Synthesis and Evaluation of Structurally Constrained Quinazolinone Derivatives as Potent and Selective Histamine H_3 Receptor Inverse Agonists

Tsuyoshi Nagase, Takashi Mizutani, Etsuko Sekino, Shiho Ishikawa, Sayaka Ito, Yuko Mitobe, Yasuhisa Miyamoto, Ryo Yoshimoto, Takeshi Tanaka, Akane Ishihara, Norihiro Takenaga, Shigeru Tokita, and Nagaaki Sato*

Tsukuba Research Institute, Merck Research Laboratories, Banyu Pharmaceutical Co., Ltd., Okubo 3, Tsukuba 300-2611, Japan

Received May 15, 2008

A series of structurally constrained derivatives of the potent H_3 inverse agonist **1** was designed, synthesized, and evaluated as histamine H_3 receptor inverse agonists. As a result, the *N*-cyclobutylpiperidin-4-yloxy group as in **2f** was identified as an optimal surrogate structure for the flexible 1-pyrrolidinopropoxy group of **1**. Subsequent optimization of the quinazolinone core of **2f** revealed that substitution at the 5-position of the quinazolinone ring influences potency. Representative derivatives **5a** and **5s** showed improved potency in a histamine release assay in rats and a receptor occupancy assay in mice.

Introduction

Histamine plays a variety of physiological roles in the CNS[a] and peripheral tissues. In the CNS, histaminergic neurons are exclusively localized in the tuberomammillary nucleus of the hypothalamus but project widely throughout the CNS.[1] There are four known G protein-coupled receptors for histamine: H_1, H_2, H_3, and H_4.[2] The histamine receptors have unique signal transduction pathways and distribution, which has led to the discovery of a variety of physiological roles for histamine. Of these receptors, H_3 is predominantly expressed in the CNS, while H_1 and H_2 are expressed in both central and peripheral tissues.[3,4] The H_4 receptor is predominantly expressed in inflammatory cells, suggesting its critical role in the regulation of inflammatory and immune responses.[5]

The H_3 receptor was discovered pharmacologically in 1983[6] and genetically identified in 1999.[3] The genetic identification of the H_3 receptor gained much attention and redirected both the detailed pharmacological characterization of the receptor and efforts from academia to the pharmaceutical industry to find drugs that bind specifically to H_3.[7] Signaling through the H_3 receptor activates G proteins that inhibit adenylate cyclase activity and reduce intracellular cAMP levels.[3,8] In the CNS, the H_3 receptor is localized on the presynaptic membrane as an autoreceptor and negatively regulates the release and synthesis of histamine.[6] In addition, the H_3 receptor is known to modulate the release of other neurotransmitters such as norepinephrine, dopamine, acetylcholine, serotonin, and GABA.[9] The H_3 receptor signals constitutively, which serves to tonically suppress target neuronal activities such as histamine release to baseline levels.[10] Agonist-induced signaling that occurs in the presence of elevated histamine levels further suppresses histamine release. While classical antagonists would interfere with histamine-mediated negative feedback, H_3 receptor inverse agonists have been demonstrated to decrease constitutive H_3 signaling, thus blocking the tonic inhibition of histamine release and further potentiating histaminergic effects. Because of the effects of H_3 signaling on multiple neuronal transmitters, it has been suggested that H_3 antagonists/inverse agonists could be effective therapeutics for several CNS-related disorders.[11] In animal models, H_3 receptor antagonists/inverse agonists have been shown to enhance wakefulness and attentive and cognitive behavior while reducing feeding and body weight.[12,13] Moreover, very recently it has been reported that **25** (BF2.649) (Figure 1), a potent and selective H_3 receptor inverse agonist, suppressed the excessive daytime sleep of narcoleptic patients.[14]

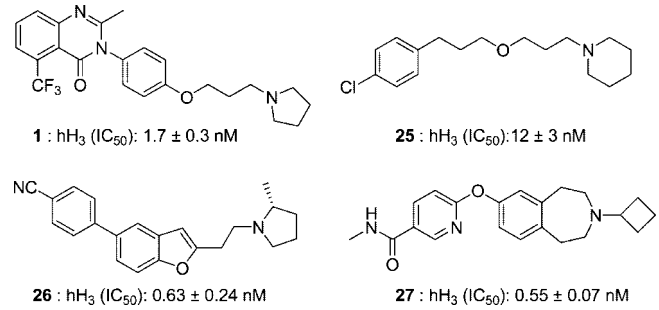


Figure 1. Structures and antagonistic activities of nonimidazole H_3 antagonists and inverse agonists. Antagonistic activities were determined by inhibition of *R*-α-methylhistamine-induced binding of [^{35}S]GTPγS at human H_3 receptor. The values represent the mean ± SE for $n \geq 3$.

First-generation imidazole-based H_3 antagonists have inhibitory activity on cytochrome P_{450} enzymes, which may result in drug−drug interactions with coadministered drugs by reducing hepatic clearance.[15] Current efforts have focused on nonimidazole classes of H_3 receptor antagonists/inverse agonists with the potential to reduce these liabilities. Since the identification of the H_3 receptor genes, various classes of nonimidazole H_3 receptor antagonists have been developed to target H_3 receptors in the CNS.[7,12,16] Several of them, including **25**,[14,17] **26** (ABT-239),[18] and **27** (GSK189254)[19] (Figure 1), have entered clinical trials to evaluate their effectiveness in treating CNS disorders such as excessive daytime sleepiness, schizophrenia, and cognitive dysfunction.

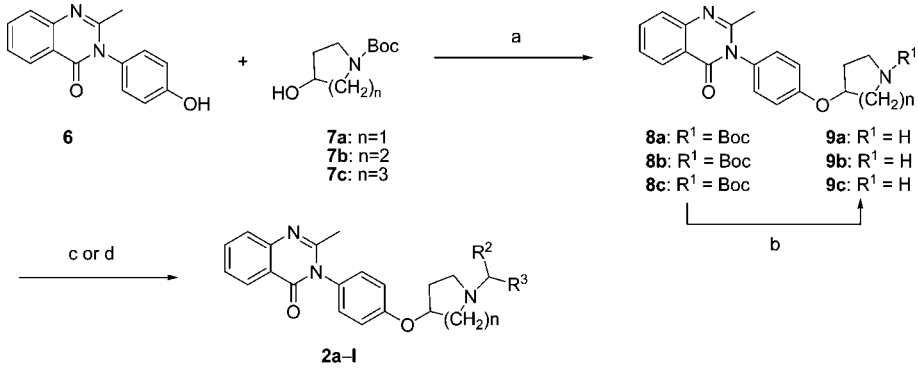
Starting from the previously reported potent H_3 inverse agonist **1** (Figure 1),[20] a series of structurally constrained analogues were prepared and evaluated, aimed at improving its potency. This approach was previously demonstrated by the

* To whom correspondence should be addressed. Phone: +81-29-877-2004. Fax: +81-29-877-2029. E-mail: nagaaki_sato@merck.com.

[a] Abbreviations: P-gp, P-glycoprotein; SD rat, Sprague−Dawley rat; SAR, structure−activity relationship; hERG, human ether-a-go-go-related gene; HEK, human embryonic kidney; CNS, central nervous system; Ts, 4-toluenesulfonyl; Ms, methanesulfonyl; DIAD, diisopropyl azodicarboxylate; DEAD, diethyl azodicarboxylate.

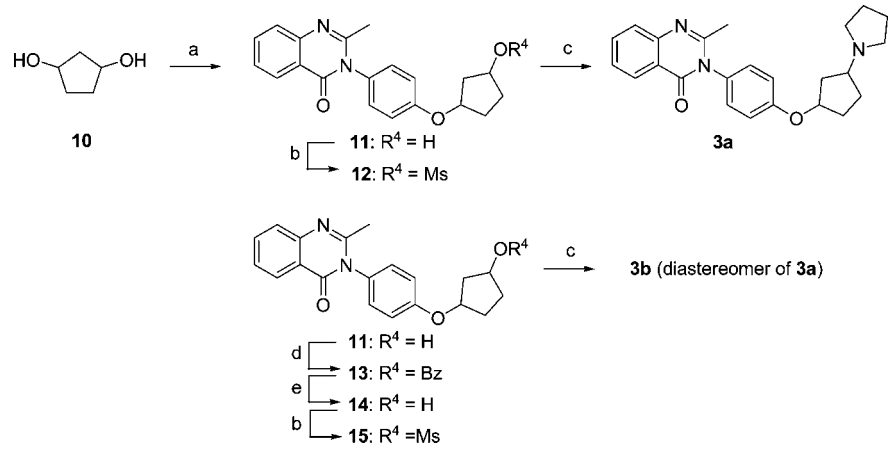
10.1021/jm800569w CCC: $40.75
Published on Web 10/08/2008

Scheme 1[a]



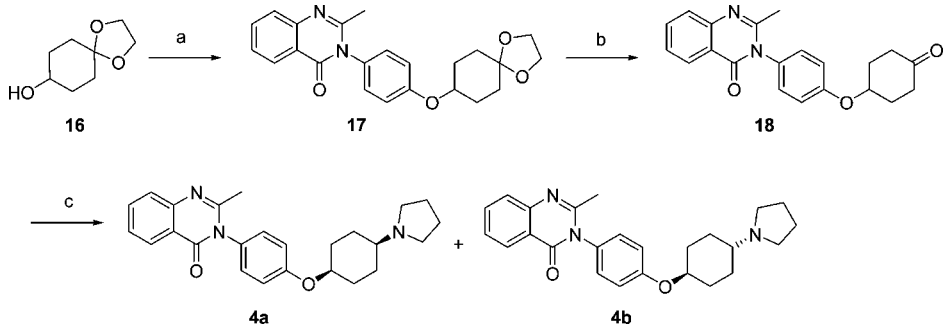
[a] Reagents: (a) DEAD, PPh_3, THF; (b) trifluoroacetic acid, $CHCl_3$; (c) R^2R^3CHI, K_2CO_3, DMF, 60 °C; (d) R^2R^3CO, $ZnCl_2$, $NaBH_3CN$, MeOH.

Scheme 2[a]



[a] Reagents: (a) **6**, DIAD, PPh_3, THF; (b) MsCl, Et_3N, CH_2Cl_2; (c) pyrrolidine, K_2CO_3, DMF, 80 °C; (d) DIAD, PPh_3, $PhCO_2H$, THF; (e) K_2CO_3, MeOH.

Scheme 3[a]



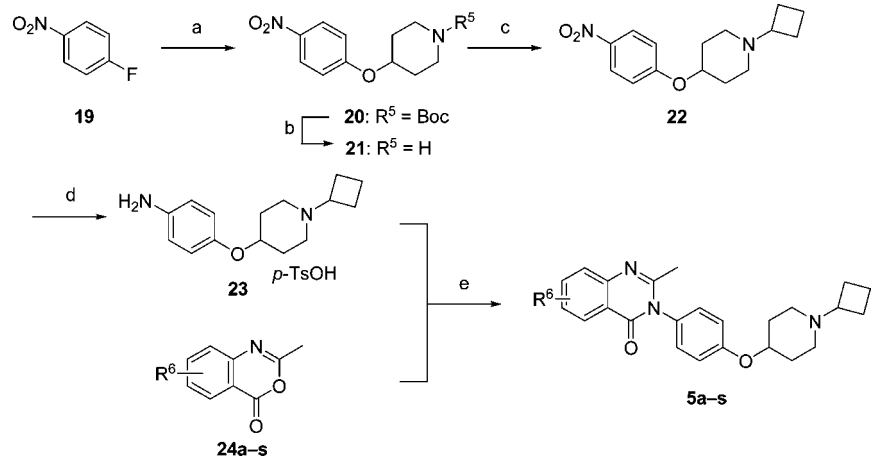
[a] Reagents: (a) **6**, DIAD, PPh_3, THF; (b) 10% HCl aq, THF; (c) pyrrolidine, $ZnCl_2$, $NaBH_3CN$, MeOH.

