find a Lead Compound

Compound affinity for the 5-HT$_{1A}$ receptor was calculated as percent inhibition of [^{3}H]-**1** binding to the 5-HT$_{1A}$ receptor in a CHO cell membrane sample expressing human 5-HT$_{1A}$ receptors.[8,12,13] Compound affinity for the 5-HT$_3$ receptor was calculated as percent inhibition of [^{3}H]BRL-43694 binding in HEK-293 cell membrane sample expressing human 5-HT$_3$ receptors. Initially, compounds were designed using a pharmacophore model, and after synthesis, compound screening and preliminary structure−activity relationship analysis were used to verify the pharmacophore model.

M. Modica et al. reported some compounds which bind to the 5-HT$_{1A}$ receptor,[11] and a subset of these compounds demonstrated an additional weak binding to the 5-HT$_3$ receptor. On the basis of this data, we synthesized a series of compounds which incorporated a thienopyrimidinone unit.

Compound **14a**, with a pyridine substituted piperazine unit, showed high affinity exclusively at the 5-HT$_{1A}$ receptor and not the 5-HT$_3$ receptor (Table 1). We next examined quinoline or phenanthridine as the aryl piperazine substitution because the parent moieties' affinity for the 5-HT$_3$ receptor could be expected to carry over into the more complex derivatives. Indeed, **5** and phenanthridinylpiperazine showed high affinity for the 5-HT$_3$ receptor (data not shown), and introduction of a bulky hydrophobic group and linker (which were necessary for 5-HT$_{1A}$ affinity) resulted in high affinity for the 5-HT$_{1A}$ receptor (**14b** and **14g**). Interestingly, compared to compound **14b** with a quinoline, the phenanthridine moiety in compound **14g** greatly decreased the affinity to 5-HT$_3$ and to a lesser degree to 5-HT$_{1A}$. From these data, we modified the pharmacophore model such that neither the substituents nor the fused aryl ring were too large. Because compound **14b**, with a quinoline moiety, had affinity to both receptors, it became our new lead compound.

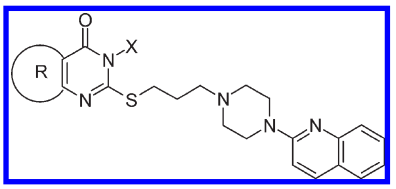
Lead Optimization

For optimization studies, the structure of the lead compound, **14b**, could be divided into three parts: aryl piperazine, linker, and bulky hydrophobic region. Since it was reported in several papers that a length of four atoms is suitable for the linker,[14,15] the linker was initially fixed to this length and compound modification focused on the other two parts of the lead structure. For the aryl piperazine, because this part must carry the 5-HT$_3$ receptor affinity, we first checked the affinity of the aryl piperazine units to the 5-HT$_3$ receptor (data not shown), and those aryl piperazines which had a high affinity were then linked with a bulky hydrophobic unit (Table 1). Compound **14c**, with a 4-methylquinolylpiperazine unit having a high affinity for the 5-HT$_3$ receptor, had high affinities for both receptors, the same as compound **14b**. On the other hand, compounds with a heterocyclic ring other than quinoline, e.g. benzothiazole (**14e**) or 3-phenylquinoxaline (**14f**), resulted in low affinities to both receptors even though the parent aryl piperazine moieties had high affinity to 5-HT$_3$. Interestingly, the compound with tetrahydroquinoline (**14d**) showed high affinity for 5-HT$_{1A}$ but greatly decreased the affinity to 5-HT$_3$. From these results, we decided to use unsubstituted or 4-methyl substituted quinoline as the optimized aryl piperazine portion of the structure.

Next, we studied the bulky hydrophobic region and the amino group at the pyrimidinone 3-position (Table 2). The amino group was found to have an important role in receptor binding as compounds without it had low affinities to 5-HT$_{1A}$ (**14i** and **14j**). Introduction of a polar group into the bulky hydrophobic region greatly improved affinity to 5-HT$_{1A}$ (**14q**); however, this strategy did not result in high affinity to the 5-HT$_3$ receptor. Thus, introduction of a polar group into this part of the structure was not suitable for our purpose. The compound **14r**, the acetylated secondary amine derivative of **14q**, had a high affinity to 5-HT$_{1A}$ receptor while somewhat maintaining affinity to the 5-HT$_3$ receptor. Fused pyridines in the structure (**14n** and **14o**) had high 5-HT$_{1A}$ affinity and moderate affinity to the 5-HT$_3$ receptor.

For secondary screening, two in vitro functional tests, a [^{35}S]GTPγS binding assay for 5-HT$_{1A}$ agonistic activity, and a guinea pig ileum contraction inhibition assay for 5-HT$_3$ antagonistic activity were carried out for those compounds with high affinities to both receptors (Table 6 and 7).[8,12,13] In the [^{35}S]GTPγS binding assay, E_{max} of compounds **14l** and **14r** in this series had about 90% and were confirmed to be full 5-HT$_{1A}$ agonists. In the contraction inhibition assay, these compound showed 90−100% inhibition at 1 μmol/L, confirming that they were 5-HT$_3$ antagonists. In particular, **14r** showed excellent results in these in vitro functional assays. In a tertiary screening, compound **14r** was studied in two other functional examinations in rats: measurement of 5-HT$_{1A}$ agonistic activity (lower lip retraction, LLR; flat body posture, FBP; change in rectal temperature, ΔT) and 5-HT$_3$ antagonistic activity (inhibition of the Bezold−Jarisch (B-J) reflex caused by 5-HT) (Table 9 and 10).[8,12,13] Compound **14r** showed disappointing results with respect to 5-HT$_{1A}$ agonist activity; therefore, we measured the compound total concentrations in the blood and brain (Table 8).[16] The compound total concentration in the brain was found to be too low to have an effect. Thus, to

Table 2. Optimization of the Lead Compound Bulky Hydrophobic and Amino Groups



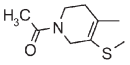
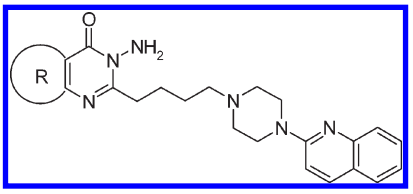
compd no.	X	R	5-HT$_{1A}$ IC$_{50}$ (nM)	5-HT$_3$ IC$_{50}$ (nM)	compd no.	X	R	5-HT$_{1A}$ IC$_{50}$ (nM)	5-HT$_3$ IC$_{50}$ (nM)
14b	NH_2		36.4	48.5	**14m**	NH_2		1.3	> 100
14h	NH_2		< 10	41.4	**14n**	NH_2		< 1	61.0
14i	H		> 100	> 100	**14o**	NH_2		< 1	64.8
14j	H		>100	44.2	**14q**	NH_2		5.2	100.0
14k	NH_2		24.4	69.9	**14r**	NH_2		2.5	77.6
14l	NH_2		5.6	63.8					

Table 3. Optimization of the Bulky Hydrophobic Portion of Methylene Linked Compounds



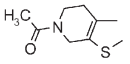
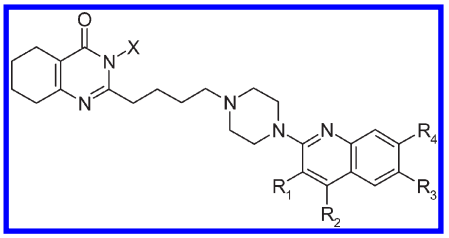
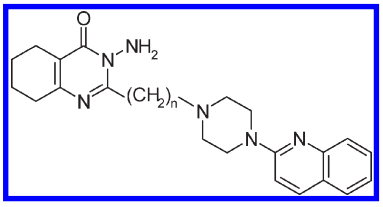
compd no.	R	5-HT$_{1A}$ IC$_{50}$ (nM)	5-HT$_3$ IC$_{50}$ (nM)	compd no.	R	5-HT$_{1A}$ IC$_{50}$ (nM)	5-HT$_3$ IC$_{50}$ (nM)
17a		< 10	26.7	**17k**		< 10	45.6
17b		< 10	45.7	**17l**		< 10	15.6
17c		< 10	50.6	**17m**		1.4	8.9
17d		< 10	99.4	**17n**		< 10	48.3
17e		< 10	> 100	**17o**		< 10	97.9
17f		< 10	> 100	**17p**		< 1	62.1
17g		< 10	> 100	**17q**		< 10	84.8
17h		18.0	> 100	**17t**		< 10	46.5
17i		< 10	83.1	**17w**		< 10	22.1
17j		13.5	> 100				

Table 4. IC_{50} Value of Tetrahydroquinazolinone Derivatives with Methylene-Type Linker



compd no.	R1	R2	R3	R4	X	5-HT_{1A} IC_{50} (nM)	5-HT_3 IC_{50} (nM)
17m	H	H	H	H	NH_2	1.4	8.9
18a	H	H	H	H	H	6.2	24.6
18b	H	CH_3	H	H	NH_2	< 10	59.1
18c	CH_3	H	H	H	NH_2	96.0	> 100
18d	CH_3	CH_3	H	H	NH_2	64.6	> 100
18e	$-(CH_2)_3-$		H	H	NH_2	62.3	> 100
18f	$-(CH_2)_4-$		H	H	NH_2	> 100	> 100
18g	OH	H	H	H	NH_2	96.4	52.6
18h	H	H	OH	H	NH_2	59.3	> 100
18i	H	H	H	OH	NH_2	46.6	55.2

Table 5. Optimization of the Methylene Linker Length



compd no.	*n*	5-HT_{1A} IC_{50} (nM)	5-HT_3 IC_{50} (nM)
19a	1	> 100	86.6
19b	2	> 100	> 100
19c	3	90.4	> 100
17m	4	1.4	8.9
19d	5	7.6	> 100

Table 6. 5-HT_{1A} Agonistic Activity in Vitro; [^{35}S]GTPγS Binding Assay

	14l	**14r**	**17b**	**17m**	**18b**
EC_{50} (nM)	182	2.5	30.7	22.3	13.7
E_{max} (%)	90.3	84.8	94.7	99.8	93.5

Table 7. 5-HT_3 Antagonistic Activity in Vitro; Guinea Pig Ileum Contraction Inhibition Assay[a]

	14b	**14l**	**14r**	**17b**	**17m**	**18b**
1 μmol/L	41.9	95.1	97.6	90.1	96.8	100
0.1 μmol/L	ND[b]	80.0	79.9	−23.2	70.4	97.1

[a] Results are listed for inhibition % of control. [b] Not determined.

improve the concentration of these compounds in the brain, we examined the effect of decreasing the number of heteroatoms in the structure.

Improvement of Compound Penetration into Brain and Affinity to Both Receptors

To reduce the number of heteroatoms, we chose to replace the sulfur atom linker with a methylene group because we believed that the aryl piperazine and pyrimidinone amino groups were necessary for receptor binding while the sulfur atom in the linker would have no influence on binding affinity. We fixed the aryl piperazine to **5** and prepared derivatives with a methylene linker that showed very high inhibition activities (Table 3). Compound **17b**, with a fused benzene ring instead of thiophene, showed high affinities to both receptors. Most of the compounds with a halogen substituted benzene ring had high affinities for the 5-HT_{1A} receptor but did not exceed compound **17b** in binding affinity to 5-HT_3. Substitution of a methyl group on the 6-position of the quinazolinone showed almost equal affinity for the unsubstituted quinazolinone (compound **17k** versus **17b**). Compound **17m**, with a tetrahydroquinazolinone, showed high affinities to both receptors as much as **17b**. As introduction of substituents on the tetrahydroquinazolinone (**17n** and **17o**) did not increase affinity and resizing the ring (**17p** and **17q**) resulted in a decrease in the affinity to 5-HT_3 receptor, the size of the tetrahydroquinazoline appeared to be optimal for affinity to both receptors. Compound **17t** that had a hydroxy group showed high affinities to both receptors and *N*-acetylated tetrahydropyridine derivative **17w** maintained high affinity for 5-HT_{1A} and increased affinity for 5-HT_3 compared with thioether type compound **14r**.