Johnson & Johnson group, in which an *N*-alkylpiperidin-4-yloxy group was systematically investigated as a structurally constrained replacement for the amimopropoxy group and an *N*-isopropylpiperidin-4-yloxy group was successfully identified.[21] In the present SAR study, additional novel structurally constrained tethers were designed and synthesized to replace the flexible aminopropoxy group of **1**, and the *N*-cyclobutylpiperidin-4-yloxy group[22] as in **2f** was identified as a useful surrogate structure. The quinazolinone core of **2f** was subsequently optimized to find potent analogues **5a**−**s**. Compounds **5a** and **5s** were profiled in vivo and shown to have good pharmacokinetic profiles and potent efficacy. In this report, the design, synthesis, SAR of the structurally constrained quinazolinone class of H_3 inverse agonists, and in vivo evaluation of the potent derivatives **5** are described.

Chemistry

The synthetic route for the derivatives reported herein is described in Schemes 1−4. Scheme 1 outlines the synthesis of compounds **9b** and **2a**−**l**. Known compounds **6**[23] and **7** were coupled by the Mitsunobu reaction[24] to give ethers **8**, followed by deprotection of the *tert*-butoxycarbonyl group to furnish amines **9**. The amino group of compounds **9** was alkylated with the desired iodoalkane (method c) or reductively alkylated with the desired ketones in the presence of sodium cyanoborohydride and zinc chloride (method d) to furnish **2a**−**l**. Preparation of

Scheme 4[a]



[a] Reagents: (a) **7b**, NaH, DMF; (b) trifluoroacetic acid, $CHCl_3$; (c) cyclobutanone, $ZnCl_2$, $NaBH_3CN$, MeOH; (d) (i) H_2, 10% Pd/C, MeOH, (ii) $TsOH \cdot H_2O$; (e) AcOH, rt.

analogue **3a** and its corresponding diastereomer **3b** is illustrated in Scheme 2. Cyclopentane-1,3-diol (**10**) and compound **6** were coupled to give alcohol **11** as a single diastereomer in 99% yield. Mesylation of the hydroxy group of **11** was followed by the displacement with pyrrolidine to furnish amine **3a** in good yield. The corresponding diastereomer **3b** was prepared by the inversion of the hydroxy group configuration in compound **11** by the Mitsunobu protocol. The benzoate group of product **13** was cleaved, and the resulting hydroxy group was mesylated and displaced with pyrrolidine to give diastereomer **3b** in good yield. The relative stereochemistry of diastereomers **3a** and **3b** has not yet been established. The preparation of compound **4** is shown in Scheme 3. Coupling reaction between 4,4-ethylenedioxycyclohexanol (**16**) and **6**, followed by hydrolysis of the ketal under acidic conditions, afforded the ketone **18**, which was reductively aminated with pyrrolidine to give amines **4a** and **4b** after the separation of the diastereomeric mixture. The quinazolinone ring-modified derivatives were conveniently prepared as depicted in Scheme 4. Compound **7b** was coupled with 4-fluoronitrobenzene (**19**) in the presence of sodium hydride followed by deprotection of the *tert*-butoxycarbonyl group to afford the amine **21**. Reductive alkylation of piperidine **21** with cyclobutanone followed by reduction of the nitro group of intermediate **22** gave the aniline **23**, which was conveniently isolated as a tosylate salt. Finally, the amino group of compound **23** was reacted with the desired substituted- or aza-benzoxazinones **24a**−**s**[20,25] to furnish the derivatives **5a**−**s**.

Results and Discussion

The compounds were tested using the [^{35}S]GTPγS binding assay in membranes isolated from cells transfected with cloned H_3 receptors.[20] All the quinazolinone derivatives reported herein reduced the basal GTPγS binding, indicating that they are inverse agonists. Selected compounds were evaluated for hERG K^+ channel inhibitory activity in the [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl]methanesulfonamide binding assay to assess cardiac QTc prolongation liability.[26] We recently discovered the quinazolinone class of histamine H_3 receptor inverse agonists and identified the clinical development candidate **1** (Figure 1).[20] The essential pharmacophore of the quinazolinone derivatives is the aminopropoxyphenyl portion that is a common structural motif recognized by a number of research groups. We envisioned that the potency of compound **1** (hH_3, IC_{50} = 1.7 nM) (Figure 1) might be further improved by imparting structural constraints to the flexible propoxy moiety. In addition to the previously studied *N*-alkylpiperidin-4-yloxy group,[21] novel structurally constrained tethers were designed to have a cyclic structure in the aminopropoxy region, conserving the three-carbon tether between the oxygen and nitrogen atoms of the aminopropoxy moiety (Table 1).

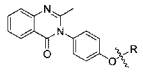
First, we prepared a variety of *N*-substituted piperidinyloxy derivatives **2a**−**h**. *N*-Substitution is essential for activity because the nonsubstituted derivative **9b** is devoid of potency. The ethyl derivative **2b** displayed the highest activity (IC_{50} = 49 nM) among the unbranched alkyl derivatives **2a**−**d**. Further extension of the alkyl chain was not attempted due to the decreased binding affinities of analogues **2c** and **2d**. The isopropyl derivative **2e** was 3-fold more potent than the ethyl derivative **2b**. The cyclobutyl derivative **2f** (IC_{50} = 1.5 nM) showed a 10-fold improvement over **2e**, while ring expansion as in analogues **2g** and **2h** resulted in decreases in potency.

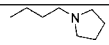
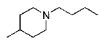
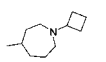
Second, the piperidine was replaced by pyrrolidine and azacycloheptane (**2i**−**l**), which affects the direction of the *N*-alkyl substituent. The cyclobutyl and cyclopentylpyrrolidine derivatives (**2i** and **2j**) displayed significantly decreased activities. The azacycloheptane derivatives **2k** and **2l** were somewhat less potent than the corresponding piperidine derivatives **2f** and **2g**. Further ring expansion was not attempted because undesirable hERG activity was observed for compounds **2k** and **2l**, possibly attributed to an increase in lipophilicity for those analogues.

Finally, the nitrogen atom was incorporated in the distal ring as in cycloalkyl analogues **3** and **4**. The cyclopentyl derivative **3a** showed a potent H_3 activity (IC_{50} = 3.4 nM) with a satisfactory hERG activity. The corresponding diastereomer **3b** was less potent. The cyclohexyl derivatives **4a** and **4b** were prepared even though they have a four-carbon tether. The *trans*-isomer **4a** showed good activity despite its four-carbon tether, and the corresponding *cis*-isomer **4b** was significantly less potent than **4a**. In this structurally constrained design, the cyclobutylpiperidinyloxy structure **2f** was identified as a potent substructure. It should be noted that racemic **3a** has the potential for additional improvement by modification of the size of the two rings.

Further optimization of **2f** by the modification of the quinazolinone group is summarized in Table 2. The fluorine-substituted derivatives **5a**−**e** displayed improved activities

Table 1. In Vitro Profiles of Compounds **1**, **2**−**4**, and **9b**[a]

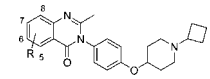


compd	R	human H_3[b] (IC_{50}, nM)	hERG[c] (IC_{50}, μM)
1		1.7 ± 0.3	>10
9b		>1000	*d*
2a		740 ± 68	*d*
2b		49 ± 9	*d*
2c		60 ± 12	*d*
2d		91 ± 11	*d*
2e		14 ± 2	*d*
2f		1.5 ± 0.3	>10
2g		3.2 ± 0.3	>10
2h		43 ± 11	*d*
2i		103 ± 29	*d*
2j		37 ± 9	*d*
2k		10 ± 1	7.3 ± 0.9
2l		7.0 ± 1.5	6.8 ± 0.8
3a		3.4 ± 0.4	>10
3b (diastreomer of **3a**)		8.6 ± 0.1	10 ± 0.7
4a		10 ± 1	8.0 ± 0.5
4b		813 ± 99	*d*

[a] The values represent the mean ± SE for $n \geq 3$. [b] Inhibition of *R*-α-methylhistamine-induced binding of [^{35}S]GTPγS at human H_3 receptor. [c] Inhibition of [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl-]methanesulfonamide binding to hERG in HEK293 cells. [d] Not determined.

compared to the parent **2f**. The 5-fluoro **5a** (IC_{50} = 0.55 nM) was the most potent derivative. In the chloro derivatives **5f**−**i**, the 5-chloro derivative **5f** was 4-fold more potent than the parent **2f**, while chloro substitution at the other positions (**5g**−**i**) was

Table 2. In Vitro Potency of Compounds **5a**−**s**[a]



compd	R	human H_3[b] (IC_{50}, nM)	hERG (IC_{50}, μM)[c]
2f	H	1.5 ± 0.3	>10
5a	5-F	0.55 ± 0.04	>10
5b	6-F	1.2 ± 0.1	>10
5c	7-F	1.3 ± 0.1	>10
5d	8-F	0.84 ± 0.12	>10
5e	6,7-diF	0.91 ± 0.06	>10
5f	5-Cl	0.34 ± 0.14	>10
5g	6-Cl	1.5 ± 0.2	5.4 ± 0.2
5h	7-Cl	3.0 ± 0.5	3.9 ± 0.3
5i	8-Cl	1.7 ± 0.1	3.3 ± 0.8
5j	5-OCH_3	1.2 ± 0.3	>10
5k	6-OCH_3	1.5 ± 0.1	>10
5l	7-OCH_3	1.8 ± 0.4	>10
5m	8-OCH_3	4.2 ± 1.0	>10
5n	5-Aza	14 ± 1	>10
5o	6-Aza	2.0 ± 0.4	>10
5p	7-Aza	1.8 ± 0.2	>10
5q	8-Aza	1.6 ± 0.2	>10
5r	5-CH_3	0.27 ± 0.08	>10
5s	5-CF_3	0.33 ± 0.05	>10

[a] The values represent the mean ± SE for $n \geq 3$. [b] Inhibition of *R*-α-methylhistamine-induced binding of [^{35}S]GTPγS at human H_3 receptor. [c] Inhibition of [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl-]methanesulfonamide binding to hERG in HEK293 cells.

Table 3. Pharmacokinetic Parameters of **5a**, **5r**, and **5s**[a]

compd	species	IV Cl_p (mL/min/kg)	PO $AUC_{0-\infty}$ (μM·h)	C_{max} (μM)	*F* (%)
5a	rat	11	6.2	1.3	53
	dog	44	0.05	0.02	4.8
	monkey	27	1.7	0.38	37
5r	rat	25	2.33	0.56	40
	dog	17	5.6	1.1	76
	monkey	17	0.08	0.02	1
5s	rat	24	1.66	0.42	33
	dog	13	1.3	0.28	46
	monkey	26	0.15	0.05	11

[a] The reported data are an average generated after 1 mg/kg iv and 3 mg/kg po doses in $n = 3$ animals/dose for rats and 0.3 mg/kg iv and 1 mg/kg po doses in $n = 3$ animals/dose for dogs and monkeys.

not effective in improving potency. The derivatives with an electron-donating methoxy substituent (**5j**−**m**) did not show noticeable improvement. Regarding the aza derivatives, the 5-aza derivative **5n** showed a significant decrease in potency while the other aza derivatives **5o**−**q** were tolerated. Encouraged by the improved potency demonstrated by the 5-chloro and fluoro derivatives (**5a** and **5f**), 5-methyl and 5-trifluoromethyl derivatives **5r** and **5s** were prepared and found to possess 5-fold more potent activities than **2f**. This enhancement of potency by the 5-substitution of the quinazolinone ring is specific to these *N*-cyclobutylpiperidinyloxy derivatives and was not observed in the flexible propoxy derivatives.[20] Compounds **5a**, **5r**, and **5s** were demonstrated to reduce basal GTPγS binding with EC_{50} values of 1.1, 0.34, and 0.71 nM, respectively, indicating that these three compounds are inverse agonists.

The representative potent 5-substituted derivatives **5a**, **5r**, and **5s** were evaluated in pharmacokinetic studies (Table 3). All three compounds showed suitable pharmacokinetic profiles for in vivo evaluation in SD rats. The systemic exposure of compound **5a** in rats was much higher ($AUC_{0-\infty}$ = 6.2 μM·h, C_{max} = 1.3 μM) than those of compounds **5r** and **5s**. The pharmacokinetic profile of compound **5a** in dogs, however, was very poor, which

Table 4. Brain Penetration and P-gp Susceptibility of **5a**, **5r**, and **5s**

	brain penetration in SD rats[a]					P-gp susceptibility[b]	
				ratio		transcellular transport ratio (B-to-A)/(A-to-B)	
compd	plasma (μM)	brain (nmol/g)	CSF (μM)	brain/plasma	CSF/brain	mouse *mdr1a*	human *MDR1*
5a	2.4	7.6	0.95	3.2	0.13	1.8	1.0
5r	1.4	3.2	0.10	2.3	0.03	1.0	0.9
5s	0.7	2.3	0.11	3.3	0.05	2.5	0.7

[a] The concentrations were determined at 2 h after 10 mg/kg oral administration. The values represent the mean for $n = 3$ animals. [b] Transcellular transport ratio ((B-to-A)/(A-to-B)) in human *MDR1*- and mouse *mdr1a*-transfected LLC-PK1 cell line. The values represent the mean for $n = 3$. The value above 3.0 indicates that a compound is a P-gp substrate.

Table 5. Binding Affinities of Compounds **5a**, **5r**, and **5s** for the Human, Rat, and Mouse H_3 Receptors[a]

	H_3 binding affinity (Ki, nM)[b]		
compd	human	rat	mouse
5a	6.8 ± 1.9	6.0 ± 0.7	8.4 ± 1.0
5r	1.7 ± 0.4	1.8 ± 0.1	3.9 ± 0.6
5s	3.6 ± 0.7	2.3 ± 0.1	3.9 ± 0.5

[a] The values represent the mean $\pm$ SE for $n \geq 3$. [b] Displacement of [^{3}H]*N*-α-methylhistamine binding to cell membranes expressing recombinant H_3 receptors.

is most likely due to the significantly higher clearance value. Compounds **5r** and **5s** showed good systemic exposure and oral bioavailability in dogs. In contrast, the pharmacokinetic profiles of **5s** and **5r** in monkeys were poor, whereas **5a** exhibited a fair profile in monkeys. It should be noted that the clearance value of **5a** in monkeys is the highest ($Cl_p = 27$ mL/min/kg) among the three derivatives, so the poor pharmacokinetic profiles of **5r** and **5s** may be caused by monkey-specific absorption problems.

The compounds were studied for their ability to penetrate the CNS using several assays. The brain and cerebrospinal fluid (CSF) levels in SD rats 2 h following 10 mg/kg oral administration of **5a**, **5r**, and **5s** were examined (Table 4). All the derivatives displayed good brain and CSF exposure. P-gp susceptibility was evaluated[28] as a pivotal factor for brain penetration, and none of them were found to be substrates for human P-gp (MDR1) (Table 4). Compounds **5a** and **5s** might be very weak substrates for mouse P-gp (mdr1a); see further discussion below regarding mouse P-gp susceptibility. Compounds **5a** and **5s** were confirmed to have excellent selectivity with respect to other histamine receptor subtypes ($IC_{50} > 10$ μM for hH_1, hH_2, and hH_4) and to a panel of 115 diverse, unrelated binding sites ($IC_{50} > 1$ μM for all the binding sites tested). The binding affinities of compounds **5a**, **5r**, and **5r** for the human, rat, and mouse H_3 receptors are summarized in Table 5. The compounds showed potent binding affinities, and no significant species difference in binding affinity was observed.

Having demonstrated excellent potency, selectivity, pharmacokinetic profile, and brain penetration, **5a** and **5s** were tested for brain histamine release in SD rats. In our histamine release assay,[29] the test compound (po) and pargyline (ip), a monoamine oxidase inhibitor, were codosed in SD rats. After 2 h, the whole brain was rapidly removed, and the concentration of *tele*-methylhistamine, a major extracellular metabolite of histamine, was measured. Significant and dose-proportional elevation of *tele*-methylhistamine was observed in the rat brain after oral administration of **5a** and **5s** (Figure 2).

Brain histamine H_3 receptor occupancy was evaluated by an ex vivo receptor occupancy method for correlation with the observed histamine elevation. The brain H_3 receptor was shown to be highly occupied (>90%) 2 h following oral administration of 1 mg/kg of **5a** and **5s**. A high degree of receptor occupancy seems to be necessary for the H_3 inverse agonists to exhibit significant elevation of brain histamine in rats.[30]

As a first step in estimating efficacious plasma levels in humans, we decided to study receptor occupancy of **5a** and **5s** in mice in detail. As shown in Table 4, **5a** and **5s** are not human P-gp substrates but may be weak mouse P-gp substrates. Hence, brain exposure and receptor occupancy studies in P-gp-deficient *mdr1a* (−/−) and wild type *mdr1a* (+/+) CF-1 mice[31] were carried out. The plasma and brain concentrations were determined 2 h after oral administration of 0.3 mg/kg of **5a** and 1 mg/kg of **5s** in *mdr1a* (−/−) and *mdr1a* (+/+) CF-1 mice (Table 6). The comparison of the brain-to-plasma ratios in *mdr1a* (−/−) and *mdr1a* (+/+) CF-1 mice clearly suggests that P-gp-mediated efflux has a considerable influence on brain penetration of **5a** and **5s** in CF-1 mice. The brain-to-plasma ratios for **5a** and **5s** in *mdr1a* (−/−) CF-1 mice were 9.4 and 13, which are about 5-fold higher than those in *mdr1a* (+/+) CF-1 mice (1.9 for **5a** and 2.6 for **5s**).

Next, the receptor occupancy−brain concentration relationships were studied in CF-1 mice (Figure 3). The brain concentrations that are required to achieve 90% receptor occupancy (brain Occ90) for **5a** and **5s** were determined to be 0.28 and 0.15 nmol/g, respectively. The plasma Occ90 values were estimated from the brain Occ90 values and the brain-to-plasma ratios (plasma Occ90 = brain Occ90/brain-to-plasma ratio), and the results are shown in Table 6. The estimated plasma Occ90 values in Table 6 suggest that the plasma Occ90 values in humans might be as low as 30 nM for **5a** and 11 nM for **5s**. On the basis of the human P-gp transcellular transport ratios for **5a** and **5s**, it is assumed that the effect of P-gp-mediated efflux is negligible in humans. Investigation of receptor occupancy in other higher species by noninvasive receptor occupancy determination methods such as the positron emission tomography is necessary to confirm these predictions. Examination of the relationship between the degree of receptor occupancy and efficacy in animal models using **5a** and **5s** is in progress.

Summary

A series of structurally constrained analogues of potent H_3 inverse agonist **1** were designed, synthesized, and evaluated as histamine H_3 receptor inverse agonists. As a result, the *N*-cyclobutylpiperidin-4-yloxy group as in compound **2f** was identified as an optimal surrogate structure for the flexible 3-(1-pyrrolidinyl)propoxy group of lead compound **1**. Subsequent optimization of the quinazolinone **2f** revealed that substitution at the 5-position of the quinazolinone ring markedly enhances in vitro potency. Representative analogues **5a** and **5s** had excellent selectivity with respect to other histamine receptor subtypes and to 115 diverse unrelated binding sites. Compounds **5a** and **5s** showed satisfactory pharmacokinetic profiles and brain penetrability in the preclinical animals. Two hours following oral administration of **5a** and **5s** in SD rats, a dose-proportional and statistically significant increase of brain histamine levels

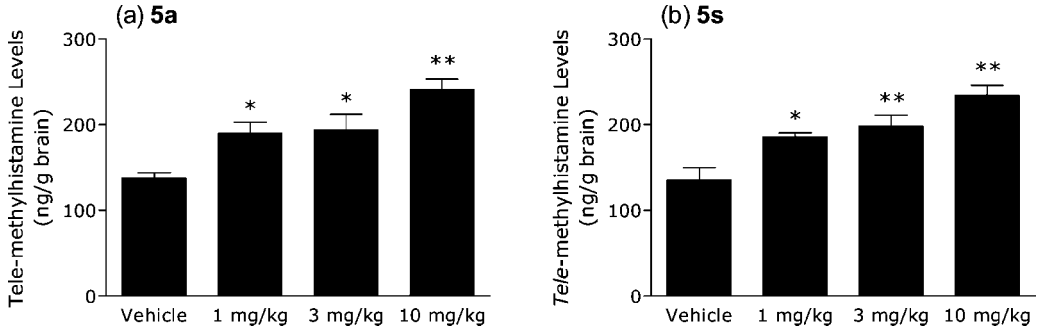


Figure 2. Brain *tele*-methylhistamine levels in SD rats after oral administration of **5a** and **5s**. Values are means ± SE, determined from five experiments. * $P < 0.05$ and ** $P < 0.01$ (ANOVA Dunnett) compared with the vehicle control.

Table 6. Brain Penetration and Plasma Occ90 Values of **5a** and **5s** in P-gp-deficient *mdr1a* (−/−) and Wild Type *mdr1a* (+/+) CF-1 Mice[a]

compd		plasma[b] (μM)	brain[b] (nmol/g)	brain/ plasma[c]	plasma Occ90[d] (nM)
5a	*mdr1a* (+/+)	0.032 ± 0.002	0.062 ± 0.002	1.9	144
	mdr1a (−/−)	0.028 ± 0.001	0.260 ± 0.039	9.4	30
5s	*mdr1a* (+/+)	0.012 ± 0.001	0.031 ± 0.002	2.6	58
	mdr1a (−/−)	0.010 ± 0.002	0.137 ± 0.014	13	11

[a] The brain and plasma concentrations were obtained 2 h following oral administration of **5a** (0.3 mg/kg) and **5s** (1 mg/kg) in mice. [b] The values represent the mean ± SE for $n = 3$. [c] The ratios were obtained from the mean values. [d] Plasma Occ90 was calculated by brain Occ90/brain to plasma ratio. The brain Occ90 values for **5a** and **5s** are 0.28 nmol/g and 0.15 nmol/g, respectively (Figure 3).

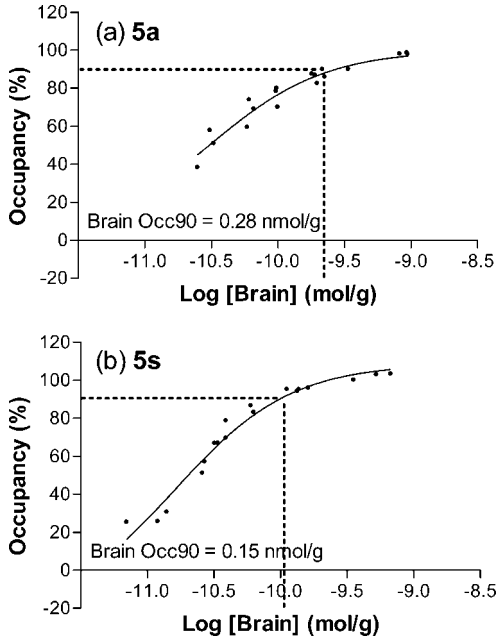


Figure 3. The brain concentration−receptor occupancy relationship of (a) **5a** and (b) **5s** in CF-1 mice. Receptor occupancy and exposure were determined 2 h following oral administration of vehicle or compound **5a** or **5s** (0.1, 0.3, 1, and 3 mg/kg). See Supporting Information for experimental details.

was observed, indicating that the brain H_3 receptors were highly occupied (>90%). The plasma Occ90 for **5s** in humans was estimated to be as low as 11 nM based on P-gp susceptibility and the receptor occupancy studies in P-gp-deficient *mdr1a* (−/−) and wild type *mdr1a* (+/+) CF-1 mice. The potential cardiovascular effects of compound **5s** were evaluated in anesthetized and ventilated dogs.[27] At 3 mg/kg iv dosing (C_{max} = 3.2 μM), no adverse treatment-related cardiovascular effects were observed. Regarding gross behavior in mice, oral administration of compound **5s** at a dose of 100 mg/kg caused no treatment-related changes in psychomotor activities, motor activities, muscle tone, CNS excitation, autonomic responses, and reflexes.[27] Further profiling of **5a** and **5s** is in progress to select an improved clinical candidate from this class, and updated results will be reported in due course.