For the quinoline part, compounds that had a substituent at the 3-position had a low affinity to both receptors (**18c−g**, Table 4) although the parent aryl piperazine moiety had a high affinity to the 5-HT_3 receptor. Thus we reconfirmed that substitution at the 3-position of the quinoline was unsuitable

Table 9. 5-HT_{1A} Agonistic Activity in Vivo; 5-HT_{1A} Receptor-Mediated Behavior and Hypothermia in Rats[a,b]

	14r[c]	**17b**	**17m**	**18b**
LLR	1.0	1.8	1.5	NE[d]
FBP	1.0	1.8	1.8	
ΔT	−0.1	−1.9	−1.4	

[a] Dose: 10 mg/kg, ip. [b] LLR = lower lip retraction, FBP = flat body posture and ΔT = change in rectal temperature. [c] Dose: 10 mg/kg, iv. [d] No effect.

Table 10. 5-HT_3 Antagonistic Activities in Vivo; Inhibition of the Bezold−Jarisch Reflex Caused by 5-HT[a]

	14r	**17b**	**17m**	**18b**[b]
inhibit %	77.0	21.9	64.0	64.5

[a] Dose: 10 μg/kg, iv. [b] Dose: 100 μg/kg, iv.

Table 8. Total Concentrations of Compound in Brain and Blood[a,b]

	2 h			4 h			8 h		
compd no.	brain conc (nM)	blood conc (nM)	brain/blood ratio	brain conc (nM)	blood conc (nM)	brain/blood ratio	brain conc (nM)	blood conc (nM)	brain/blood ratio
14l	63.1 ± 12.1	21.2 ± 4.25	2.99 ± 0.261	32.2 ± 6.32	10.6 ± 2.28	3.05 ± 0.151	13.0 ± 5.55	5.17 ± 2.42	2.53 ± 0.092
14r	18.7 ± 3.61	56.0 ± 5.41	0.332 ± 0.036	12.9 ± 1.79	36.1 ± 1.02	0.357 ± 0.044	2.96 ± 1.32	8.98 ± 4.66	0.338 ± 0.003
17b	685 ± 268	97.4 ± 34.3	7.11 ± 1.38	367 ± 68.4	55.5 ± 6.88	6.59 ± 0.643	204 ± 105	28.3 ± 18.5	7.65 ± 1.28
17m	160 ± 42.3	66.5 ± 32.2	2.56 ± 0.488	70.2 ± 11.0	16.8 ± 6.29	4.40 ± 0.873	56.4 ± 12.7	9.97 ± 4.74	6.54 ± 2.83
17w	15.1 ± 4.36	51.0 ± 7.00	0.294 ± 0.071	19.5 ± 2.85	55.8 ± 12.9	0.355 ± 0.040	13.4 ± 1.12	43.7 ± 4.02	0.307 ± 0.004

[a] Each value represents the mean ± SD of three rats. [b] Dose: 3 mg/kg, po.

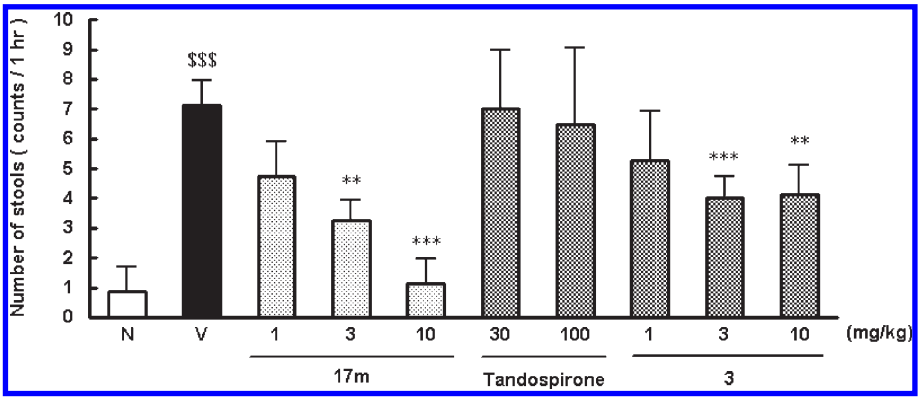


Figure 3. The effects of **17m**, tandospirone, and **3** on stress-induced defecation in rats.[12] The upper bodies of lightly anesthetized rats were wrapped with adhesive tape, and the animals were returned to the observation cages. The number of feces dropped on the tray was counted 1 h after the wrapping. Vehicle or test drugs were administered orally 1 h before the restraint; $n = 8$ per group. Values represent mean (±SD). \$\$\$ $P < 0.001$ versus normal (N); Wilcoxon test. **, $P < 0.01$, ***, $P < 0.001$ versus vehicle (V); nonparametric Dunnett's multiple comparison test. Normal group represents animals without wrapping.

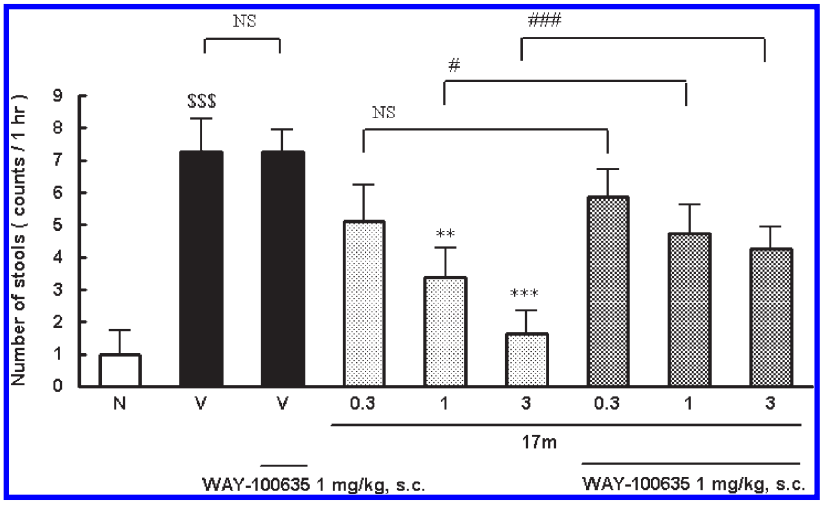


Figure 4. The influence of 5-HT_{1A} antagonist, WAY-100635, on the inhibitory effect of **17m** in stress-induced defecation.[12] Vehicle or **17m** was injected intraperitoneally 20 min following subcutaneous injection with saline (containing diluted hydrochloric acid) or WAY-100635. Five minutes later, lightly anesthetized rats were wrapped for 1 h and the number of feces was counted. $n = 8$ per group. Values represent mean (±SD). \$\$\$ $P < 0.001$ versus normal (N); Wilcoxon test. **, $P < 0.01$, ***, $P < 0.001$ versus vehicle (V); nonparametric Dunnett's multiple comparison test. # $P < 0.05$, ### $P < 0.001$ between indicated two groups; Wilcoxon test. NS means nonsignificant. Normal group represents animals without wrapping.

for binding to the 5-HT_{1A} receptor as well as the 5-HT_3 receptor. Deaminated compound (**18a**) showed somewhat lower affinity than **17m** for 5-HT_{1A}, and 4-methylquinoline derivative (**18b**) had moderate affinity for 5-HT_3. Compounds **18h** and **18i** with a hydroxyl group at R3 or R4 had greatly decreased affinity to 5-HT_{1A}.

Optimization of the linker length was carried out (Table 5). Affinity to the 5-HT_{1A} receptor was maintained to some degree at $n = 5$ (**19d**), but the affinity to both receptors was maximized for $n = 4$ (**17m**).

In secondary screens, compounds with high affinities to both receptors in the methylene linker class were subjected to in vitro functional examinations ([^{35}S]GTPγS binding assay and a guinea pig ileum contraction inhibition assay, Table 6 and 7). In the [^{35}S]GTPγS binding assay, every compound in this series (**17b**, **17m**, and **18b**) was confirmed to be a full 5-HT_{1A} agonist, and for the contraction inhibition assay, almost every compound showed 90−100% inhibition at 1 μmol/L, confirming that they were 5-HT_3 antagonists.

Total concentrations of compounds **17b**, **17m**, and **17w** in the blood and brain were measured (Table 8).[16] Compounds **17b** and **17m** had significantly increased concentrations both in the blood and brain (versus **14l**), with compound **17b** having a very high concentration in brain compared to **17m**. The conversion from S to CH_2 in the linker improved the compound pharmacokinetics without decreasing the affinity to either receptor.

Compounds **17b** and **17m** were found to have excellent in vivo activity (Tables 9 and 10). In the B-J reflex assay, compound **17m** inhibited bradycardia at a very low dosage. Compound **18b** had no effect on LLR, FBP, and ΔT. Though compound **18b** had affinity to 5-HT_{1A} and agonistic activity in vitro, the 5-HT_{1A} agonistic action was not seen in vivo. Because its B-J reflection was about one order weaker than **17m**, we thought that its blood level would be low and the concentration in brain also would be low along with it (concentration of **18b** in brain and blood was not measured). Therefore, it seemed that 5-HT_{1A} agonistic effect was not seen.

As compounds **17b** and **17m** showed excellent results, we checked their selectivity to other receptors (Table 11).[17,18] **17m** had a low affinity to these other receptors including the α1 receptor, but **17b**, an aromatized type of **17m**, had some degree

Table 11. In Vitro Binding (IC_{50}[a]) to Other Receptors

compd no.	rat α_1	rat α_2	hD_2[b]	rat 5-HT_2	gp 5-HT_4[c]
17b	63% at 0.1 μM	42% at 0.1 μM	ND[d]	78% at 0.1 μM	ND
17m	> 0.1	> 0.1	> 0.1	≈0.1	> 1

[a] IC_{50}; μM. [b] h = human. [c] gp = guinea pig. [d] Not determined.

Table 12. In Vivo Pharmacokinetic Data for Compound **17m** in Rat

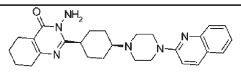
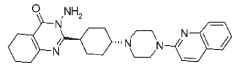
dose	T_{max} (hr)	$AUC_{0\text{-}t}$ (ng·h/mL)	$T_{1/2}$ (h)	C_{max} (ng/mL)	MRT (h)	F (%)
po[a]	0.5	705	2.054	241.488	3.100	25.5

[a] Dose: 10 mg/kg, po.

Table 13. Binding Inhibition Constants (K_i Values) of Compound **17m**

	5-HT_{1A}	5-HT_3
K_i (mol/L)	$7.008 \times 10^{-10} \pm 0.3309 \times 10^{-10}$	$4.720 \times 10^{-9} \pm 0.2270 \times 10^{-9}$

Table 14. Inhibition Activities of Tetrahydroquinazolinone Derivatives with a Rigid Linker

Compd. No.	structure	5-HT_{1A} inhibition (%) 1000nM	5-HT_{1A} inhibition (%) 100nM	5-HT_3 inhibition (%) 1000nM	5-HT_3 inhibition (%) 100nM
26a		91.5	42.3	87.3	19.6
26b		96.3	76.9	18.4	1.4

of affinity to several receptors. This illustrates that the bulky hydrophobic region is important for receptor binding selectivity. The pharmacokinetic data for **17m** show it is orally bioavailable (Table 12). Binding inhibition constants (K_i values) were calculated (Table 13). K_i values of **17m** for human 5-HT_{1A} receptors were $7.008 \times 10^{-10} \pm 0.3309 \times 10^{-10}$ mol/L and for human 5-HT_3 receptors were $4.720 \times 10^{-9} \pm 0.2270 \times 10^{-9}$ mol/L.

Finally, we examined **17m** in the wrap restraint-induced stress model of IBS (Figure 3).[12] Compound **17m** reduced the restraint stress-induced defecation as **3** did. It is thought that at the doses used in this assay, **17m** reduced the defecation at least partially through 5-HT_3 antagonism. It is interesting that, although **3** only partially inhibited the restraint stress-induced defecation, **17m** completely normalized this process. Moreover, the effect of **17m** was partly inhibited by a 5-HT_{1A} antagonist, WAY-100635 (Figure 4).[12] These results indicate that stimulation of the 5-HT_{1A} receptors by **17m** also reduces the restraint stress-induced defecation. Taken together, these data suggest that the suppression of restraint stress-induced defecation by **17m** was the result of contributions from both 5-HT_{1A} agonism and 5-HT_3 antagonism.