Experimental Section

Chemistry. General Procedures. Unless otherwise noted, all solvents, chemicals, and reagents were obtained commercially and used without purification. The ^{1}H NMR spectra were obtained at 400 MHz on a MERCURY-400 (Varian) or JMN-AL400 (JEOL) spectrometer, with chemical shift (δ, ppm) reported relative to TMS as an internal standard. Mass spectra were recorded with electron-spray ionization (ESI) or atmospheric pressure chemical ionization (APCI) on a Waters micromass ZQ, micromass Quattro II, or micromass Q-Tof-2 instrument. Flash chromatography was carried out with prepacked silica gel columns (KP-Sil silica) from Biotage. Preparative thin-layer chromatography was performed on a TLC Silica gel 60 F (Merck KGaA). Preparative HPLC purification was carried out on a YMC-Pack *Pro* C18 (YMC, 50 mm × 30 mm id), eluting with a gradient of CH_3CN/0.1% aqueous CF_3CO_2H at a flow rate of 40 mL/min. Purity of target compounds was determined by HPLC with the two different eluting methods as follows. Analytical HPLC was performed on a SPELCO Ascentis Express (4.6 mm × 150 mm id), eluting with a gradient of (A) 0.1% H_3PO_4/CH_3CN = 95/5 to 10/90 over 7 min followed by 10/90 isocratic over 1 min and (B) 10 mM potassium phosphate buffer (pH 6.6)/CH_3CN = 95/5 to 20/80 over 7 min followed by 20/80 isocratic over 1 min (detection at 210 nm). High resolution mass spectra were recorded with electron-spray ionization on a micromass Q-Tof-2 instrument.

General Procedure for the Preparation of 2a–e via Alkylation. 3-(4-[(1-Ethyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (2b). To a mixture of **9b** (50 mg, 0.15 mmol) and potassium carbonate (42 mg, 0.30 mmol) in dry DMF (1 mL) was added ethyl iodide (23 mg, 0.15 mmol) under a nitrogen atmosphere, and the mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between ethyl acetate and 1 N NaOH. The layers were separated, and the aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with 1 N NaOH and brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography with 3% $CHCl_3$ in methanol to give 2b as a colorless solid (25 mg, 46%); HPLC purity (99.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (3H, J = 6.8 Hz), 1.83−1.95 (2H, m), 2.02−2.10 (2H, m), 2.26 (3H, s), 2.26−2.39 (2H, m), 2.45 (2H, q, J = 7.2 Hz), 2.72−2.81 (2H, m), 4.35−4.43 (1H, m), 7.03 (2H, d, J = 8.8 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.44 (1H, t, J = 8.0 Hz), 7.65 (1H, d, J = 8.0 Hz), 7.74 (1H, t, J = 8.0 Hz), 8.25 (1H, d, J = 8.0 Hz); MS (ESI) m/z 364 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{22}H_{26}N_3O_2$, 364.2025; found, 364.2020.

2-Methyl-3-(4-[(1-methyl-4-piperidinyl)oxy]phenyl)-4(3*H*)-quinazolinone (2a). Compound **2a** was prepared from methyl iodide and **9b** using the procedure described for **2b** as a colorless solid (13% yield); HPLC purity (95.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91−1.99 (2H, br m), 2.06−2.18 (2H, br m), 2.27 (3H, s), 2.36−2.51 (2H, br m), 2.39 (3H, s), 2.74−2.82 (2H, br m), 4.40−4.46 (1H, br m), 7.05 (2H, d, J = 8.8 Hz), 7.16 (2H, d, J = 8.8 Hz), 7.47 (1H, t, J = 7.8 Hz), 7.67 (1H, d, J = 7.8 Hz), 7.77 (1H, td, J = 7.8, 1.5 Hz), 8.27 (1H, dd, J = 7.8, 1.5 Hz). MS (ESI) m/z 350 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{21}H_{24}N_3O_2$, 350.1869; found, 350.1865.

2-Methyl-3-(4-[(1-propyl-4-piperidinyl)oxy]phenyl)-4(3*H*)-quinazolinone (2c). Compound **2c** was prepared from *n*-propyl iodide and **9b** using the procedure described for **2b** as a colorless solid (48% yield); HPLC purity (95.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ; 0.93 (3H, t, J = 7.3 Hz), 1.55−1.69 (2H, br m), 1.88−1.97 (2H, br m), 2.06−2.18 (2H, br m), 2.27 (3H, s), 2.31−2.49 (4H, br m), 2.76−2.85 (2H, br m), 4.37−4.46 (1H, br m), 7.04 (2H, d, J = 8.8 Hz), 7.15 (2H, d, J = 8.8 Hz), 7.47 (1H, td, J = 7.8, 1.5 Hz), 7.67 (1H, d, J = 7.8 Hz), 7.77 (1H, td, J = 7.8, 1.5 Hz), 8.27 (1H, dd, J = 7.8, 1.5 Hz). MS (ESI) m/z 378 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{23}H_{28}N_3O_2$, 378.2168; found, 378.2182.

3-(4-[(1-Butyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (2d). Compound **2d** was prepared from *n*-butyl iodide and **9b** using the procedure described for **2b** as a colorless solid (43% yield); HPLC purity (99.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (3H, t, J = 7.2 Hz), 1.28−1.39 (2H, m), 1.46−1.54 (2H, m), 1.83−1.93 (2H, m), 2.01−2.10 (2H, m), 2.26 (3H, s), 2.26−2.39 (4H, m), 2.72−2.81 (2H, m), 4.33−4.41 (1H, m), 7.03 (2H, d, J = 8.8 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.44 (1H, t, J = 8.0 Hz), 7.65 (1H, d, J = 8.0 Hz), 7.74 (1H, t, J = 8.0 Hz), 8.25 (1H, d, J = 8.0 Hz); MS (ESI) m/z 392 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{30}N_3O_2$, 392.2338; found, 392.2337.

3-(4-[(1-Isopropyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (2e). Compound **2e** was prepared from *i*-propyl iodide and **9b** using the procedure described for **2b** as a colorless solid (36% yield); HPLC purity (98.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (6H, d, J = 6.4 Hz), 1.82−1.92 (2H, m), 2.02−2.11 (2H, m), 2.26 (3H, s), 2.37−2.46 (2H, m), 2.72−2.84 (3H, m), 4.31−4.40 (1H, m), 7.03 (2H, d, J = 8.8 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.44 (1H, t, J = 8.0 Hz), 7.65 (1H, d, J = 8.0 Hz), 7.74 (1H, t, J = 8.0 Hz), 8.25 (1H, d, J = 8.0 Hz). MS (ESI) m/z 378 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{23}H_{28}N_3O_2$, 378.2182; found, 378.2186.

Representative Procedure for the Preparation of 2f−l via Reductive Amination. 3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (2f). To a stirred solution of **9b** (370 mg, 1.10 mmol) and cyclobutanone (155 mg, 2.20 mmol) in methanol (10 mL) was added a solution of zinc chloride (409 mg, 3.00 mmol) and sodium cyanoborohydride (189 mg, 3.00 mmol) in methanol (6 mL) at room temperature, and the mixture was stirred at room temperature for 1 h. The resulting mixture was concentrated, and the residue was partitioned between ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with water, dried over sodium sulfate, and concentrated. The residue was purified by preparative HPLC to provide **2f** as a colorless solid (165 mg, 39%); HPLC purity (99.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.76 (2H, m), 1.84−1.95 (4H, m), 1.99−2.10 (4H, m), 2.14−2.23 (2H, m), 2.26 (3H, s), 2.59−2.67 (2H, m), 2.70−2.79 (1H, m), 4.33−4.41 (1H, m), 7.01 (2H, d, J = 8.8 Hz), 7.44 (1H, t, J = 8.0 Hz), 7.65 (1H, d, J = 8.4 Hz), 7.74 (1H, t, J = 7.6 Hz), 8.24 (1H, d, J = 7.6 Hz). MS (ESI) m/z 390 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{28}N_3O_2$, 390.2182; found, 390.2185.

3-(4-[(1-Cyclopentyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (2g). Compound **2g** was prepared from cyclopentanone and **9b** using the procedure described for **2f** as a colorless solid (42% yield); HPLC purity (95.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25−1.48 (2H, m), 1.52−1.61 (2H, m), 1.66−1.73 (2H, m), 1.83−1.93 (4H, m), 2.01−2.12 (2H, m), 2.25 (3H, s), 2.32−2.40 (2H, m), 2.49−2.58 (1H, m), 2.79−2.95 (2H, m), 4.33−4.41 (1H, m), 7.03 (2H, d, J = 8.8 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.44 (1H, t, J = 7.2 Hz), 7.65 (1H, d, J = 7.6 Hz), 7.74 (1H, t, J = 7.2 Hz), 8.25 (1H, d, J = 7.6 Hz). MS (ESI) m/z 418 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{26}H_{32}N_3O_2$, 418.2495; found, 418.2505.

3-(4-[(1-Cyclohexyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (2h). Compound **2h** was prepared from cyclohexanone and **9b** using the procedure described for **2f** as a colorless solid (48% yield); HPLC purity (99.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18−1.30 (6H, m), 1.78−1.90 (6H, m), 2.01−2.08 (2H, m), 2.26 (3H, s), 2.27−2.36 (1H, m), 2.42−2.51 (2H, m), 2.81−2.89 (2H, m), 4.30−4.38 (1H, m), 7.03 (2H, d, J = 8.8 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.44 (1H, t, J = 8.0 Hz), 7.65 (1H, d, J = 8.0 Hz), 7.74 (1H, t, J = 8.0 Hz), 8.25 (1H, d, J = 8.0 Hz). MS (ESI) m/z 418 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{26}H_{32}N_3O_2$, 418.2495; found, 418.2505.

3-(4-[(1-Cyclobutyl-3-pyrrolidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (2i). Compound **2i** was prepared from cyclobutanone and **9a** using the procedure described for **2f** as a beige solid (21% yield); HPLC purity (97.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68−1.78 (2H, m), 1.94−2.07 (5H, m), 2.25 (3H, s), 2.30−2.35 (1H, m), 2.46−2.52 (1H, m), 2.71−2.76 (2H, m), 2.86−2.91 (1H, m), 2.93−3.00 (1H, m), 4.83−4.87 (1H, m), 6.96 (2H, d, J = 8.8 Hz), 7.12 (2H, d, J = 8.8 Hz), 7.44 (1H, t, J = 8.1 Hz), 7.64 (1H, d, J = 8.1 Hz), 7.74 (1H, t, J = 8.1 Hz), 8.24 (1H, d, J = 8.1 Hz). MS (ESI) m/z 376 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{23}H_{26}N_3O_2$, 376.2025; found, 376.2019.

3-(4-[(1-Cyclopentyl-3-pyrrolidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (2j). Compound **2j** was prepared from cyclopentanone and **9a** using the procedure described for **2f** as a colorless oil (18% yield); HPLC purity (97.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45−1.57 (4H, m), 1.66−1.88 (4H, m), 1.98−2.05 (1H, m), 2.25 (3H, s), 2.31−2.36 (1H, m), 2.46−2.52 (1H, m), 2.52−2.61 (1H, m), 2.79−2.84 (2H, m), 3.00−2.97 (1H, m), 4.82−4.87 (1H, m), 6.96 (2H, d, J = 8.8 Hz), 7.12 (2H, d, J = 8.8 Hz), 7.44 (1H, t, J = 8.1 Hz), 7.64 (1H, d, J = 8.1 Hz), 7.74 (1H, t, J = 8.1 Hz), 8.24 (1H, d, J = 8.1 Hz). MS (ESI) m/z 390 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{28}N_3O_2$, 390.2182; found, 390.2185.