Many compounds, with an affinity to the 5-HT_{1A} receptor, generally have a long shape in which an aryl piperazine derivative is linked to a bulky hydrophobic region such as in compound **17m**. However, 5-HT_3 antagonists in general do not have such a long structure. In considering why **17m** displayed affinity to both receptors, we postulated that **17m** had an extended conformation when binding to 5-HT_{1A} and had a bent conformation when binding to 5-HT_3. To verify this hypothesis, we synthesized two conformationally restricted compounds with a cyclohexane ring linker (Table 14). Although both rigid linker compounds had decreased affinity for the 5-HT receptors, compound **26b** with a *trans*-cyclohexane locked linker (extended shape) has a particular affinity for the 5-HT_{1A} receptor and it has no affinity for the 5-HT_3 receptor. On the other hand, compound **26a** with a *cis*-cyclohexane locked linker (bended shape) shows some affinity for 5-HT_3 and has decreased affinity for 5-HT_{1A} compared with **26b**. Thus, we believe that **17m** acts selectively on the 5-HT receptor subtypes via two different conformations.

Conclusion

Our initial aim was to search for compounds with both gastrointestinal motor inhibition and antianxiety effects for the treatment of diarrhea type IBS through dual 5-HT_3 antagonist and 5-HT_{1A} agonist activity. By superposition of the pharmacophores of 5-HT_{1A} agonists and 5-HT_3 antagonists and use of the aryl piperazine moiety as a common structural feature, we discovered compound **14b**, which had affinity to both receptors. We further optimized this lead compound to discover quinazolinone derivatives with a sulfur containing linker. These showed high affinity to both receptors but did not show in vivo activity. To improve the blood−brain barrier penetration, we designed a compound which replaces sulfur in the linker with a methylene group. These compounds showed high affinity to both receptors, and in addition, the compound total concentrations in brain and blood were improved. In this compound series, **17m** has a pronounced affinity to both receptors. **17m** showed 5-HT_{1A} agonistic/5-HT_3 antagonistic activity concurrently in in vitro/vivo functional assays. In the stress-induced defecation IBS model, **17m** significantly inhibited stress-induced defecation. Pretreatment with WAY-100635, a 5-HT_{1A} antagonist, significantly attenuated but did not abolish the inhibitory effect of **17m**. These results suggested that **17m** exerted an inhibitory effect via both 5-HT_{1A} agonist and 5-HT_3 antagonist activities. This compound can be expected to contribute to the improvement of compliance of taking medicine and the reduction of patient's medical cost. From these results, **17m** was selected for advanced evaluation as a treatment for IBS.

Experimental Section

Unless otherwise noted, all nonaqueous reactions were carried out under an Ar atmosphere using commercial grade solvents and reagents. ^{1}H NMR spectra were recorded on a JEOL JNM-ECP 400. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane as an internal standard, using the following abbreviations: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet; br, broad. Coupling constants (J) are reported in hertz (Hz) where relevant. Mass spectrometric analyses were obtained with a Shimadzu GC/MS QP-5000, with electrospray ionization methodology. The purities of the compounds were examined by HPLC (≥95%) using a Waters 2695 HPLC system.

Synthesis of 5, General Procedure of Synthesis of Aryl Piperazine (12). Anhydrous piperazine (4.31 g, 50.0 mmol) was dissolved in ethylene glycol (30 mL), and 2-chloroquinoline (818 mg, 5.00 mmol) was added. The mixture was stirred at 140 °C for 2 h. After cooling, saturated aqueous sodium hydrogencarbonate solution was added and the system was extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (chloroform:methanol = 2:1) to provide 1.09 g

(100%) of 2-piperazin-1-ylquinoline (**5**). ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.59 (dd, J = 1.5, 8.0 Hz, 1H), 7.53 (ddd, J = 1.5, 7.0, 8.4 Hz, 1H), 7.26−7.22 (m, 1H), 6.97 (d, J = 9.2 Hz, 1H), 3.70 (t, J = 5.0 Hz, 4H), 3.01 (t, J = 5.0 Hz, 4H). Mass, m/z: 213 (M^+), 145 (base).

2-[4-(3-Chloropropyl)piperazin-1-yl]quinoline, General Procedure for the Synthesis of Alkylchloride Derivatives (13). Compound **5** (853 mg, 4.00 mmol) was dissolved in acetone (5 mL), and aqueous sodium hydroxide (160 mg in 5 mL) was added. 1-Bromo-3-chloropropane (0.5 mL) was added dropwise to the solution, and stirring was continued overnight at room temperature. The reaction was diluted with diethyl ether, and the organic layer was washed with saturated aqueous sodium hydrogencarbonate solution and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (chloroform:methanol = 50:1) to provide 1.10 g (95%) of **13**. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.59 (dd, J = 1.4, 8.0 Hz, 1H), 7.53 (ddd, J = 1.5, 7.1, 8.5 Hz, 1H), 7.22 (ddd, J = 1.1, 6.9, 8.0 Hz, 1H), 6.98 (d, J = 9.2 Hz, 1H), 3.75 (t, J = 5.1 Hz, 4H), 3.61 (t, J = 6.5 Hz, 2H), 2.63−2.43 (m, 6H), 2.04−1.97 (m, 2H). Mass, m/z: 289 (M^+), 157 (base).

3-Amino-5,6-dimethyl-2-[3-(4-quinolin-2-ylpiperazin-1-yl)-propylthio]-3*H*-thieno[2,3-*d*]pyrimidin-4-one (14b), General Procedure for the Synthesis of Thioether Linked Compounds. A mixture of potassium 3-amino-5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-thiolate (**9**) (80 mg, 0.30 mmol), prepared from ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate, and 2-[4-(3-chloropropyl)piperazin-1-yl]quinoline (104 mg, 0.36 mmol) in ethanol (5 mL) were heated to reflux for 4.5 h. After cooling the reaction mixture, chloroform was added, followed by washing with saturated brine. The organic layer was dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (chloroform:methanol = 100:1) to provide 72 mg (50%) of **14b**. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 8.7 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.54−7.52 (m, 1H), 7.23−7.20 (m, 1H), 6.99 (d, J = 9.2 Hz, 1H), 4.77 (s, 2H), 3.79 (t, J = 5.1 Hz, 4H), 3.19 (t, J = 7.3 Hz, 2H), 2.62 (t, J = 5.1 Hz, 4H), 2.56 (t, J = 7.0 Hz, 2H), 2.45 (s, 3H), 2.36 (s, 3H), 2.00 (q, J = 7.3 Hz, 2H). Mass, m/z: 480 (M^+), 157 (base).

3-Amino-5,6-dimethyl-2-[3-(4-pyridin-2-ylpiperazin-1-yl)propylthio]-3*H*-thieno[2,3-*d*]pyrimidin-4-one (14a). This compound was synthesized using the same procedure as for **14b** starting with pyridyl piperazine and ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate. ^{1}H NMR ($CDCl_3$) δ: 8.19 (ddd, J = 0.6, 1.9, 4.9 Hz, 1H), 7.48 (ddd, J = 1.9, 7.2, 8.9 Hz, 1H), 6.65 (d, J = 8.5 Hz, 1H), 6.62 (ddd, J = 0.8, 4.9, 7.1 Hz, 1H), 4.77 (s, 2H), 3.57 (t, J = 5.0 Hz, 4H), 3.18 (t, J = 7.1 Hz, 2H), 2.59 (t, J = 5.1 Hz, 4H), 2.54 (t, J = 7.0 Hz, 2H), 2.44 (s, 3H), 2.36 (s, 3H), 1.98 (q, J = 7.2 Hz, 2H). Mass, m/z: 430 (M^+), 107 (base).

3-Amino-5,6-dimethyl-2-[3-[4-(4-methylquinolin-2-yl)piperazin-1-yl]propylthio]-3*H*-thieno[2,3-*d*]pyrimidin-4-one (14c). This compound was synthesized using the same procedure as for **14b** starting with ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate and 2-chlorolepidine. ^{1}H NMR ($CDCl_3$) δ: 7.77 (dd, J = 1.1, 8.1 Hz, 1H), 7.71 (d, J = 7.7 Hz, 1H), 7.53 (ddd, J = 1.5, 7.0, 8.4 Hz, 1H), 7.25−7.22 (m, 1H), 6.84 (d, J = 3.7 Hz, 1H), 4.78 (s, 2H), 3.78 (t, J = 5.1 Hz, 4H), 3.20−3.17 (m, 2H), 2.62−2.60 (m, 4H), 2.60 (s, 3H), 2.58−2.54 (m, 2H), 2.44 (d, J = 0.7 Hz, 3H), 2.36 (d, J = 0.7 Hz, 3H), 1.99 (q, J = 7.3 Hz, 2H). Mass, m/z: 494 (M^+), 171 (base).

3-Amino-5,6-dimethyl-2-[3-[4-(5,6,7,8-tetrahydroquinolin-2-yl)-piperazin-1-yl]propylthio]-3*H*-thieno[2,3-*d*]pyrimidin-4-one (14d). This compound was synthesized using the same procedure as for **14b** starting with ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate and 2-chloro-5,6,7,8-tetrahydroquinoline. ^{1}H NMR ($CDCl_3$) δ: 7.17 (d, J = 8.4 Hz, 1H), 6.43 (d, J = 8.4 Hz, 1H), 4.77 (s, 2H), 3.50 (t, J = 5.1 Hz, 4H), 3.17 (t, J = 7.3 Hz, 2H), 2.75 (t, J = 5.6 Hz, 2H), 2.63−2.52 (m, 8H), 2.44 (s, 3H), 2.35 (d, J = 7.0 Hz, 3H), 1.97 (q, J = 7.3 Hz, 1H), 1.86−1.80 (m, 2H), 1.78−1.75 (m, 2H). Mass, m/z: 484 (M^+), 161 (base).

3-Amino-2-[3-(4-benzothiazol-2-ylpiperazin-1-yl)propylthio]-5,6-dimethyl-3*H*-thieno[2,3-*d*]pyrimidin-4-one (14e). This compound was synthesized using the same procedure as for **14b** starting with 2-chlorobenzothiazol and ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate. ^{1}H NMR ($CDCl_3$) δ: 7.61−7.59 (m, 1H), 7.57−7.55 (m, 1H), 7.29 (ddd, J = 1.4, 7.5, 7.9 Hz, 1H), 7.08 (ddd, J = 1.2, 7.5, 7.9 Hz, 1H), 4.77 (s, 2H), 3.68 (t, J = 5.1 Hz, 4H), 3.18 (t, J = 7.4 Hz, 2H), 2.62 (t, J = 5.1 Hz, 4H), 2.56−2.54 (m, 2H), 2.44 (d, J = 0.7 Hz, 3H), 2.36 (d, J = 0.9 Hz, 3H), 2.00−1.94 (m, 2H). Mass, m/z: 486 (M^+), 128 (base).

3-Amino-5,6-dimethyl-2-[3-[4-(3-phenylquinoxalin-2-yl)piperazin-1-yl]propylthio]-3*H*-thieno[2,3-*d*]pyrimidin-4-one (14f). This compound was synthesized using the same procedure as for **14b** starting with ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate and 2-chloro-3-phenylquinoxaline. ^{1}H NMR ($CDCl_3$) δ: 7.96−8.01 (m, 2H), 7.84 (dd, J = 1.5, 8.4 Hz, 1H), 7.60 (ddd, J = 1.1, 6.9, 8.1 Hz, 1H), 7.41−7.52 (m, 5H), 4.75 (s, 2H), 3.29−3.37 (m, 4H), 3.14 (t, J = 7.0 Hz, 2H), 2.47−2.53 (m, 4H), 2.43 (s, 3H), 2.35 (s, 3H), 1.91 (q, J = 7.0 Hz, 2H), 1.24 (t, J = 7.0 Hz, 2H). Mass, m/z: 557 (M^+), 128 (base).

3-Amino-5,6-dimethyl-2-[3-(4-phenanthridin-6-ylpiperazin-1-yl)-propylthio]-3*H*-thieno[2,3-*d*]pyrimidin-4-one (14g). This compound was synthesized using the same procedure as for **14b** starting with 6-chlorophenanthridine and ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate. ^{1}H NMR ($CDCl_3$) δ: 8.55 (d, J = 8.4 Hz, 1H), 8.42 (dd, J = 1.1, 8.1 Hz, 1H), 8.21 (d, J = 7.3 Hz, 1H), 7.92 (dd, J = 1.1, 8.1 Hz, 1H), 7.79−7.75 (m, 1H), 7.63−7.60 (m, 2H), 7.50−7.46 (m, 1H), 3.56 (br s, 4H), 3.21 (t, J = 7.0 Hz, 2H), 2.79 (br s, 4H), 2.64 (t, J = 7.0 Hz, 2H), 2.44 (s, 3H), 2.35 (s, 3H). Mass, m/z: 530 (M^+), 207 (base).

3-Amino-2-[3-(4-quinolin-2-ylpiperazin-1-yl)propylthio]-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (14h). This compound was synthesized using the same procedure as for **14b** starting with ethyl 2-amino-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylate and 2-chloroquinoline. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 8.7 Hz, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.59 (dd, J = 1.3, 8.0 Hz, 1H), 7.53 (m, 1H), 7.24−7.20 (m, 1H), 6.99 (d, J = 9.2 Hz, 1H), 4.77 (s, 2H), 3.79 (t, J = 5.1 Hz, 4H), 3.21−3.17 (m, 2H), 2.99−2.95 (m, 2H), 2.75−2.71 (m, 2H), 2.62 (t, J = 5.1 Hz, 4H), 2.56 (t, J = 7.0 Hz, 2H), 1.99 (q, J = 7.3 Hz, 2H), 1.91−1.80 (m, 4H). Mass, m/z: 506 (M^+), 157 (base).

5,6-Dimethyl-2-[3-(4-quinolin-2-yl-piperazin-1-yl)propylthio]-3*H*-thieno[2,3-*d*]pyrimidin-4-one (14i). This compound was synthesized using the same procedure as for **14b** starting with potassium 5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-2-thiolate, which was prepared from ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 8.8 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.59 (dd, J = 1.2, 8.0 Hz, 1H), 7.54−7.50 (m, 1H), 7.22 (ddd, J = 1.2, 6.9, 8.0 Hz, 1H), 6.98 (d, J = 9.2 Hz, 1H), 3.89−3.80 (m, 4H), 3.29 (t, J = 6.9 Hz, 2H), 2.67 (t, J = 4.9 Hz, 4H), 2.62 (t, J = 6.8 Hz, 2H), 2.43 (d, J = 0.8 Hz, 3H), 2.43 (d, J = 0.8 Hz, 3H), 2.08−1.98 (m, 2H). Mass, m/z: 465 (M^+), 157 (base).

2-[3-(4-Quinolin-2-ylpiperazin-1-yl)propylthio]-5,6,7,8-tetrahydro-3*H*-benzo[4,5]thieno[2,3-*d*]pyrimidin-4-one (14j). This compound was synthesized using the same procedure as for **14i** starting with ethyl 2-amino-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylate. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.0 Hz, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.55−7.51 (m, 1H), 7.24−7.18 (m, 1H), 6.99 (d, J = 9.0 Hz, 1H), 3.89 (br s, 4H), 3.30 (t, J = 6.8 Hz, 2H), 3.01−2.92 (m, 2H), 2.78−2.70 (m, 2H), 2.66 (br s, 4H), 2.68−2.58 (m, 2H), 1.89−1.78 (m, 2H), 1.59−1.50 (m, 2H). Mass, m/z: 491 (M^+), 157 (base).

3-Amino-2-[3-(4-quinolin-2-ylpiperazin-1-yl)propylthio]-3*H*-quinazolin-4-one (14k). This compound was synthesized using the same procedure as for **14b** starting with anthranilic acid ethyl ester and 2-chloroquinoline. ^{1}H NMR ($CDCl_3$) δ: 8.22−8.19 (m, 1H), 7.89 (d, J = 9.2 Hz, 1H), 7.73−7.69 (m, 1H), 7.61−7.51 (m, 3H), 7.41−7.36 (m, 1H), 7.24−7.20 (m, 1H), 6.99 (d, J = 9.2 Hz, 1H), 4.82 (s, 2H),

3.79 (t, J = 4.8 Hz, 4H), 3.26 (t, J = 7.3 Hz, 2H), 2.65−2.58 (m, 6H), 2.05 (q, J = 7.0 Hz, 2H). Mass, m/z: 446 (M^+), 157 (base).

3-Amino-2-[3-(4-quinolin-2-ylpiperazin-1-yl)propylthio]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (14l). This compound was synthesized using the same procedure as for **14b** starting with ethyl 2-aminocyclohex-1-ene-1-carboxylate and 2-chloroquinoline. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.0 Hz, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.59 (d, J = 7.9 Hz, 1H), 7.53 (ddd, J = 1.6, 7.0, 8.7 Hz, 1H), 7.24−7.20 (m, 1H), 6.98 (d, J = 8.3 Hz, 1H), 4.80 (s, 2H), 3.77 (t, J = 4.8 Hz, 4H), 3.14 (t, J = 7.4 Hz, 4H), 2.62−2.60 (m, 4H), 2.58−2.45 (m, 6H), 1.99−1.95 (m, 2H), 1.80−1.72 (m, 4H). Mass, m/z: 450 (M^+), 157 (base).

3-Amino-7-nitro-2-[3-(4-quinolin-2-ylpiperazin-1-yl)propylthio]-3*H*-quinazolin-4-one (14m). This compound was synthesized using the same procedure as for **14b** starting with 7-nitroanthranilic acid and 2-chloroquinoline. ^{1}H NMR (DMSO-d_6) δ: 8.29 (d, J = 8.9 Hz, 1H), 8.23 (d, J = 2.2 Hz, 1H), 8.12 (dd, J = 2.2, 8.9 Hz, 1H), 8.02 (d, J = 9.2 Hz, 1H), 7.67 (d, J = 7.2 Hz, 1H), 7.56−7.40 (m, 2H), 7.23−7.18 (m, 2H), 5.84 (s, 2H), 3.72 (t, J = 4.8 Hz, 4H), 3.17 (t, J = 7.3 Hz, 2H), 2.55−2.48 (m, 6H), 1.93 (t, J = 7.3 Hz, 2H). Mass, m/z: 491 (M^+), 157 (base).

3-Amino-2-[3-(4-quinolin-2-ylpiperazin-1-yl)propylthio]-3*H*-pyrido[3,2-*d*]pyrimidin-4-one (14n). This compound was synthesized using the same procedure as for **14b** starting with 3-aminopyridine-2-carboxylic acid methyl ester and 2-chloroquinoline. ^{1}H NMR ($CDCl_3$) δ: 8.92 (dd, J = 1.8, 4.4 Hz, 1H), 8.55 (dd, J = 1.8, 8.1 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.59 (dd, J = 1.1, 8.1 Hz, 1H), 7.53 (ddd, J = 1.5, 7.0, 8.4 Hz, 1H), 7.37−7.34 (m, 1H), 7.24−7.20 (m, 1H), 6.98 (d, J = 9.1 Hz, 1H), 4.84 (s, 2H), 3.78 (t, J = 5.1 Hz, 4H), 3.38 (t, J = 7.3 Hz, 2H), 2.64−2.59 (m, 6H), 2.07 (q, J = 7.3 Hz, 2H). Mass, m/z: 447 (M^+), 157 (base).

3-Amino-2-[3-(4-quinolin-2-ylpiperazin-1-yl)propylthio]-3*H*-pyrido[2,3-*d*]pyrimidin-4-one (14o). This compound was synthesized using the same procedure as for **14b** starting with 2-aminonicotinic acid ethyl ester and 2-chloroquinoline. ^{1}H NMR ($CDCl_3$) δ: 8.77 (dd, J = 1.5, 4.4 Hz, 1H), 7.92 (dd, J = 1.5, 8.4 Hz, 1H), 7.89 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.64−7.59 (m, 2H), 7.54 (ddd, J = 1.5, 7.0, 8.4 Hz, 1H), 7.25−7.21 (m, 1H), 6.99 (d, J = 9.2 Hz, 1H), 4.94 (s, 2H), 3.79 (t, J = 5.1 Hz, 4H), 3.27 (t, J = 7.3 Hz, 2H), 2.64 (t, J = 5.1 Hz, 4H), 2.60 (t, J = 7.3 Hz, 2H), 2.05 (q, J = 7.3 Hz, 2H). Mass, m/z: 447 (M^+), 157 (base).

3-Amino-7-*tert*-butoxycarbonyl-2-[3-(4-quinolin-2-ylpiperazin-1-yl)propylthio]-3,5,6,8-tetrahydro-4*H*-pyrido[4′,3′:4,5]thieno[2,3-*d*]-pyrimidine (14p). This compound was synthesized using the same procedure as for **14b** starting with 2-chloroquinoline and 2-amino-4,7-dihydro-5*H*-thieno[2,3-*c*]pyridine-3,6-dicarboxylic acid 6-*tert*-butyl ester 3-ethyl ester. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.61−7.58 (m, 1H), 7.53 (ddd, J = 1.5, 7.0, 8.4 Hz, 1H), 7.24−7.20 (m, 1H), 6.99 (d, J = 9.2 Hz, 1H), 4.78 (s, 2H), 4.60 (br s, 2H), 3.79 (t, J = 5.1 Hz, 4H), 3.73−3.70 (m, 2H), 3.22−3.18 (m, 2H), 3.06 (br s, 2H), 2.62 (t, J = 5.1 Hz, 4H), 2.58−2.54 (m, 2H), 2.00 (q, J = 7.3 Hz, 2H). Mass, m/z: 607 (M^+), 157 (base).

3-Amino-2-[3-(4-quinolin-2-ylpiperazin-1-yl)propylthio]-5,6,7,8-tetrahydro-3*H*-pyrido[4′,3′:4,5]thieno[2,3-*d*]pyridin-4-one Trihydrochloride (14q). 14p (0.10 g, 0.16 mmol) was added to 6 mL of 4N hydrochloric acid dioxane solution and was stirred for 2.5 h. Evaporation of the solvent under reduced pressure gave 105 mg (100%) of **14q**. ^{1}H NMR (DMSO-d_6) δ: 9.82 (br s, 1H), 8.46 (d, J = 9.5 Hz, 1H), 8.32 (br s, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.78 (m, 1H), 7.58 (d, J = 9.2 Hz, 1H), 7.50 (m, 1H), 4.87 (d, J = 8.2 Hz, 2H), 4.33 (br s, 2H), 4.20−3.85 (m, 4H), 3.70 (d, J = 5.4 Hz, 2H), 3.39 (br s, 2H), 3.26 (br s, 2H), 3.17−3.13 (m, 4H), 2.23−2.16 (m, 2H). Mass, m/z: 507 (M^+), 157 (base).

7-Acetyl-3-amino-2-[3-(4-quinolin-2-ylpiperazin-1-yl)propylthio]-5,6,7,8-tetrahydro-3*H*-pyrido[4′,3′:4,5]thieno[2,3-*d*]pyridin-4-one (14r). 14q (0.20 g, 0.33 mmol) and 133 mg of triethylamine (1.31 mmol) were added to 10 mL of tetrahydrofuran. To the ice cooled mixture, acetyl chloride (28 mg, 0.35 mmol) was added dropwise and the reaction was stirred for 30 min followed by concentration under reduced pressure. The residue was purified by silica gel column chromatography (chloroform:methanol = 25:1) to provide 150 mg (83%) of **14r**. ^{1}H NMR ($CDCl_3$) δ: 7.99 (d, J = 8.9 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.59 (d, J = 7.7 Hz, 1H), 7.55−7.51 (m, 1H), 7.24−7.21 (m, 1H), 6.99 (d, J = 9.2 Hz, 1H), 4.78 (br s, 4H), 3.79 (t, J = 4.6 Hz, 4H), 3.75 (d, J = 5.8 Hz, 2H), 3.22−3.19 (m, 2H), 3.15−3.07 (m, 2H), 2.67 (t, J = 4.6 Hz, 4H), 2.58−2.55 (m, 2H), 2.21 (s, 3H), 2.04−1.96 (m, 2H). Mass, m/z: 549 (M^+), 157 (base).