3-(4-[(1-Cyclobutyl-4-azepanyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (2k). Compound **2k** was prepared from cyclobutanone and **9c** using the procedure described for **2f** as a pale-yellow oil (28% yield); HPLC purity (99.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57−2.18 (12H, m), 2.24 (3H, s), 2.39−2.50 (2H, m), 2.53−2.62 (2H, m), 2.85−2.95 (1H, m), 4.54−4.61 (1H, m), 6.96 (2H, d, J = 8.8 Hz), 7.10 (2H, d, J = 8.8 Hz), 7.43 (1H, t, J = 8.0 Hz), 7.63 (1H, d, J = 7.2 Hz), 7.72 (1H, t, J = 7.2 Hz), 8.23 (1H, d, J = 8.0 Hz). MS (ESI) m/z 404 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{25}H_{30}N_3O_2$, 404.2338; found, 404.2346.

3-(4-[(1-Cyclopentyl-4-azepanyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (2l). Compound **2l** was prepared from cyclopentanone and **9c** using the procedure described for **2f** as a colorless oil (39% yield); HPLC purity (96.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55−2.22 (14H, br m), 2.26 (3H, s), 2.38−2.49 (1H, br m), 2.89−3.25 (4H, br m), 4.69−4.72 (1H, br m), 7.01 (2H, d, J = 9.3 Hz), 7.16 (2H, d, J = 8.8 Hz), 7.45−7.49 (1H, m), 7.67 (1H, d, J = 7.8 Hz), 7.75−7.79 (1H, m), 8.27 (1H, dd, J = 8.0, 1.2 Hz). MS (ESI) m/z 418 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{26}H_{32}N_3O_2$, 418.2495; found, 418.2491.

2-Methyl-3-[4-([3-(1-pyrrolidinyl)cyclopentyl]oxy)phenyl]-4(3*H*)-quinazolinone (3a). A mixture of **12** (550 mg, 1.33 mmol), pyrrolidine (474 mg, 6.7 mmol), and potassium carbonate (277 mg, 2.0 mmol) in dry DMF (10 mL) was stirred at 80 °C for 14 h under a nitrogen atmosphere. The resulting mixture was poured into water and extracted with ethyl acetate three times. The combined organic extracts were washed with water, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography with 5% methanol in $CHCl_3$ to give **3a** as a colorless solid (143 mg, 28%); HPLC purity (99.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57−1.67 (1H, m), 1.79−1.82 (4H, m), 1.85−1.92 (2H, m), 2.03−2.08 (1H, m), 2.14−2.19 (1H, m), 2.22−2.30 (1H, m), 2.24 (3H, s), 2.53−2.56 (4H, br m), 2.76−2.83 (1H, m), 4.81−4.86 (1H, m), 6.96 (2H, d, J = 8.8 Hz), 7.11 (2H,

d, $J = 8.8$ Hz), 7.44 (1H, t, $J = 8.1$ Hz), 7.66 (1H, d, $J = 8.1$ Hz), 7.73 (1H, t, $J = 8.1$ Hz), 8.23 (1H, d, $J = 8.1$ Hz). MS (ESI) *m/z* 390 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{28}N_3O_2$, 390.2182; found, 390.2166.

2-Methyl-3-[4-([3-(1-pyrrolidinyl)cyclopentyl]oxy)phenyl]-4(3*H*)-quinazolinone (3b, Diastereomer of 3a). Compound **3b** was prepared from **15** and pyrrolidine using the procedure described for **3a** as a colorless solid (47% yield); HPLC purity (98.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.77−1.96 (9H, m), 2.01−2.07 (1H, m), 2.26 (3H, s), 2.43−2.50 (1H, m), 2.56−2.61 (4H, br m), 4.80−4.74 (1H, m), 6.96−7.01 (2H, m), 7.13 (2H, d, $J = 8.8$ Hz), 7.44−7.48 (1H, m), 7.67 (1H, d, $J = 8.3$ Hz), 7.74−7.78 (1H, m), 8.27 (1H, dd, $J = 8.0$, 1.2 Hz). MS (ESI) *m/z* 390 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{28}N_3O_2$, 390.2182; found, 390.2179.

***cis*-2-Methyl-3-(4-([4-(1-pyrrolidinyl)cyclohexyl]oxy)phenyl)-4(3*H*)-quinazolinone (4a) and *trans*-2-Methyl-3-(4-([4-(1-pyrrolidinyl)cyclohexyl]oxy)phenyl)-4(3*H*)-quinazolinone (4b).** Compounds **4a** (*cis*-isomer) and **4b** (*trans*-isomer) were synthesized from **18** and pyrrolidine using a similar procedure described for **2f**. Purification by silica gel column chromatography with 20% → 50% ethyl acetate in hexanes followed by preparative HPLC afforded **4a** as a colorless solid (260 mg, 46%) and **4b** as a colorless solid (180 mg, 32%). The stereochemistry of each isomer was determined by NOE experiments. For *cis*-isomer (**4a**), HPLC purity (96.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40−1.66 (4H, m), 1.77−1.81 (4H, m), 2.04−2.10 (3H, m), 2.12−2.20 (2H, m), 2.25 (3H, s), 2.60−2.63 (4H, br s), 4.19−4.26 (1H, m), 7.00 (2H, d, $J = 8.8$ Hz), 7.11 (2H, d, $J = 8.8$ Hz), 7.44 (1H, t, $J = 8.1$ Hz), 7.65 (1H, d, $J = 8.1$ Hz), 7.74 (1H, t, $J = 8.1$ Hz), 8.25 (1H, d, $J = 8.1$ Hz). MS (ESI) *m/z* 404 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{25}H_{30}N_3O_2$, 404.2338; found, 404.2351. For *trans*-isomer (**4b**), HPLC purity (98.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59−1.83 (10H, br m), 2.10−2.15 (3H, m), 2.26 (3H, s), 2.62 (4H, br s), 4.53−4.56 (1H, br m), 7.03 (2H, d, $J = 9.3$ Hz), 7.13 (2H, d, $J = 8.8$ Hz), 7.44−7.48 (1H, m), 7.67 (1H, d, $J = 7.8$ Hz), 7.74−7.78 (1H, m), 8.27 (1H, dd, $J = 8.0$, 1.2 Hz). MS (ESI) *m/z* 404 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{25}H_{30}N_3O_2$, 404.2338; found, 404.2343.

Representative Procedure for the Preparation of 5a−s. 3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-5-(trifluoromethyl)-4(3*H*)-quinazolinone (5s). A mixture of **23** tosylate (20.0 g, 47.8 mmol), 2-methyl-5-(trifluoromethyl)-4*H*-3,1-benzoxazin-4-one (**24s**; 10.95 g, 47.8 mmol) in acetic acid (110 mL) was stirred at room temperature for 30 h. The resulting mixture was concentrated, and the residue was partitioned between ethyl acetate and 2 N NaOH. The layers were separated, and the aqueous layer was extracted with ethyl acetate twice. The combined organic extracts were washed with 2 N NaOH and brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel chromatography with 0% → 2% methanol in $CHCl_3$ followed by recrystallization from ethyl acetate to afford **5s** as a colorless solid (13.7 g, 63%); HPLC purity (99.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.77 (2H, m), 1.82−1.96 (4H, m), 1.98−2.09 (4H, m), 2.13−2.23 (2H, m), 2.26 (3H, s), 2.58−2.66 (2H, m), 2.70−2.79 (1H, m), 4.33−4.40 (1H, m), 7.01 (2H, d, $J = 8.8$ Hz), 7.12 (2H, d, $J = 8.8$ Hz), 7.77 (1H, d, $J = 8.0$ Hz), 7.82−7.88 (2H, m). MS (ESI) *m/z* 458 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{25}H_{27}N_3O_2F_3$, 458.2055; found, 458.2045.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-5-fluoro-2-methyl-4(3*H*)-quinazolinone (5a). Compound **5a** was prepared from 5-fluoro-2-methyl-4*H*-3,1-benzoxazin-4-one (**24a**) and **23** tosylate using the procedure described for **5s** as a colorless solid (50% yield); HPLC purity (99.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.75 (2H, m), 1.83−1.93 (4H, m), 2.00−2.11 (4H, m), 2.13−2.24 (2H, m), 2.24 (3H, s), 2.60−2.68 (2H, m), 2.72−2.79 (1H, m), 4.36−4.41 (1H, m), 7.04 (2H, d, $J = 8.8$ Hz), 7.08−7.14 (3H, m), 7.46 (1H, d, $J = 8.0$ Hz), 7.65−7.71 (1H, m). MS (ESI) *m/z* 408 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{27}N_3O_2F$, 408.2087; found, 408.2101.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-6-fluoro-2-methyl-4(3*H*)-quinazolinone (5b). Compound **5b**was prepared from 6-fluoro-2-methyl-4*H*-3,1-benzoxazin-4-one (**24b**) and **23** tosylate using the procedure described for **5s** as a colorless solid (48% yield); HPLC purity (97.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.75 (2H, m), 1.84−1.92 (4H, m), 2.01−2.12 (4H, m), 2.16−2.24 (2H, m), 2.25 (3H, s), 2.61−2.70 (2H, m), 2.72−2.81 (1H, m), 4.36−4.42 (1H, m), 7.04 (2H, d, $J = 8.8$ Hz), 7.14 (2H, d, $J = 8.8$ Hz), 7.45−7.51 (1H, m), 7.68 (1H, dd, $J = 4.8$, 8.8 Hz), 7.89 (1H, dd, $J = 3.2$, 8.0 Hz). MS (ESI) *m/z* 408 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{27}N_3O_2F$, 408.2087; found, 408.2094.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-7-fluoro-2-methyl-4(3*H*)-quinazolinone (5c). Compound **5c** was prepared from 7-fluoro-2-methyl-4*H*-3,1-benzoxazin-4-one (**24c**) and **23** tosylate using the procedure described for **5s** as a colorless solid (72% yield); HPLC purity (99.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.75 (2H, m), 1.82−1.96 (4H, m), 2.00−2.11 (4H, m), 2.14−2.24 (2H, m), 2.25 (3H, s), 2.60−2.68 (2H, m), 2.72−2.79 (1H, m), 4.36−4.41 (1H, m), 7.04 (2H, d, $J = 8.4$ Hz), 7.12−7.20 (3H, m), 7.31 (1H, dd, $J = 2.0$,9.6 Hz), 8.27 (1H, dd, $J = 6.0$, 8.4 Hz). MS (ESI) *m/z* 408 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{27}N_3O_2F$, 408.2087; found, 408.2090.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-8-fluoro-2-methyl-4(3*H*)-quinazolinone (5d). Compound **5d** was prepared from 8-fluoro-2-methyl-4*H*-3,1-benzoxazin-4-one (**24d**) and **23** tosylate using the procedure described for **5s** as a colorless solid (60% yield); HPLC purity (99.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66−1.75 (2H, m), 1.84−1.95 (4H, m), 2.02−2.11 (4H, m), 2.17−2.28 (2H, m), 2.31 (3H, s), 2.61−2.68 (2H, m), 2.75−2.80 (1H, m), 4.37−4.43 (1H, m), 7.05 (2H, d, $J = 9.3$ Hz), 7.14 (2H, d, $J = 9.3$ Hz), 7.42−7.37 (1H, m), 7.47−7.52 (1H, m), 8.05 (1H, d, $J = 7.8$ Hz). MS (ESI) *m/z* 408 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{27}N_3O_2F$, 408.2087; found, 408.2106.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-6,7-difluoro-2-methyl-4(3*H*)-quinazolinone (5e). Compound **5e** was prepared from 6,7-difluoro-2-methyl-4*H*-3,1-benzoxazin-4-one (**24e**) and **23** tosylate using the procedure described for **5s** as a colorless solid (67% yield); HPLC purity (99.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66−1.75 (2H, m), 1.83−1.95 (4H, m), 2.00−2.11 (4H, m), 2.16−2.24 (2H, m), 2.24 (3H, s), 2.60−2.68 (2H, m), 2.72−2.79 (1H, m), 4.35−4.42 (1H, m), 7.04 (2H, d, $J = 8.4$ Hz), 7.13 (2H, d, $J = 8.4$ Hz), 7.44 (1H, dd, $J = 6.8$, 10.4 Hz), 8.01 (1H, dd, $J = 8.4$, 9.6 Hz). MS (ESI) *m/z* 426 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{26}N_3O_2F_2$, 426.1993; found, 426.1998.