Synthesis of 3-Amino-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (17m). General Procedure for the Synthesis of Methylene Linker Derivatives. Step 1. Synthesis of Ethyl 2-(5-Bromopentanoylamino)cyclohex-1-enecarboxylate (15). To an ice cooled mixture of 42.3 g of ethyl 2-aminocyclohex-1-enecarboxylate (**6**, 250 mmol) and 40.0 g of pyridine (506 mmol) in 150 mL of tetrahydrofuran, 5-bromovaleryl chloride (54.9 g, 275 mmol) was added dropwise. After stirring the reaction mixture overnight at room temperature, ethyl acetate was added, followed by washing sequentially with saturated aqueous sodium hydrogencarbonate solution, 10% aqueous citric acid, and saturated brine. The product was dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane:ethyl acetate = 8:1), to provide 76.6 g (92%) of **15**. ^{1}H NMR ($CDCl_3$) δ:11.62 (br s, 1H), 4.22−4.09 (m, 2H), 3.42 (t, J = 6.9 Hz, 2H), 2.97−2.94 (m, 2H), 2.34 (t, J = 7.0 Hz, 2H), 2.32−2.23 (m, 2H), 1.94−1.88 (m, 2H), 1.85−1.79 (m, 2H), 1.65−1.56 (m, 4H), 1.30 (t, J = 7.0 Hz, 3H). Mass, m/z: 333 (M^+), 55 (base).

Step 2. Synthesis of Ethyl 2-[5-(4-Quinolin-2-ylpiperazin-1-yl)pentanoylamino]cyclohex-1-enecarboxylate (16). 15 (66.5 g, 200 mmol), 46.9 g of 2-piperazin-1-ylquinoline (220 mmol), and 22.3 g of triethylamine (220 mmol) were dissolved in 350 mL of toluene, and the mixture was heated at reflux overnight. The solvent was evaporated under reduced pressure, and ethyl acetate was added to the residue, followed by washing with saturated aqueous sodium hydrogencarbonate solution. The organic layer was dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure. Purification of the residue by silica gel column chromatography (*n*-hexane:ethyl acetate:methanol = 1:6:0.2) provided 79.8 g (86%) of **16**. ^{1}H NMR ($CDCl_3$) δ:11.61 (br s, 1H), 7.87 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.58−7.56 (m, 1H), 7.51 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.20 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.16 (q, J = 6.9 Hz, 2H), 3.74 (t, J = 5.0 Hz, 4H), 2.97 (t, J = 5.0 Hz, 2H), 2.56 (t, J = 5.0 Hz, 4H), 2.43−2.90 (m, 6H), 1.74−1.70 (m, 2H), 1.68 (m, 4H), 1.28 (t, J = 6.9 Hz, 2H). Mass, m/z: 464 (M^+), 157 (base).

Step 3. Synthesis of 3-Amino-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-5,6,7,8-tetrahydro-3H-quinazolin-4-one (17m). To a solution of 8.0 g of **16** (17.2 mmol) in 120 mL of ethanol, 60 mL of hydrazine monohydrate was added, followed by stirring under reflux for 4 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in chloroform, washed with saturated aqueous sodium hydrogencarbonate solution and saturated brine, and dried over anhydrous magnesium sulfate. Evaporation of the solvent under reduced pressure gave a residue which was purified by silica gel column chromatography (chloroform:methanol = 50:1) to provide 3.8 g (51%) of **17m**. ^{1}H NMR ($CDCl_3$) δ: 7.87 (d, J = 9.3 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.58 (d, J = 7.7 Hz, 1H), 7.52 (ddd, J = 1.5, 6.9, 7.5 Hz, 1H), 7.25−7.19 (m, 1H), 6.97 (d, J = 8.9 Hz, 1H), 4.93 (s, 2H), 3.74 (t, J = 5.0 Hz, 4H), 2.92 (t, J = 7.7 Hz, 2H), 2.58−2.55 (m, 6H), 2.52−2.49 (m, 2H), 2.44 (t, J = 7.3 Hz, 2H), 1.81−1.63 (m, 8H). Mass, m/z: 432 (M^+), 157 (base).

3-Amino-5,6-dimethyl-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-thieno[2,3-*d*]pyrimidin-4-one (17a). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 9.2 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.58 (dd,

J = 1.2, 8.1 Hz, 1H), 7.52 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.23−7.19 (m, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.88 (s, 2H), 3.76 (t, J = 5.0 Hz, 4H), 3.04 (t, J = 7.7 Hz, 2H), 2.57 (t, J = 5.0 Hz, 4H), 2.46−2.44 (m, 2H), 2.46 (d, J = 0.8 Hz, 3H), 2.37 (d, J = 0.8 Hz, 3H), 1.88−1.82 (m, 2H), 1.72−1.66 (m, 2H). Mass, m/z: 462 (M^+), 157 (base).

3-Amino-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17b). This compound was synthesized using the same procedure as for **17m** starting with ethyl anthranilate. ^{1}H NMR ($CDCl_3$) δ: 8.24 (dd, J = 1.2, 8.1 Hz, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.73 (ddd, J = 1.6, 6.9, 8.5 Hz, 1H), 7.71−7.65 (m, 2H), 7.59 (dd, J = 1.2, 7.9 Hz, 1H), 7.52 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 7.44 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 7.21 (ddd, J = 1.2, 6.4, 8.1 Hz, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.91 (s, 2H), 3.75 (t, J = 4.8 Hz, 4H), 3.07 (t, J = 7.7 Hz, 2H), 2.58 (t, J = 5.0 Hz, 4H), 2.48 (t, J = 7.3 Hz, 2H), 1.95−1.87 (m, 2H), 1.76−1.69 (m, 2H). Mass, m/z: 428 (M^+), 157 (base).

3-Amino-5-fluoro-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17c). This compound was synthesized using the same procedure as for **17m** starting with methyl 2-amino-6-fluorobenzoate. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.66−7.62 (m, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.54−7.51 (m, 1H), 7.45 (d, J = 8.5 Hz, 1H), 7.23−7.19 (m, 1H), 7.11−7.06 (m, 1H), 6.97 (d, J = 8.9 Hz, 1H), 4.86 (s, 2H), 3.76 (br s, 4H), 3.06 (t, J = 7.7 Hz, 2H), 2.59 (br s, 4H), 2.50−2.46 (m, 2H), 1.92−1.87 (m, 2H), 1.72−1.70 (m, 2H). Mass, m/z: 446 (M^+), 157 (base).

3-Amino-6-fluoro-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17d). This compound was synthesized using the same procedure as for **17m** starting with methyl 2-amino-5-fluorobenzoate. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.2 Hz, 1H), 7.87 (dd, J = 2.7, 8.5 Hz, 1H), 7.72−7.65 (m, 2H), 7.59 (dd, J = 1.2, 8.1 Hz, 1H), 7.53 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.46 (dt, J = 2.7, 8.5 Hz, 1H), 7.22 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 6.98 (d, J = 9.2 Hz, 1H), 4.92 (s, 2H), 3.76 (t, J = 5.0 Hz, 4H), 3.07 (t, J = 7.7 Hz, 2H), 2.59 (t, J = 5.0 Hz, 4H), 2.49 (t, J = 7.7 Hz, 2H), 1.91 (q, J = 7.7 Hz, 2H), 1.73 (q, J = 7.7 Hz, 2H). Mass, m/z: 446 (M^+), 157 (base).

3-Amino-7-fluoro-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17e). This compound was synthesized using the same procedure as for **17m** starting with methyl 2-amino-4-fluorobenzoate. ^{1}H NMR ($CDCl_3$) δ: 8.25 (dd, J = 6.2, 8.9 Hz, 1H), 7.89 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.59 (dd, J = 1.2, 8.1 Hz, 1H), 7.53 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.31 (dd, J = 2.7, 9.6 Hz, 1H), 7.22 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 7.16 (dt, J = 2.3, 8.9 Hz, 1H), 6.98 (d, J = 9.2 Hz, 1H), 4.89 (s, 2H), 3.76 (t, J = 5.0 Hz, 4H), 3.07 (t, J = 7.7 Hz, 2H), 2.59 (t, J = 5.0 Hz, 4H), 2.49 (t, J = 7.7 Hz, 2H), 1.91 (q, J = 7.7 Hz, 2H), 1.73 (q, J = 7.7 Hz, 2H). Mass, m/z: 446 (M^+), 157 (base).

3-Amino-5-chloro-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17f). This compound was synthesized using the same procedure as for **17m** starting with methyl 2-amino-6-chlorobenzoate. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.59−7.50 (m, 4H), 7.44 (dd, J = 2.7, 6.6 Hz, 1H), 7.23−7.19 (m, 1H), 6.97 (d, J = 8.9 Hz, 1H), 4.86 (s, 2H), 3.76 (t, J = 5.0 Hz, 4H), 3.05 (t, J = 7.7 Hz, 2H), 2.59 (t, J = 5.0 Hz, 4H), 2.48 (t, J = 7.7 Hz, 2H), 1.89 (q, J = 7.7 Hz, 2H), 1.75−1.69 (m, 2H). Mass, m/z: 462 (M^+), 157 (base).

3-Amino-6-chloro-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17g). This compound was synthesized using the same procedure as for **17m** starting with methyl 2-amino-5-chlorobenzoate. ^{1}H NMR ($CDCl_3$) δ: 8.19 (d, J = 2.3 Hz, 1H), 7.87 (d, J = 9.2 Hz, 1H), 7.68 (d, J = 9.2 Hz, 1H), 7.66 (dd, J = 2.3, 8.4 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.56−7.59 (m, 1H), 7.52 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.21 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 6.96 (d, J = 9.2 Hz, 1H), 4.90 (br s, 2H), 3.75 (t, J = 5.0 Hz, 4H), 3.05 (t, J = 7.4 Hz, 2H), 2.58 (t, J = 5.0 Hz, 4H), 2.47 (t, J = 7.4 Hz, 2H),1.89 (q, J = 7.4 Hz, 2H),1.71 (q, J = 7.4 Hz, 2H). Mass, m/z: 462 (M^+), 446, 157 (base).

3-Amino-7-chloro-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17h). This compound was synthesized using the same procedure as for **17m** starting with methyl 2-amino-4-chlorobenzoate. ^{1}H NMR ($CDCl_3$) δ: 8.16 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.67 (d, J = 1.9 Hz, 1H), 7.58 (d, J = 8.1 Hz, 1H), 7.52 (ddd, J = 1.5, 6.9, 8.1 Hz, 1H), 7.43 (d, J = 1.9 Hz, 1H), 7.38 (d, J = 2.3 Hz, 1H), 7.21 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.89 (s, 2H), 3.76 (t, J = 5.0 Hz, 4H), 3.06 (t, J = 7.3 Hz, 2H), 2.58 (t, J = 5.0 Hz, 4H), 2.48 (t, J = 7.3 Hz, 2H), 1.90 (q, J = 7.7 Hz, 2H), 1.71 (q, J = 7.3 Hz, 2H). Mass, m/z: 462 (M^+), 157 (base).

3-Amino-8-chloro-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17i). This compound was synthesized using the same procedure as for **17m** starting with methyl 2-amino-3-chlorobenzoate. ^{1}H NMR ($CDCl_3$) δ: 8.16 (dd, J = 1.5, 8.1 Hz, 1H), 7.88 (d, J = 9.2 Hz, 1H), 7.81 (dd, J = 1.5, 8.1 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.58 (d, J = 7.7 Hz, 1H), 7.52 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.38−7.34 (m, 1H), 7.23−7.19 (m, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.93 (s, 2H), 3.76 (t, J = 5.0 Hz, 4H), 3.12 (t, J = 7.7 Hz, 2H), 2.60 (t, J = 5.0 Hz, 4H), 2.50 (t, J = 7.7 Hz, 2H), 1.95 (q, J = 7.7 Hz, 2H), 1.75 (q, J = 7.7 Hz, 2H). Mass, m/z: 462 (M^+), 157 (base).

3-Amino-5-methyl-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17j). This compound was synthesized using the same procedure as for **17m** starting with methyl 2-amino-6-methylbenzoate. ^{1}H NMR ($CDCl_3$) δ: 8.08 (d, J = 8.1 Hz, 1H), 7.88 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.59−7.54 (m, 2H), 7.54−7.50 (m, 1H), 7.32 (t, J = 7.7 Hz, 1H), 7.23−7.20 (m, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.89 (s, 2H), 3.75 (t, J = 5.0 Hz, 4H), 3.07 (t, J = 7.3 Hz, 2H), 2.61 (s, 3H), 2.58 (t, J = 5.0 Hz, 4H), 2.49 (t, J = 7.7 Hz, 2H), 1.93 (q, J = 7.7 Hz, 2H), 1.77−1.70 (m, 2H). Mass, m/z: 442 (M^+), 157 (base).

3-Amino-6-methyl-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17k). This compound was synthesized using the same procedure as for **17m** starting with methyl 2-amino-5-methylbenzoate. ^{1}H NMR ($CDCl_3$) δ: 8.02 (s, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.59−7.50 (m, 4H), 7.23−7.19 (m, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.90 (s, 2H), 3.75 (t, J = 5.0 Hz, 4H), 3.06 (t, J = 1.7 Hz, 2H), 2.58 (t, J = 5.0 Hz, 4H), 2.49−2.44 (m, 2H), 2.48 (s, 3H), 1.90 (q, J = 7.7 Hz, 2H), 1.72 (q, J = 7.7 Hz, 2H). Mass, m/z: 442 (M^+), 157 (base).

3-Amino-8-methyl-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3*H*-quinazolin-4-one (17l). This compound was synthesized using the same procedure as for **17m** starting with methyl 2-amino-3-methylbenzoate. ^{1}H NMR ($CDCl_3$) δ: 8.08 (dd, J = 0.8, 8.1 Hz, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.58 (ddd, J = 1.5, 6.2, 7.7 Hz, 1H), 7.52 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.32 (t, J = 7.7 Hz, 1H), 7.23−7.19 (m, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.88 (s, 2H), 3.76 (t, J = 5.0 Hz, 4H), 3.07 (t, J = 7.7 Hz, 2H), 2.59 (t, J = 5.4 Hz, 4H), 2.50 (t, J = 7.7 Hz, 2H), 1.95 (q, J = 7.3 Hz, 2H), 1.77−1.73 (m, 2H). Mass, m/z: 442 (M^+), 157 (base).

3-Amino-6-methyl-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (17n). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-amino-5-methylcyclohex-1-enecarboxylate. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.59 (dd, J = 1.2, 8.1 Hz, 1H), 7.53 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.22 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 6.98 (d, J = 9.2 Hz, 1H), 4.95 (s, 2H), 3.76 (t, J = 5.0 Hz, 4H), 2.93 (t, J = 7.7 Hz, 2H), 2.77−2.69 (m, 1H), 2.67−2.60 (m, 2H), 2.58 (t, J = 5.0 Hz, 4H), 2.45 (t, J = 7.3 Hz, 2H), 2.09−1.98 (m, 1H), 1.92−1.62 (m, 6H), 1.45−1.32 (m, 1H), 1.08 (d, J = 6.6 Hz, 3H). Mass, m/z: 446 (M^+), 157 (base).

3-Amino-7-methyl-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (17o). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-amino-4-methylcyclohex-1-enecarboxylate. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.59 (dd, J = 1.2, 8.1 Hz, 1H), 7.53 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.22 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 6.98 (d, J = 9.2 Hz, 1H), 4.95 (s, 2H), 3.76 (t, J = 5.0 Hz, 4H), 2.93 (t, J = 6.9 Hz, 2H), 2.74−2.63 (m, 2H), 2.58 (t, J = 5.0 Hz, 4H), 2.49−2.37 (m, 3H), 2.28−2.18 (m, 1H), 1.92−1.75

(m, 4H), 1.72−1.59 (m, 2H), 1.34−1.22 (m, 1H), 1.06 (d, J = 6.6 Hz, 3H). Mass, m/z: 446 (M^+), 157 (base).

3-Amino-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-5,6,7-trihydro-3*H*-cyclopenta[d]pyrimidin-4-one (17p). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-aminocyclopent-1-enecarboxylate. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 9.3 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.58 (d, J = 7.7 Hz, 1H), 7.52 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.21 (ddd, J = 1.2, 7.0, 8.1 Hz, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.97 (s, 2H), 3.75 (t, J = 5.0 Hz, 4H), 2.97 (t, J = 7.7 Hz, 2H), 2.86−2.80 (m, 4H), 2.57 (t, J = 5.0 Hz, 4H), 2.45 (t, J = 7.5 Hz, 2H), 2.08 (t, J = 7.7 Hz, 2H), 1.84−1.78 (m, 2H), 1.70−1.61 (m, 2H). Mass, m/z: 418 (M^+), 402, 157 (base).

3-Amino-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-5,6,7,8,9-pentahydro-3*H*-cyclohepta[d]pyrimidin-4-one (17q). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-aminocyclohept-1-enecarboxylate. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 9.3 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H) 7.58 (d, J = 9.1 Hz, 1H), 7.52 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 7.22−7.20 (m, 1H), 6.97 (d, J = 9.3 Hz, 1H), 5.00 (s, 2H), 3.75 (t, J = 5.0 Hz, 4H), 2.91 (t, J = 7.3 Hz, 2H), 2.75 (t, J = 8.5 Hz, 2H), 2.58 (t, J = 5.0 Hz, 4H), 2.45 (t, J = 7.3 Hz, 2H), 1.83−1.78 (m, 4H), 1.70−1.60 (m, 6H). Mass, m/z: 446 (M^+), 430, 157 (base).

3-Amino-6,6-ethylenedioxy-2-[4-(4-quinolin-2-ylpiperazin-1-yl)-butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (17r). This compound was synthesized using the same procedure as for **17m** starting with 2-amino-5,5-ethylenedioxycyclohex-1-enecarboxylic acid ethyl ester. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.59 (dd, J = 1.2, 7.7 Hz, 1H), 7.53 (ddd, J = 1.5, 6.9, 8.1 Hz, 1H), 7.22 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 6.98 (d, J = 9.2 Hz, 1H), 4.94 (s, 2H), 4.07−3.98 (m, 4H), 3.76 (t, J = 5.0 Hz, 4H), 2.94 (t, J = 7.7 Hz, 2H), 2.85 (t, J = 6.9 Hz, 2H), 2.75 (s, 2H), 2.58 (t, J = 5.0 Hz, 4H), 2.45 (t, J = 7.3 Hz, 2H), 1.96 (t, J = 6.9 Hz, 2H), 1.80 (q, J = 7.7 Hz, 2H), 1.72−1.59 (m, 2H). Mass, m/z: 490 (M^+), 157 (base).

3-Amino-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-3,4,5,6,7,8-hexahydro-4,6-dioxaquinazoline (17s). To 40 mL of 6N hydrochloric acid, 2.72 g of **17r** (5.54 mmol) was added and the reaction was heated at reflux for an hour. After cooling, the reaction mixture was neutralized with saturated aqueous sodium hydrogencarbonate solution, extracted with methylene chloride, dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (methanol:methylene chloride = 2:23) to provide 1.77 g (71%) of **17r**. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.60 (dd, J = 1.2, 8.1 Hz, 1H), 7.53 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.23 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 6.98 (d, J = 9.2 Hz, 1H), 5.01 (s, 2H), 3.76 (t, J = 5.0 Hz, 4H), 3.39 (s, 2H), 3.06−2.95 (m, 4H), 2.66 (t, J = 7.3 Hz, 2H), 2.59 (t, J = 5.0 Hz, 4H), 2.47 (t, J = 7.3 Hz, 2H), 1.89−1.79 (m, 2H), 1.74−1.64 (m, 2H). Mass, m/z: 446 (M^+), 157 (base).

3-Amino-6-hydroxy-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (17t). A solution of 1.00 g of **17s** (2.24 mmol) in 25 mL of methanol was added dropwise to an ice-cooled mixture of 500 mg of sodium borohydride (13.2 mmol) in 25 mL of methanol. The ice bath was removed, and the reaction mixture was stirred at room temperature for 3 h, then concentrated under reduced pressure. The residue was diluted with water, extracted with methylene chloride, washed with water, dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (methanol:methylene chloride = 1:9) to provide 520 mg (52%) of **17t**. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.53 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.22 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.97 (s, 2H), 4.24−4.16 (m, 1H), 3.75 (t, J = 5.0 Hz, 4H), 2.93 (t, J = 7.7 Hz, 2H), 2.88−2.76 (m, 2H), 2.69−2.49 (m, 6H), 2.45 (t, J = 7.3 Hz, 2H), 2.01−1.61 (m, 6H). Mass, m/z: 448 (M^+), 157 (base).

3-Amino-7-*tert*-butyloxycarbonyl-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-5,6,7,8-tetrahydro-3*H*-pyrido[4′,3′:4,5]thieno-[2,3-*d*]-pyrimidin-4-one (17u). This compound was synthesized using the same procedure as for **17m** starting with 2-amino-4,7-dihydro-5*H*-thieno[2,3-*c*]pyridine-3,6-dicarboxylic acid 6-*tert*-butyl ester 3-ethyl ester. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.56−7.60 (m, 1H), 7.52 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.21 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 6.97 (d, J = 9.3 Hz, 1H), 4.88 (s, 2H), 4.60 (br s, 2H), 3.68−3.78 (m, 6H), 3.00−3.12 (m, 4H), 2.55−2.60 (m, 4H), 2.46 (t, J = 7.0 Hz, 2H), 1.82−1.91 (m, 2H), 1.66−1.73 (m, 2H), 1.48 (s, 9H). Mass, m/z: 589 (M^+), 489, 445, 157 (base).

7-Acetyl-3-amino-2-[4-(4-quinolin-2-ylpiperazin-1-yl)butyl]-5,6,7,8-tetrahydro-3*H*-pyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4-one (17w). This compound was synthesized using the same procedure as for **14r** starting with compound **17u**. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.58 (dd, J = 1.1, 8.5 Hz, 1H), 7.52 (ddd, J = 1.1, 6.9, 8.5 Hz, 1H), 7.22 (ddd, J = 1.1, 6.9, 8.5 Hz, 1H), 6.97 (d, J = 9.2 Hz, 1H), 4.90 (br s, 2H), 4.79 (s, 2H), 3.72−3.80 (m, 6H), 3.13−3.18 (m, 2H), 3.04 (t, J = 7.0 Hz, 4H), 2.60 (t, J = 5.0 Hz, 4H), 2.48 (t, J = 7.0 Hz, 2H), 2.20 (s, 3H), 1.87 (q, J = 7.0 Hz, 2H), 1.70 (q, J = 7.0 Hz, 2H). Mass, m/z: 531 (M^+), 387, 157 (base).

2-[4-(4-Quinolin-2-ylpiperazin-1-yl)butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (18a). To a ice cold solution of **17m** (500 mg, 1.16 mmol) in acetic acid (5 mL), sodium nitrite (88 mg, 1.27 mmol) was added and then stirred at room temperature for 3 h. After neutralization with saturated aqueous sodium hydrogencarbonate solution, the solution was extructed with chloroform. Organic layer was dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (chloroform:methanol = 10:1) gave **18a** (338 mg, 70%). ^{1}H NMR ($CDCl_3$) δ: 12.27 (br s, 1H), 7.87 (d, J = 9.2 Hz, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.59−7.57 (m, 1H), 7.54−7.49 (m, 1H), 7.21 (ddd, J = 1.2, 6.9, 8.1 Hz, 1H), 6.96 (d, J = 9.2 Hz, 1H), 3.80−3.78 (m, 4H), 2.69−2.65 (m, 2H), 2.62−2.57 (m, 6H), 2.50−2.43 (m, 4H), 1.86−1.60 (m, 8H). Mass, m/z: 417 (M^+), 204, 157 (base).

3-Amino-2-[4-[4-(4-methylquinolin-2-yl)piperazin-1-yl]butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (18b). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-aminocyclohex-1-enecarboxylate and 2-chlorolepizine. ^{1}H NMR ($CDCl_3$) δ: 7.74 (d, J = 8.5 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.51 (ddd, J = 1.2, 7.0, 8.1 Hz, 1H), 7.22 (d, J = 8.1 Hz, 1H), 6.82 (s, 1H), 4.94 (s, 2H), 3.74 (t, J = 5.0 Hz, 4H), 2.92 (t, J = 7.3 Hz, 2H), 2.57 (s, 3H), 2.56−2.54 (m, 6H), 2.53−2.50 (m, 2H), 2.44 (t, J = 7.3 Hz, 2H), 1.82−1.62 (m, 8H). Mass, m/z: 446 (M^+), 171 (base).

3-Amino-2-[4-[4-(3-methylquinolin-2-yl)piperazin-1-yl]butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (18c). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-aminocyclohex-1-enecarboxylate and 2-chloro-3-methylquinoline. ^{1}H NMR ($CDCl_3$) δ: 7.83 (d, J = 8.5 Hz, 1H), 7.77 (s, 1H), 7.60 (d, J = 8.1 Hz, 1H), 7.52 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.31 (ddd, J = 1.1, 6.9, 8.1 Hz, 1H), 4.98 (s, 2H), 3.35−3.33 (m, 4H), 2.93 (t, J = 7.3 Hz, 2H), 2.64 (br s, 4H), 2.59 (t, J = 6.2 Hz, 2H), 2.53−2.50 (m, 2H), 2.47 (t, J = 7.3 Hz, 2H), 2.42 (d, J = 0.8 Hz, 3H), 1.83−1.65 (m, 8H). Mass, m/z: 446 (M^+), 171 (base).