5-Chloro-3-(4-[(1-cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (5f). Compound **5f** was prepared from 5-chloro-2-methyl-4*H*-3,1-benzoxazin-4-one (**24f**) and **23** tosylate using the procedure described for **5s** as a colorless solid (53% yield); HPLC purity (99.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.77 (2H, m), 1.82−1.96 (4H, m), 1.99−2.09 (4H, m), 2.13−2.23 (2H, m), 2.23 (3H, s), 2.58−2.66 (2H, m), 2.69−2.79 (1H, m), 4.33−4.40 (1H, m), 7.00 (2H, d, $J = 8.8$ Hz), 7.12 (2H, d, $J = 8.8$ Hz), 7.43 (1H, dd, $J = 1.6$, 7.2 Hz), 7.52−7.61 (2H, m). MS (ESI) *m/z* 424 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{27}N_3O_2Cl$, 424.1792; found, 424.1785.

6-Chloro-3-(4-[(1-cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (5g). Compound **5g** was prepared from 6-chloro-2-methyl-4*H*-3,1-benzoxazin-4-one (**24g**) and **23** tosylate using the procedure described for **5s** as a colorless solid (20% yield); HPLC purity (98.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66−1.79 (2H, m), 1.83−1.99 (4H, m), 2.00−2.12 (4H, m), 2.15−2.30 (5H, m), 2.59−2.71 (2H, m), 2.72−2.84 (1H, m), 4.40 (1H, br s), 7.04 (2H, d, $J = 8.8$ Hz), 7.13 (2H, d, $J = 8.8$ Hz), 7.61 (1H, d, $J = 8.8$ Hz), 7.69 (1H, dd, $J = 8.8$, 2.4 Hz), 8.22 (1H, d, $J = 2.4$ Hz). MS (ESI) *m/z* 424 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{27}N_3O_2Cl$, 424.1792; found, 424.1794.

7-Chloro-3-(4-[(1-cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (5h). Compound **5h** was prepared from 7-chloro-2-methyl-4*H*-3,1-benzoxazin-4-one (**24h**) and **23** tosylate using the procedure described for **5s** as a colorless solid (61% yield); HPLC purity (99.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.74

(2H, m), 1.84−1.93 (4H, m), 2.01−2.08 (4H, m), 2.15−2.22 (2H, m), 2.25 (3H, s), 2.60−2.67 (2H, m), 2.71−2.79 (1H, m), 4.35−4.40 (1H, m), 7.04 (2H, d, J = 9.3 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.41 (1H, dd, J = 8.5, 2.2 Hz), 7.66 (1H, d, J = 2.0 Hz), 8.19 (1H, d, J = 8.8 Hz). MS (ESI) m/z 424 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{27}N_3O_2Cl$, 424.1792; found, 424.1803.

8-Chloro-3-(4-[(1-cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (5i). Compound **5i** was prepared from 8-chloro-2-methyl-4*H*-3,1-benzoxazin-4-one (**24i**) and **23** tosylate using the procedure described for **5s** as a colorless solid (69% yield); HPLC purity (99.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.76 (2H, m), 1.85−1.93 (4H, m), 2.01−2.08 (4H, m), 2.15−2.22 (2H, m), 2.33 (3H, s), 2.60−2.67 (2H, m), 2.71−2.79 (1H, m), 4.41−4.36 (1H, m), 7.04 (2H, d, J = 8.8 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.38 (1H, t, J = 8.0 Hz), 7.84 (1H, dd, J = 7.8, 1.5 Hz), 8.19 (1H, dd, J = 8.3, 1.5 Hz). MS (ESI) m/z 424 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{24}H_{27}N_3O_2Cl$, 424.1792; found, 424.1786.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-5-methoxy-2-methyl-4(3*H*)-quinazolinone (5j). Compound **5j** was prepared from 5-methoxy-2-methyl-4*H*-3,1-benzoxazin-4-one (**24j**) and **23** tosylate using the procedure described for **5s** as a beige solid (82% yield); HPLC purity (99.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.77 (2H, m), 1.82−1.96 (4H, m), 1.98−2.09 (4H, m), 2.12−2.23 (2H, m), 2.21 (3H, s), 2.58−2.67 (2H, m), 2.69−2.79 (1H, m), 3.94 (3H, s), 4.33−4.40 (1H, m), 6.85 (1H, d, J = 8.8 Hz), 6.99 (2H, d, J = 8.8 Hz), 7.08 (2H, d, J = 8.8 Hz), 7.21 (1H, d, J = 8.0 Hz), 7.62 (1H, t, J = 8.0 Hz). MS (ESI) m/z 420 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{25}H_{30}N_3O_3$, 420.2287; found, 420.2289.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-6-methoxy-2-methyl-4(3*H*)-quinazolinone (5k). Compound **5k** was prepared from 6-methoxy-2-methyl-4*H*-3,1-benzoxazin-4-one (**24k**) and **23** tosylate using the procedure described for **5s** as a colorless solid (69% yield); HPLC purity (99.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64−1.74 (2H, m), 1.85−1.96 (4H, m), 2.01−2.13 (4H, m), 2.15−2.24 (2H, m), 2.23 (3H, s), 2.60−2.72 (2H, m), 2.72−2.81 (1H, m), 3.91 (3H, s), 4.35−4.42 (1H, brs), 7.04 (2H, d, J = 6.8 Hz), 7.13 (2H, d, J = 6.8 Hz), 7.36 (1H, dd, J = 3.2, 9.2 Hz), 7.61 (1H, d, J = 9.2 Hz), 7.63 (1H, d, J = 3.2 Hz). MS (ESI) m/z 420 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{25}H_{30}N_3O_3$, 420.2287; found, 420.2302.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-7-methoxy-2-methyl-4(3*H*)-quinazolinone (5l). Compound **5l** was prepared from 7-methoxy-2-methyl-4*H*-3,1-benzoxazin-4-one (**24l**) and **23** tosylate using the procedure described for **5s** as a pale-yellow solid (68% yield); HPLC purity (99.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65−1.76 (2H, m), 1.83−1.95 (4H, m), 2.00−2.09 (4H, m), 2.15−2.23 (2H, m), 2.24 (3H, s), 2.60−2.68 (2H, m), 2.71−2.79 (1H, m), 3.93 (3H, s), 4.35−4.41 (1H, m), 7.06−7.01 (4H, m), 7.14 (2H, d, J = 9.3 Hz), 8.16 (1H, d, J = 8.8 Hz). MS (ESI) m/z 420 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{25}H_{30}N_3O_3$, 420.2287; found, 420.2294.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-8-methoxy-2-methyl-4(3*H*)-quinazolinone (5m). Compound **5m** was prepared from 8-methoxy-2-methyl-4*H*-3,1-benzoxazin-4-one (**24m**) and **23** tosylate using the procedure described for **5s** as a beige solid (87% yield); HPLC purity (98.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67−1.74 (2H, m), 1.84−1.93 (4H, m), 2.00−2.09 (4H, m), 2.15−2.22 (2H, m), 2.32 (3H, s), 2.61−2.66 (2H, m), 2.71−2.79 (1H, m), 4.04 (3H, s), 4.36−4.41 (1H, m), 7.03 (2H, d, J = 8.8 Hz), 7.14 (2H, d, J = 8.8 Hz), 7.22 (1H, dd, J = 8.0, 1.2 Hz), 7.40 (1H, t, J = 8.0 Hz), 7.85 (1H, dd, J = 8.0, 1.2 Hz). MS (ESI) m/z 420 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{25}H_{30}N_3O_3$, 420.2287; found, 420.2295.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methylpyrido[3,2-*d*]pyrimidin-4(3*H*)-one (5n). Compound **5n** was prepared from 2-methyl-4*H*-pyrido[3,2-*d*][1,3]oxazin-4-one (**24n**) and **23** tosylate using the procedure described for **5s** as a pale-yellow solid (49% yield); HPLC purity (95.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67−1.74 (2H, m), 1.96−1.84 (4H, m), 2.03−2.10 (4H, m), 2.17−2.25 (2H, m), 2.27 (3H, s), 2.59−2.71 (2H, m), 2.73−2.79 (1H, m), 4.36−4.42 (1H, m), 7.05 (2H, td, J = 2.0, 8.8 Hz), 7.17 (2H, td, J = 2.0, 8.8 Hz), 7.67 (1H, dd, J = 8.3, 4.4 Hz), 8.00 (1H, dd, J = 8.3, 1.5 Hz), 8.84 (1H, dd, J = 4.4, 1.5 Hz). MS (ESI) m/z 391 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{23}H_{27}N_4O_2$, 391.2134; found, 391.2144.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methylpyrido[4,3-*d*]pyrimidin-4(3*H*)-one (5o). Compound **5o** was prepared from 2-methyl-4*H*-pyrido[4,3-*d*][1,3]oxazin-4-one (**24o**) and **23** tosylate using the procedure described for **5s** as a pale-yellow solid (27% yield); HPLC purity (97.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.77 (2H, m), 1.82−1.96 (4H, m), 1.99−2.11 (4H, m), 2.14−2.24 (2H, m), 2.29 (3H, s), 2.60−2.68 (2H, m), 2.70−2.80 (1H, m), 4.33−4.41 (1H, m), 7.03 (2H, d, J = 8.8 Hz), 7.11 (2H, d, J = 8.8 Hz), 7.47 (1H, d, J = 5.2 Hz), 8.82 (1H, d, J = 5.2 Hz), 9.45 (1H, s). MS (ESI) m/z 391 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{23}H_{27}N_4O_2$, 391.2134; found, 391.2122.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one (5p). Compound **5p** was prepared from 2-methyl-4*H*-pyrido[3,4-*d*][1,3]oxazin-4-one (**24p**) and **23** tosylate using the procedure described for **5s** as a colorless solid (11% yield); HPLC purity (98.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.77 (2H, m), 1.82−1.95 (4H, m), 1.99−2.11 (4H, m), 2.15−2.23 (2H, m), 2.29 (3H, s), 2.60−2.69 (2H, m), 2.73−2.83 (1H, m), 4.36−4.43 (1H, m), 7.03 (2H, d, J = 8.8 Hz), 7.12 (2H, d, J = 8.8 Hz), 8.01 (1H, dd, J = 0.8, 5.6 Hz), 8.65 (1H, d, J = 5.2 Hz), 9.09 (1H, d, J = 0.8 Hz). MS (ESI) m/z 391 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{23}H_{27}N_4O_2$, 391.2134; found, 391.2134.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (5q). Compound **5q** was prepared from 2-methyl-4*H*-pyrido[2,3-*d*][1,3]oxazin-4-one (**24q**) and **23** tosylate using the procedure described for **5s** as a colorless solid (8% yield); HPLC purity (99.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.77 (2H, m), 1.82−1.95 (4H, m), 1.99−2.11 (4H, m), 2.15−2.23 (2H, m), 2.34 (3H, s), 2.60−2.68 (2H, m), 2.70−2.80 (1H, m), 4.33−4.41 (1H, m), 7.03 (2H, d, J = 8.8 Hz), 7.12 (2H, d, J = 8.8 Hz), 7.39 (1H, dd, J = 4.4, 8.0 Hz), 8.56 (1H, dd, J = 2.0, 7.6 Hz), 8.96 (1H, dd, J = 2.4, 4.8 Hz). MS (ESI) m/z 391 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{23}H_{27}N_4O_2$, 391.2134; found, 391.2122.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2,5-dimethyl-4(3*H*)-quinazolinone (5r). Compound **5r** was prepared from 2,5-dimethyl-4*H*-3,1-benzoxazin-4-one (**24r**) and **23** tosylate using the procedure described for **5s** as a colorless solid (70% yield); HPLC purity (99.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.75 (2H, m), 1.82−1.96 (4H, m), 1.99−2.10 (4H, m), 2.13−2.22 (2H, m), 2.22 (3H, s), 2.58−2.67 (2H, m), 2.69−2.79 (1H, m), 2.81 (3H, s), 4.33−4.40 (1H, m), 7.02 (2H, d, J = 8.8 Hz), 7.11 (2H, d, J = 8.8 Hz), 7.19 (1H, d, J = 8.0 Hz), 7.48 (1H, d, J = 8.0 Hz), 7.57 (1H, t, J = 8.0 Hz). MS (ESI) m/z 404 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{25}H_{30}N_3O_2$, 404.2338; found, 404.2343.