3-Amino-2-[4-[4-(3,4-dimethylquinolin-2-yl)piperazin-1-yl]butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (18d). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-aminocyclohex-1-enecarboxylate and 2-chloro-3,4-dimethylquinoline. ^{1}H NMR ($CDCl_3$) δ: 7.87 (d, J = 7.3 Hz, 1H), 7.83 (d, J = 7.3 Hz, 1H), 7.53 (t, J = 8.1 Hz, 1H), 7.37−7.35 (m, 1H), 4.99 (s, 2H), 3.27 (br s, 4H), 2.93 (t, J = 7.7 Hz, 2H), 2.64 (br s, 4H), 2.59 (t, J = 5.8 Hz, 2H), 2.55 (s, 3H), 2.52 (t, J = 6.2 Hz, 2H), 2.47−2.46 (m, 2H), 2.37 (s, 3H), 1.81−1.67 (m, 8H). Mass, m/z: 460 (M^+), 185 (base).

3-Amino-2-[4-[4-(2,3-dihydro-1*H*-cyclopenta[*c*]quinolin-4-yl)-piperazin-1-yl]butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (18e).

This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-aminocyclohex-1-enecarboxylate and 4-chloro-2,3-dihydro-1*H*-cyclopenta[*c*]quinoline. ^{1}H NMR ($CDCl_3$) δ: 7.80 (d, J = 8.5 Hz, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.53−7.49 (m, 1H), 7.30−7.26 (m, 1H), 4.96 (s, 2H), 3.57 (br s, 4H), 3.17 (t, J = 7.3 Hz, 2H), 3.04 (t, J = 7.3 Hz, 2H), 2.93 (t, J = 7.7 Hz, 2H), 2.60−2.57 (m, 6H), 2.52 (t, J = 6.2 Hz, 2H), 2.47−2.44 (m, 2H), 2.22 (q, J = 7.3 Hz, 2H), 1.82−1.65 (m, 8H). Mass, m/z: 472 (M^+), 197 (base).

3-Amino-2-[4-[4-(7,8,9,10-tetrahydrophenanthridin-6-yl)piperazin-1-yl]butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (18f). This compound was synthesized using the same procedure as for **17m** starting with 6-chloro-7,8,9,10-tetrahydrophenanthridine. ^{1}H NMR ($CDCl_3$) δ: 7.84−7.80 (m, 2H), 7.53 (t, J = 8.1 Hz, 1H), 7.37−7.33 (m, 1H), 4.99 (s, 2H), 3.31 (br s, 4H), 3.10 (t, J = 6.6 Hz, 2H), 2.95−2.91 (m, 2H), 2.76 (t, J = 5.8 Hz, 2H), 2.62 (br s, 4H), 2.60−2.57 (m, 2H), 2.52 (t, J = 6.2 Hz, 2H), 2.49−2.45 (m, 2H), 1.98−1.93 (m, 2H), 1.82−1.65 (m, 10H). Mass, m/z: 486 (M^+), 211 (base).

3-Amino-2-[4-[4-(3-hydroxyquinolin-2-yl)piperazin-1-yl]butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (18g). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-aminocyclohex-1-enecarboxylate and 2-chloro-3-hydroxyquinoline. ^{1}H NMR ($CDCl_3$) δ: 7.86 (d, J = 8.9 Hz, 1H), 7.62 (d, J = 8.1 Hz, 1H), 7.47 (t, J = 6.9 Hz, 1H), 7.42 (s, 1H), 7.38−7.35 (m, 1H), 4.96 (s, 2H), 3.30 (m, 4H), 2.95−2.91 (m, 2H), 2.67 (br s, 4H), 2.59 (t, J = 6.2 Hz, 2H), 2.54−2.47 (m, 4H), 1.85−1.64 (m, 8H). Mass, m/z: 448 (M^+), 432, 289, 173 (base).

3-Amino-2-[4-[4-(6-hydroxyquinolin-2-yl)piperazin-1-yl]butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (18h). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-aminocyclohex-1-enecarboxylate and 2-chloro-6-hydroxyquinoline. ^{1}H NMR (DMSO-d_6) δ: 9.36 (br s, 1H), 7.87 (d, J = 9.2 Hz, 1H), 7.44 (d, J = 8.9 Hz, 1H), 7.15 (d, J = 9.2 Hz, 1H), 7.09 (dd, J = 2.7, 9.2 Hz, 1H), 6.96 (d, J = 2.7 Hz, 1H), 5.75 (s, 2H), 3.30 (br s, 4H), 2.85−2.81 (m, 2H), 2.60−2.30 (m, 10H), 1.76−1.54 (m, 8H). Mass, m/z: 289, 173 (base).

3-Amino-2-[4-[4-(7-hydroxyquinolin-2-yl)piperazin-1-yl]butyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (18i). This compound was synthesized using the same procedure as for **17m** starting with ethyl 2-aminocyclohex-1-enecarboxylate 2-chloro-7-hydroxyquinoline. ^{1}H NMR (DMSO-d_6) δ: 9.69 (br s, 1H), 7.86 (d, J = 9.2 Hz, 1H), 7.50 (d, J = 8.9 Hz, 1H), 6.95 (d, J = 9.2 Hz, 1H), 6.83 (d, J = 1.9 Hz, 1H), 6.76 (dd, J = 2.3, 8.5 Hz, 1H), 5.75 (s, 2H), 3.62 (br s, 4H), 2.85−2.81 (m, 2H), 2.60−2.30 (m, 10H), 1.76−1.52 (m, 8H). Mass, m/z: 432, 173 (base).

3-Amino-2-[2-(4-quinolin-2-ylpiperazin-1-yl)methyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (19a). This compound was synthesized using the same procedure as for **17m** with chloroacetyl chloride instead of 5-bromovaleryl chloride. ^{1}H NMR ($CDCl_3$) δ: 7.90 (d, J = 8.9 Hz, 1H), 7.68−7.67 (m, 1H), 7.60 (d, J = 7.7 Hz, 1H), 7.54−7.52 (m, 1H), 7.26−7.24 (m, 1H), 6.96 (d, J = 9.2 Hz, 1H), 6.40 (br s, 2H), 3.73 (br s, 6H), 3.73 (t, J = 5.0 Hz, 4H), 2.64−2.56 (m, 4H), 1.81−1.75 (m, 4H). Mass, m/z: 366 (M^+), 107 (base).

3-Amino-2-[2-(4-quinolin-2-ylpiperazin-1-yl)ethyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (19b). This compound was synthesized using the same procedure as for **17** with 3-chloropropionyl chloride instead of 5-bromovaleryl chloride. ^{1}H NMR ($CDCl_3$) δ: 7.89 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7,52 (ddd, J = 1.5, 6.9, 8.5 Hz, 1H), 7.24−7.20 (m, 1H), 6.96 (d, J = 9.2 Hz, 1H), 5.71 (s, 2H), 3.72 (t, J = 5.0 Hz, 4H), 3.14 (t, J = 6.5 Hz, 2H), 2.86 (t, J = 6.5 Hz, 2H), 2.69 (t, J = 5.0 Hz, 4H), 2.59 (t, J = 6.2 Hz, 2H), 2.53 (t, J = 6.2 Hz, 2H), 1.81−1.72 (m, 4H). Mass, m/z: 404 (M^+), 157 (base).

3-Amino-2-[3-(4-quinolin-2-ylpiperazin-1-yl)propyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (19c). This compound was synthesized using the same procedure as for **17m** with 4-chlorobutyryl chloride instead of 5-bromovaleryl chloride. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.58 (d, J = 7.7 Hz, 1H), 7.54−7.50 (m, 1H), 7.24−7.20 (m, 1H), 6.95 (d, J = 9.2 Hz, 1H), 5.57 (s, 2H), 3.70 (t, J = 5.0 Hz, 4H), 3.01−2.98 (m, 2H), 2.61−2.50 (m, 8H), 2.47−2.44 (m, 2H), 2.05 (q, J = 6.9 Hz, 2H), 1.82−1.70 (m, 4H). Mass, m/z: 418 (M^+), 157 (base).

5-Amino-2-[5-(4-quinolin-2-ylpiperazin-1-yl)pentyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (19d). This compound was synthesized using the same procedure as for **17m** with 6-bromohexanoyl chloride instead of 5-bromovaleryl chloride. ^{1}H NMR ($CDCl_3$) δ: 7.87 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7,54−7.50 (m, 1H), 7,23−7.19 (m, 1H), 6.97 (d, J = 8.9 Hz, 1H), 4.86 (s, 2H), 3.75 (t, J = 5.0 Hz, 4H), 2.89 (t, J = 7.7 Hz, 2H), 2.60−2.55 (m, 6H), 2.53−2.50 (m, 2H), 2.40 (t, J = 7.7 Hz, 2H), 1.81−1.71 (m, 6H), 1.64−1.57 (m, 2H), 1.50−1.44 (m, 2H). Mass, m/z: 446 (M^+), 157 (base).

Ethyl *cis*-4-(4-Benzylpiperazin-1-yl)cyclohexanecarboxylate (20). To the ethanol (25 mL) solution of ethyl *cis*-4-aminocyclohexane carboxylate hydrochloride (208 mg, 1.00 mmol), *N*-benzyl-bis(2-chloroethyl)amine (232 mg, 1.00 mmol), and sodium hydrogencarbonate (300 mg, 3.57 mmol) were added and the mixture was heated at reflux overnight. After concentration, the residue was purified by silica gel column chromatography (*n*-hexane:ethyl acetate = 1:2−1:8) to provide 233 mg (68%) of **20**. ^{1}H NMR ($CDCl_3$) δ: 7.34−7.21 (m, 5H), 4.12 (q, J = 7.3 Hz, 2H), 3.50 (s, 2H), 2.70−2.30 (m, H), 2.30−2.11 (m, 2H), 1.69−1.45 (m, 8H), 1.24 (t, J = 7.3 Hz, 3H). Mass, m/z: 330 (M^+), 91 (base).

Ethyl *cis*-4-(4-Quinolin-2-ylpiperazin-1-yl)cyclohexanecarboxylate (22). To the mixed solution of **20** (920 mg, 2.78 mmol) and ammonium formate (4.40 g, 69.6 mmol) in 100 mL of ethanol, palladium carbon (10%) (900 mg) was added and the mixture was heated at reflux for an hour. After filtration, the filtrate was vaporated under reduced pressure to give a mixture of deprotected piperazine derivative **21**. The residue was dissolved in 2-propanol (100 mL), and to the solution, triethylamine (6.20 g, 61.2 mmol) and 2-chloroquinoline (9.10 g, 55.6 mmol) were added. The mixture was heated at reflux overnight. After concentration, the residue was dissolved with chloroform, washed with saturated aqueous sodium hydrogencarbonate solution, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was purified by silica gel flush column chromatography (*n*-hexane:ethyl acetate = 1:5) to provide 237 mg (23%) of the *N*-substituted compound **5** derivative **22**.

***cis*-4-(4-Quinolin-2-ylpiperazin-1-yl)cyclohexanecarboxylic Acid (23).** To the solution of **22** (0.17 g, 0.46 mmol) in ethanol (1.0 mL), 1 N aqueous sodium hydroxide solution (1.0 mL) was added and the mixture was stirred at 80 °C for 30 minuites. After cooling and neutralization with 1N hydrogen chloride solution, the precipitate was collected, washed with water, and dried to give the carboxylic acid **23** (137 mg, 87%). ^{1}H NMR (DMSO-d_6) δ: 8.02 (d, J = 9.2 Hz, 1H), 7.70−7.68 (m, 1H), 7.56−7.51 (m, 2H), 7.23−7.19 (m, 2H), 3.66 (t, J = 5.0 Hz,4H), 2.60−2.50 (m, 4H), 1.99−1.96 (m, 2H), 1.56−1.47 (m, 8H). Mass, m/z: 339 (M^+), 157 (base).

2-{[*cis*-4-(4-Quinolin-2-ylpiperazin-1-yl)cyclohexanecarbonyl]-amino}cyclohex-1-enecarboxylic Acid Ethyl Ester (24). To the ice-cold mixture of ethyl 2-aminocyclohex-1-enecarboxylate (**6**) (0.15 g, 0.88 mmol) in pyridine, phosphorus trichloride (34 mg, 0.25 mmol) was added. After 15 miniuites, **23** (75 mg, 0.22 mmol) was added at room temperature to the mixture, and then was stirred at same temperature for 6 h. Chloroform was added, washed with saturated aqueous sodium hydrogencarbonate solution, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (chloroform:methanol = 20:1) to provide 150 mg (quant) of ester **24**. ^{1}H NMR ($CDCl_3$) δ: 11.72 (br s, 1H), 7.86 (d, J = 9.1 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H), 7.57 (d, J = 7.7 Hz, 1H), 7.53−7.49 (m, 1H), 7.22−7.18 (m, 1H), 6.96 (d, J = 9.2 Hz, 1H), 4.21−4.12 (m, 2H), 3.75−3.72 (m, 4H), 3.00−2.97 (m, 2H), 2.65 (t, J = 5.0 Hz, 4H), 2.50−2.45 (m, 1H), 2.32−2.26 (m, 4H), 2.19−2.12 (m, 1H), 1.83−1.75 (m, 2H), 1.70−1.45 (m, 8H), 1.31−1.25 (m, 3H). Mass, m/z: 490 (M^+), 157 (base).

2-{[*cis*-4-(4-Quinolin-2-ylpiperazin-1-yl)cyclohexanecarbonyl]-amino}cyclohex-1-enecarboxylic Acid (25). To the solution of

24 (0.10 g, 0.20 mmol) in ethanol (4.0 mL), 1N aqueous sodium hydroxide solution (4.0 mL) was added and the mixture was stirred at 60 °C for 1.5 h. After cooling and neutralization with 1N hydrogen chloride solution, the precipitate was collected, washed with water, and dried to give the carboxylic acid **25** (78 mg, 83%). ^{1}H NMR (DMSO-d_6) δ: 11.88 (br s, 1H), 8.02 (d, J = 9.2 Hz, 1H), 7.70−7.69 (m, 1H), 7.57−7.49 (m, 2H), 7.24−7.19 (m, 2H), 3.67 (br s, 4H), 2.85−2.84 (m, 2H), 2.63−2.40 (m,4H), 2.25−2.23 (m, 4H), 2.05−1.90 (m, 2H), 1.70−1.54 (m, 10H). Mass, m/z: 462(M^+), 157(base).

3-Amino-2-[*cis*-4-(4-Quinolin-2-yl-piperazin-1-yl)cyclohexyl]-5,6,7,8-tetrahydro-3*H*-quinazolin-4-one (26a) and Its Trans Isomer (26b). To the ice colded solution of **25** (70 mg, 0.15 mmol) in pyridine (4.0 mL), acetic anhydride (300 mg) was added.The mixture was warmed to room temperature and stirred for an hour. Again the mixture was cooled with ice bath, and 2-propanol (10 mL) was added to the mixtre. At the same temperature, hydrazine hydrate (2 mL) was added to the mixture. The reaction mixture was warmed to room temperature and stirred for an hour. After concentration, the residue was purified by silica gel column chromatography (chloroform:methanol = 40:1) to provide 39 mg (56%) of **26a** and 17 mg (24%) of **26b**.

***cis*-Isomer (26a).** ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.58 (dd, J = 1.2, 8.1 Hz,1H), 7.54−7.50 (m, 1H), 7.23−7.19 (m, 1H), 6.98 (d, J = 8.9 Hz, 1H), 4.83 (s, 2H), 3.77−3.74 (m, 4H), 3.44 (br s, 1H), 2.67 (br s, 4H), 2.60 (t, J = 6.2 Hz, 2H), 2.51 (t, J = 6.2 Hz, 2H), 2.35 (br s 1H), 2.11−2.05 (m, 4H), 1.80−1.58 (m, 8H). Mass, m/z: 458(M^+), 157(base).

***trans*-Isomer (26b).** ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.59−7.57 (m,1H), 7.54−7.50 (m, 1H), 7.23−7.19 (m, 1H), 6.98 (d, J = 8.9 Hz, 1H), 4.81 (s, 2H), 3.78−3.75 (m, 4H), 3.29−3.23 (m, 1H), 2.73 (t, J = 5.0 Hz 4H), 2.58−2.55 (m, 2H), 2.52−2.49 (m, 3H), 2.08−1.98 (m, 4H), 1.77−1.60 (m, 8H). Mass, m/z: 458(M^+), 157(base).

Evaluation of Biological Activity. Measurement of Compound Affinity to Human 5-HT$_{1A}$ Receptor (in Vitro).[8,12,13] A 0.25 mL (about 50 units) sample of CHO cell membrane expressing human 5-HT$_{1A}$ receptor (PerkinElmer, Inc.) was added to 24.75 mL of incubation buffer solution A (an aqueous solution of 50 mmol/l of Tris-hydrochloric acid, 10 mmol/L of magnesium sulfate, 0.5 mmol/L of EDTA, and 0.1% ascorbic acid, with the pH adjusted to 7.4 with 1 N aqueous sodium hydroxide solution at 27 °C) and was labeled as membrane sample suspension A. Each test compound was dissolved in DMSO to give a 270 μmol/L solution and was diluted to a prescribed concentration with the incubation buffer solution A to provide a compound solution. A piece of polypropylene tube was charged with 20 μL of [^{3}H]-**1** (the concentration of the [^{3}H]-**1** had been adjusted in advance to render its concentration in the reaction mixture to be 0.25 nmol/L) and 20 μL of a compound solution. A further 500 μL of membrane sample suspension A was added to the tube, followed by incubation at 27 °C for 60 min. The reaction was terminated by rapid filtration of the reaction mixture using Brandel cell harvester through a GF/C filter which had been previously immersed in a solution of 0.3% polyethyleneimine in incubation buffer solution A. The filter was twice washed with about 5 mL of 50 mmol/L of Tris-hydrochloric acid which had been cooled to 4 °C. Residual radioactivity on the filter was measured with a liquid scintillation counter (Aloka Co., LSC-5100), and percent inhibition of [^{3}H]-**1** binding to 5-HT$_{1A}$ receptor by each test compound was calculated. Buffer A or the test drugs, 20 μL, [^{3}H]-**1** solution, 20 μL, and the human 5-HT$_{1A}$ receptor membrane preparation, 500 μL, (including 1 unit) were placed in tubes and mixed to prepare reaction mixtures in duplicate or triplicate.

Measurement of Compound Affinity to Human 5-HT$_3$ Receptor (in Vitro).[8,12,13] A 0.05 mL (about 50 microassay) sample of HEK-293 cell membrane expressing human 5-HT$_3$ receptor (purchased from BIOLINKS KK) was added to 24.95 mL of incubation buffer solution B (an aqueous solution of 50 mmol/l of Tris-hydrochloric acid, 5 mmol/L of magnesium chloride, and 1 mmol/L of EDTA, with the pH adjusted to 7.5 with 1 N aq NaOH at 25 °C) and homogenized to provide membrane sample suspension B. Each test compound was dissolved in DMSO to give a 270 μmol/L solution and was diluted to a prescribed concentration with the incubation buffer solution B to provide a compound solution. A piece of polypropylene tube was charged with 20 μL of [^{3}H]BRL-43694 (the concentration of [^{3}H]BRL-43694 had been adjusted in advance to render its concentration in the reaction mixture to be 0.5 nmol/L) and 20 μL of a compound solution. A further 500 μL of membrane sample suspension B was added to the tube, followed by incubation at 25 °C for 60 min. The reaction was terminated by rapid filtration of the reaction mixture using a Brandel cell harvester through a GF/B filter, which had been previously immersed in a solution of 0.5% polyethyleneimine in incubation buffer solution B. The filter was twice washed with about 5 mL of 50 mmol/L of Tris-hydrochloric acid which had been cooled to 4 °C. Residual radioactivity on the filter was measured with a liquid scintillation counter (Aloka Co., LSC-5100), and percent inhibition of [^{3}H]BRL-43694 binding to 5-HT$_3$ receptor by each test compound was calculated. Buffer B or the test drugs, 20 μL, [^{3}H]BRL-43694 solution, 20 μL, and the human 5-HT$_3$ receptor membrane preparation, 500 μL (including 1 unit), were placed in tubes and mixed to prepare reaction mixtures in dupricate or triplicate.

5-HT$_{1A}$ Agonist-induced [^{35}S]GTPγS Binding Assays.[19] Human 5-HT$_{1A}$ receptor (Cloned Human Serotonin Receptor Subtype 1A, produced in CHO cells, PerkinElmer, Inc.) was thawed on ice and diluted with incubation buffer (20 mM HEPES/3 mM $MgCl_2$/120 mM NaCl, pH 7.4 at 30 °C). The membranes were incubated with GDP (20 μM) and the test drugs at a volume of 900 μL for 20 min at 30 °C and then were placed on ice for 15 min. [^{35}S]GTPγS (100 pM) was added to the incubation tubes to yield a final volume of 1 mL, and the tubes were further incubated for 30 min at 30 °C. Incubation was terminated by filtering the mixtures through GF/B filters using a Brandel cell harvester. The filters were washed twice with 5 mL of cold wash buffer (20 mM HEPES/3 mM $MgCl_2$, pH 7.4 at 4 °C). Radioactivity retained on the filters was counted by a liquid scintillation counter (Aloka Co., LSC-5100).

Inhibition of 5-HT$_3$ Receptor Mediated Contraction in Guinea Pig Ileum.[20] The ileum preparations were suspended in an organ bath containing Tyrode solution (137 mM NaCl/3 mM KCl/2 mM $CaCl_2$/1 mM $MgCl_2$/12 mM $NaHCO_3$/0.4 mM NaH_2PO_4/6 mM D-(+)-glucose), warmed to 37 °C and aerated with a mixture of 5% CO_2 and 95% O_2. Isotonic contractions under a loading tension of 1 g were recorded using an isotonic force transducer. Experiments were started after stable contractions induced by 10 μM 2-methyl-5-HT were obtained at least 3 times. The vehicle (DMSO) or the test drug was added to the organ bath and the preparations were exposed to the vehicle or test drug for 20 min. Then 2-methyl-5-HT (10 μM) was added to the organ bath and the contractions were recorded.

5-HT$_{1A}$ Receptor-mediated Behavior and Hypothermia in Rats. Rats were acclimated to the test environment for 2 weeks prior to testing and were conditioned to the test procedures during this period. On the day of the experiment, rats were acclimated to the test cage for 1 h. Test compound or vehicle (saline containing diluted hydrochloric acid) was injected intraperitoneally, and then 5-HT$_{1A}$ receptor-mediated behavior (lower lip retraction and flat body posture) was measured in the test cage. Behavioral responses were measured at 5, 10, 20, and 30 min after administration using a 0−3 scale as previously described in the literature.[21] The rectal temperature was recorded before and at 30 min after administration of the test drug using a thermistoprobe that was inserted into the rectum 3 cm from the anal orifice. The difference between the temperatures measured before and after administration was designated as the index of hypothermia.

5-HT-induced Bradycardia (von Bezold−Jarisch Reflex) in Rats. The surgical procedures and mean heart rate recordings were performed as follows: animals were anesthetized with urethane at a dose of 1.25 g/kg, ip, and then polyethylene cannulas were inserted into the right common carotid artery and vein to measure the blood pressure and to administer 5-HT, respectively. The duodenum was incised, decorticated 2−3 cm from the stomach and cannulated for intraduodenal administration of the test drugs. Blood pressure was monitored using a pressure amplifier (AP-601G; Nihon Kohden Co., Tokyo), and the mean heart rate was recorded by a tachometer (AT-601G; Nihon Kohden Co., Tokyo) triggered by blood pressure pulsation. 5-HT was injected intravenously at 300 μg/kg to evoke a transient bradycardia (B-J reflex). Following recovery to normal blood pressure and heart rate, the test drugs and vehicle (0.5% Tween 80) were administered into the duodenum. Thirty minutes later, 5-HT was readministered intravenously and the bradycardia was assessed.

Supporting Information Available: IC_{50} values of **17m** for receptors and transporters. This material is available free of charge via the Internet at http://pubs.acs.org.

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