3-(4-[(1-*tert*-Butoxycarbonyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (8b). To a stirred solution of **6** (1.0 g, 3.96 mmol), *N*-(*tert*-butoxycarbonyl)-4-piperidinol (**7b**; 956 mg, 4.75 mmol), and triphenylphosphine (1.56 g, 5.94 mmol) in THF (2 mL) was added diethyl azodicarboxylate (1.17 mL, 5.94 mmol) dropwise at 0 °C under a nitrogen atmosphere. The mixture was allowed to warm to room temperature and stirred for 48 h. The resulting mixture was concentrated, and the residual oil was taken up into diethyl ether. The resulting precipitates were removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography with 30% ethyl acetate in hexanes to give **8b** as a light-brown solid (1.10 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (9H, s), 1.76−1.84 (2H, br m), 1.92−1.99 (2H, br m), 2.26 (3H, s), 3.34 (2H, br m), 3.66−3.75 (2H, br m), 4.48−4.56 (1H, m), 7.03 (2H, d, J = 9.2 Hz), 7.14 (2H, d, J = 9.2 Hz), 7.51−7.55 (1H, m), 7.63 (1H, d, J = 8.0 Hz), 7.72−7.77 (1H, m), 8.25 (1H, dd, J = 8.0, 1.2 Hz). MS (ESI) m/z 436 $(M + H)^+$.

3-(4-[(1-*tert*-Butoxycarbonyl-3-pyrrolidinyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (8a). Compound **8a** was prepared from compound **6** and *N*-(*tert*-butoxycarbonyl)-3-pyrrolidinol (**7a**) using the procedure described for **8b** as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (9H, s), 2.10−2.19 (1H, br m), 2.22−2.29 (1H, br m), 2.27 (3H, s), 3.50−3.70 (4H, br m), 4.93−4.96 (1H, br m),

7.03 (2H, d, J = 7.3 Hz), 7.18 (2H, d, J = 7.3 Hz), 7.45−7.49 (1H, m), 7.68 (1H, d, J = 7.3 Hz), 7.77 (1H, t, J = 7.3 Hz), 8.27 (1H, dd, J = 8.0, 1.2 Hz). MS (ESI) m/z 422 $(M + H)^+$.

3-(4-[(1-*tert*-Butoxycarbonyl-4-azepanyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (8c). Compound **8c** was prepared from compound **6** and *N*-(*tert*-butoxycarbonyl)-4-azepanol (**7c**) using the procedure described for **8b** as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (9H, s), 1.65−1.70 (2H, br m), 1.93−2.02 (3H, m), 2.06−2.16 (1H, m), 2.25 (3H, s), 3.25−3.37 (1H, m), 3.40−3.64 (3H, m), 4.46−4.50 (1H, m), 6.98 (2H, d, J = 8.8 Hz), 7.13 (2H, d, J = 8.0 Hz), 7.44 (1H, t, J = 8.4 Hz), 7.64 (1H, d, J = 8.0 Hz), 7.71−7.75 (1H, m), 8.24 (1H, d, J = 8.0 Hz). MS (ESI) m/z 450 $(M + H)^+$.

2-Methyl-3-[4-(4-piperidinyloxy)phenyl]-4(3*H*)-quinazolinone (9b). To a stirred solution of **8b** (1.10 g, 2.53 mmol) in $CHCl_3$ (10 mL) was added trifluoroacetic acid (10 mL), and the mixture was stirred at room temperature for 30 min. The resulting mixture was concentrated, and the residue was partitioned between ethyl acetate and 2 N NaOH. The layers were separated, and the aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with 2 N NaOH and brine, dried over sodium sulfate, and concentrated. The residue was vacuum-dried to give **9b** as a slightly purple solid (0.83 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67−1.81 (2H, m), 2.01−2.10 (2H, m), 2.26 (3H, s), 2.72−2.80 (2H, m), 3.13−3.20 (2H, m), 4.40−4.47 (1H, m), 7.03 (2H, d, J = 8.8 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.44 (1H, t, J = 8.0 Hz), 7.65 (1H, d, J = 7.6 Hz), 7.74 (1H, t, J = 7.6 Hz), 8.25 (1H, d, J = 8.0 Hz). MS (ESI) m/z 336 $(M + H)^+$.

2-Methyl-3-[4-(3-pyrrolidinyloxy)phenyl]-4(3*H*)-quinazolinone (9a). Compound **9a** was prepared from **8a** using the procedure described for **9b** as a beige solid (34% over 2 steps from **6**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01−2.20 (2H, m), 2.27 (3H, s), 2.94−3.01 (1H, br m), 3.06−3.11 (1H, br m), 3.19−3.28 (2H, m), 4.87−4.90 (1H, br m), 7.01 (2H, d, J = 9.3 Hz), 7.16 (2H, d, J = 9.3 Hz), 7.45−7.49 (1H, m), 7.67 (1H, d, J = 7.8 Hz), 7.75−7.79 (1H, m), 8.27 (1H, dd, J = 8.0, 1.2 Hz). MS (ESI) m/z 322 $(M + H)^+$.

3-[4-(4-Azepanyloxy)phenyl]-2-methyl-4(3*H*)-quinazolinone (9c). Compound **9c** was prepared from **8c** using the procedure described for **9b** as a brown oil (40% over 2 steps from **6**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57−1.68 (1H, m), 1.83−2.22 (5H, m), 2.25 (3H, s), 2.84−3.06 (4H, m), 4.57−4.62 (1H, m), 6.99 (2H, d, J = 8.8 Hz), 7.12 (2H, d, J = 8.8 Hz), 7.43 (1H, t, J = 8.0 Hz), 7.64 (1H, d, J = 8.0 Hz), 7.74 (1H, t, J = 8.0 Hz), 8.24 (1H, d, J = 6.4 Hz). MS (ESI) m/z 350 $(M + H)^+$.

3-(4-[(3-Hydroxycyclopentyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (11). To a stirred solution of **6** (1.0 g, 3.96 mmol), cyclopentane-1,3-diol (**10**; 810 mg, 7.93 mmol), and triphenylphosphine (1.56 g, 5.95 mmol) in THF (10 mL) was added diisopropyl azodicarboxylate (1.16 mL, 5.95 mmol) dropwise at 0 °C under a nitrogen atmosphere, and the mixture was stirred at room temperature for 14 h. The resulting mixture was partitioned between water and ethyl acetate. The layers were separated, and the aqueous layer was extracted with ethyl acetate three times. The combined organic layers were washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography with 50% → 100% ethyl acetate in hexanes to afford **11** (1.32 g, 99%) as a beige solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91−2.20 (6H, m), 2.26 (3H, s), 4.38−4.42 (1H, br m), 4.85−4.89 (1H, br m), 7.03 (2H, d, J = 8.8 Hz), 7.16 (2H, d, J = 8.8 Hz), 7.46 (1H, td, J = 7.4, 1.1 Hz), 7.67 (1H, d, J = 7.8 Hz), 7.74−7.79 (1H, m), 8.27 (1H, dd, J = 7.8, 1.0 Hz). MS (ESI) m/z 337 $(M + H)^+$.

3-[4-((3-[(Methylsulfonyl)oxy]cyclopentyl)oxy)phenyl]-2-methyl-4(3*H*)-quinazolinone (12). To a stirred solution of **11** (520 mg, 1.55 mmol) and triethylamine (0.33 mL, 2.32 mmol) in dichloromethane (10 mL) was added mesyl chloride (0.12 mL, 1.55 mmol) dropwise at 0 °C. After being stirred at 0 °C for 5 min, the mixture was allowed to warm to room temperature and stirred for an additional 10 min. The mixture was diluted with ethyl acetate and washed with water, dried over magnesium sulfate, and concentrated to give **12** as a pale-yellow solid (553 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.05−2.30 (5H, m), 2.26 (3H, s), 2.44−2.51 (1H, m), 3.02 (3H, s), 4.80−4.83 (1H, m), 5.16−5.23 (1H, m), 6.99 (2H, d, J = 9.2 Hz), 7.15 (2H, d, J = 9.2 Hz), 7.42−7.46 (1H, m), 7.65 (1H, d, J = 8.0 Hz), 7.74 (1H, t, J = 8.4 Hz), 8.24 (1H, d, J = 8.0 Hz). MS (ESI) m/z 415 $(M + H)^+$.

3-[4-(2-Methyl-4-*oxo*-3(4*H*)-quinazolinyl)phenoxy]cyclopentyl benzoate (13). To a stirred solution of **11** (200 mg, 0.595 mmol), benzoic acid (109 mg, 0.892 mmol), and triphenylphosphine (234 mg, 0.892 mmol) in THF (2 mL) was added diisopropyl azodicarboxylate (173 μL, 0.892 mmol) dropwise at 0 °C under an nitrogen atmosphere. After being stirred at 0 °C for 10 min, the mixture was allowed to warm to room temperature and stirred for an additional 1 h. The resultant mixture was partitioned between water and ethyl acetate. The layers were separated, and the aqueous layer was extracted with ethyl acetate three times. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography with 40% → 100% ethyl acetate in hexanes to give **13** (377 mg) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98−2.10 (2H, m), 2.23−2.46 (4H, m), 2.27 (3H, s), 4.96−5.03 (1H, br m), 5.59−5.63 (1H, m), 7.02 (2H, d, J = 8.3 Hz), 7.16 (2H, d, J = 8.8 Hz), 7.44−7.49 (3H, m), 7.57 (1H, t, J = 7.3 Hz), 7.68 (1H, d, J = 7.8 Hz), 7.75−7.79 (1H, m), 8.04 (2H, dd, J = 8.5, 1.2 Hz), 8.28 (1H, dd, J = 7.8, 1.0 Hz). MS (ESI) m/z 441 $(M + H)^+$.

3-(4-[(3-Hydroxycyclopentyl)oxy]phenyl)-2-methyl-4(3*H*)-quinazolinone (14, Diastereomer of 11). A mixture of **13** (227 mg, 0.36 mmol) and potassium carbonate (71.2 mg, 0.515 mmol) in methanol (5 mL) was stirred at room temperature for 8 h under a nitrogen atmosphere. The resulting mixture was partitioned between ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with 10% aqueous $NaHCO_3$ and brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography with 60% → 100% ethyl acetate in hexanes to give **14** as a pale-yellow solid (80 mg, 66% over 2 steps from **11**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68−1.75 (1H, m), 1.90−1.97 (1H, m), 2.07−2.21 (3H, m), 2.24−2.33 (1H, m), 2.26 (3H, s), 4.56−4.60 (1H, m), 4.93−4.98 (1H, m), 6.99 (2H, d, J = 8.8 Hz), 7.14 (2H, d, J = 8.8 Hz), 7.44−7.48 (1H, m), 7.67 (1H, d, J = 7.3 Hz), 7.74−7.79 (1H, m), 8.27 (1H, dd, J = 8.0, 1.2 Hz). MS (ESI) m/z 337 $(M + H)^+$.

3-[4-((3-[(Methylsulfonyl)oxy]cyclopentyl)oxy)phenyl]-2-methyl-4(3*H*)-quinazolinone (15, Diastereomer of 12). Compound **15** was prepared from **14** using the procedure described for **12** as a colorless solid (98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01−2.13 (2H, m), 2.17−2.30 (2H, m), 2.26 (3H, s), 2.37−2.47 (2H, m), 3.04 (3H, s), 4.95−5.00 (1H, m), 5.32−5.37 (1H, m), 6.99 (2H, d, J = 8.8 Hz), 7.16 (2H, d, J = 9.3 Hz), 7.44−7.49 (1H, m), 7.68 (1H, d, J = 7.8 Hz), 7.75−7.79 (1H, m), 8.27 (1H, dd, J = 8.0, 1.2 Hz). MS (ESI) m/z 415 $(M + H)^+$.

3-[4-(1,4-Dioxaspiro[4.5]dec-8-yloxy)phenyl]-2-methyl-4(3*H*)-quinazolinone (17). Compound **17** was prepared from **6** and 4,4-ethylenedioxycyclohexanol (**16**) using a similar procedure described for **8b** as a pale-purple solid, which was used for the next reaction without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62−1.69 (2H, m), 1.91−2.01 (6H, m), 2.27 (3H, s), 3.96−4.01 (4H, m), 4.45−4.49 (1H, m), 7.05 (2H, d, J = 9.3 Hz), 7.15 (2H, d, J = 9.3 Hz), 7.46 (1H, t, J = 7.6 Hz), 7.67 (1H, d, J = 7.8 Hz), 7.74−7.79 (1H, m), 8.27 (1H, dd, J = 8.0, 1.2 Hz); MS (ESI) m/z 393 $(M + H)^+$.

2-Methyl-3-(4-[(4-oxocyclohexyl)oxy]phenyl)-4(3*H*)-quinazolinone (18). To a solution of **17** (1.59 g) in THF (5 mL) was added 10% hydrochloric acid (10 mL), and the mixture was stirred at room temperature for 3 h. After being neutralized with 2 N NaOH, the mixture was extracted with ethyl acetate three times. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography with 5% methanol in $CHCl_3$ to give **18** as a light-orange solid (511 mg, 49% yield for 2 steps). ^{1}H NMR

(400 MHz, $CDCl_3$) δ 2.08−2.16 (2H, m), 2.27 (3H, s), 2.30−2.40 (4H, m), 2.67−2.76 (2H, m), 4.73−4.78 (1H, m), 7.09 (2H, d, J = 8.4 Hz), 7.18 (2H, d, J = 8.8 Hz), 7.45 (1H, t, J = 8.0 Hz), 7.65 (1H, d, J = 7.2 Hz), 7.73−7.77 (1H, m), 8.25 (1H, dd, J = 8.0, 1.2 Hz). MS (ESI) m/z 349 $(M + H)^+$.

1-*tert*-Butoxycarbonyl-4-(4-nitrophenoxy)piperidine (20). To a stirred solution of *N*-(*tert*-butoxycarbonyl)-4-piperidinol (**7b**; 10.0 g, 49.7 mmol) in DMF (50 mL) was added sodium hydride (60% dispersion in mineral oil, 3.0 g, 75 mmol) portionwise at 0 °C under a nitrogen atmosphere. After being stirred at 0 °C for 15 min, the mixture was allowed to warm to room temperature and stirred for an additional 30 min. To the mixture was added a solution of 4-fluoronitrobenzene (7.71 g, 55 mmol) in DMF (10 mL) dropwise at 0 °C. The mixture was allowed to warm to room temperature and stirred for 15 h. After being quenched by the addition of water (15 mL), the mixture was concentrated. The residue was partitioned between ethyl acetate (120 mL) and water (120 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with water twice, dried over sodium sulfate, and concentrated. The residual solid was suspended in a mixture of diisopropylether/hexanes (v/v = 1/4), and the precipitates were collected by filtration and vacuum-dried to provide **20** as a pale-yellow solid (12.4 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (9H, s), 1.75−1.82 (2H, m), 1.93−1.99 (2H, m), 3.36−3.42 (2H, m), 3.66−3.73 (2H, m), 4.58−4.62 (2H, m), 6.96 (2H, d, J = 8.0 Hz), 8.20 (2H, d, J = 8.0 Hz). MS (ESI) m/z 323 $(M + H)^+$.

4-(4-Nitrophenoxy)piperidine (21). Compound **20** (10.9 g, 33.8 mmol) was dissolved in 30 mL of trifluoroacetic acid, and the mixture was stirred at room temperature for 1 h. The resulting mixture was concentrated, and the residue was partitioned between ethyl acetate (150 mL) and 2 N NaOH (100 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with 1 N NaOH and brine, dried over sodium sulfate, and concentrated to give **21** as a brown oil (7.5 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70−1.79 (2H, m), 2.03−2.09 (2H, m), 2.77−2.83 (2H, m), 3.14−3.20 (2H, m), 4.49−4.55 (1H, m), 6.95 (2H, d, J = 8.0 Hz), 8.19 (2H, d, J = 8.0 Hz). MS (ESI) m/z 223 $(M + H)^+$.

1-Cyclobutyl-4-(4-nitrophenoxy)piperidine (22). To a mixture of **21** (7.5 g, 33.8 mmol), zinc chloride (4.61 g, 33.8 mmol), and cyclobutanone (3.55 g, 50.7 mmol) in methanol (100 mL) was added sodium cyanoborohydride (3.19 g, 50.7 mmol) portionwise at room temperature, and the mixture was stirred at room temperature for 5 h. The resulting mixture was concentrated, and the residue was partitioned between ethyl acetate and 2 N NaOH. The layers were separated, and the aqueous layer was extracted with ethyl acetate twice, and the combined organic layers were washed with 1 N NaOH and brine, dried over sodium sulfate, and concentrated. The resulting solid was suspended in a mixture of diisopropylether/hexanes (v/v = 1/4), and the precipitates were collected by filtration and vacuum-dried to give **22** (7.5 g, 80%) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.75−1.86 (1H, m), 1.87−1.94 (1H, m), 2.07−2.17 (2H, br m), 2.22−2.35 (6H, br m), 2.92−3.02 (4H, br m), 3.28−3.34 (1H, m), 4.76 (1H, br s), 6.99 (2H, d, J = 9.2 Hz), 8.18 (2H, d, J = 9.2 Hz). MS (ESI) m/z 277 $(M + H)^+$.

4-[(1-Cyclobutyl-4-piperidinyl)oxy]aniline *p*-Toluenesulfonate (23 Tosylate). Compound **22** (7.5 g, 27 mmol) was hydrogenated over 3.0 g of 10% Pd/C in methanol (100 mL) under a hydrogen atmosphere (1 atm) at room temperature for 13 h. The mixture was filtered through a pad of celite, and the filtrate was concentrated. The residue was vacuum-dried to give the aniline **23** as a pale-brown oil (6.5 g, 97%). To a stirred solution of **23** (6.5 g, 26.4 mmol) in ethyl acetate (100 mL) was added a solution of *p*-toluenesulfonic acid monohydrate (26.4 mmol) in EtOH (20 mL) at room temperature. The mixture was heated to reflux for 10 min to dissolve the precipitates. After being cooled to room temperature, the resulting precipitates were collected and vacuum-dried to provide **23** tosylate as a beige solid (10.3 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 4/1) δ 1.68−1.79 (1H, m), 1.81−1.90 (1H, m), 2.09−2.15 (2H, m), 2.19−2.27 (2H, m), 2.34−2.43 (5H, m), 2.52−2.61 (2H, m), 2.86−2.93 (2H, m), 3.34−3.44 (3H, m), 4.52 (1H, brs), 6.70−6.75 (4H, m), 7.21 (2H, d, J = 7.8 Hz), 7.79 (2H, d, J = 7.8 Hz). MS (ESI) m/z 247 $(M + H)^+$.

Cloning and Expression of the Histamine H_3 Receptor. Human histamine H_3 receptor (GenBank accession no. AB045369) was cloned as previously described.[32] The DNAs were inserted into the mammalian expression vector pCAGGS[33] (pCAGGS-hH_3), and cells, stably expressing the human histamine H_3 receptors, were generated by transfecting pCAGGS-hH_3 into CHO-K1 cells. HEK293/CRE-β-lactamase cells expressing rat histamine H_3 receptor were prepared as previously described.[34]

Ligand Binding Assay. Membrane preparations from the cells expressing histamine H_3 receptors and [^{3}H] *N*-α-methylhistamine binding studies were performed as previously described.[34]

[^{35}S]GTPγS Functional Binding Assay. [^{35}S]GTPγS functional binding assays were carried out with a minor modification to the method described previously.[34] In brief, membranes were incubated with 200 pM [^{35}S]GTPγS in 50 mM Tris-HCl, 100 mM NaCl, 5 mM $MgCl_2$, and 10 μM GDP (pH 7.4) containing 1.0 mg of wheat germ agglutinin-coated SPA beads for 3 h at 25 °C in the presence or absence of various concentrations of compounds. Membrane-bound radioactivity was detected by scintillation proximetry with a TopCount microplate scintillation counter (Packard, Meriden, CT).

Brain Histamine Release Assay. Brain *tele*-methylhistamine levels were measured as previously described.[29] In brief, 2 h after treatment with orally administered vehicle (0.5% methylcellulose) or compounds **5a** and **5s** (1, 3, and 10 mg /kg), male SD rats (Charles River, Tokyo, Japan) were decapitated and the brains were homogenized. After the solid phase extraction, *tele*-methylhistamine was analyzed by high performance liquid chromatography with fluorometry.

Receptor Occupancy by Ex Vivo Autoradiography. Male SD rats received vehicle or compounds **5a** and **5s** (1 mg /kg) by oral gavage. Animals were decapitated 2 h following oral dosing. In other studies, female *mdr1a* (+/+) and *mdr1a* (−/−) CF-1 mice (Charles River, Tokyo, Japan) were administered vehicle or compound **5a** (0.1, 0.3, and 1 mg /kg) or **5s** (0.3, 1, and 3 mg/kg) by oral gavage and decapitated 2 h following oral dosing. Terminal blood samples were collected and whole brains were rapidly removed in all studies. Whole brain tissue was dissected from half of each brain (for ex vivo autoradiography), and the other half of the brain was kept for pharmacokinetic analysis of brain concentrations of **5a** and **5s**. All dissected tissue samples were frozen in dry ice-cold isopentane and stored at −80 °C until use. Serial coronal sections, 20 μm in thickness, of the anterior striatal (0.2−1.0 mm anterior to Bregma) regions were cut by cryostat microtome and stored at −80 °C. Striatal sections were incubated with 400 pM of 3-([1,1,1-^{3}H]methyl)-2-(4-([3-(1-pyrrolidinyl)propyl]oxy)phenyl)-4(3*H*)-quinazolinone (a selective histamine H_3 receptor inverse agonist radioligand) in sodium phosphate buffer for 10 min at room temperature. Nonspecific binding was defined by the presence of *R*-α-methylhistamine (10 μM). Following incubation, the striatum sections were washed, air-dried, and exposed to beta-Imager (Biospace, Paris, France) for 6 h. Autoradiographic images were quantified as photostimulated luminescence (cpm) per mm^2 by beta-Imager. Blood samples were centrifuged to separate the plasma, and the brain samples were homogenized by ultrasonification with 4 volumes of water. The plasma and brain homogenate samples were deproteinized with ethanol containing an internal standard. Compounds **5a** and **5s** and the internal standard were detected by LC-MS/MS in a positive ionization mode using the electrospray ionization probe, and their precursor to product ion combinations were monitored in Multiple Reaction Monitoring mode.

Pharmacokinetics. Pharmacokinetic characterizations were conducted in male SD rats, male Beagle dogs, and male rhesus monkeys following single oral and single intravenous administration. In the three species, single doses of **5a**, **5r**, and **5s** were administered either intravenously in a vehicle of PEG400/EtOH/H_2O = 50/10/40 or orally by gavage in a vehicle of 0.5% methylcellurose aqueous suspension. Doses of 1 (iv) and 3 (po) mg/kg for rats and 0.3 (iv)

and 1 (po) mg/kg for dogs and monkeys were used. Blood samples for the determination of drug plasma concentrations were obtained at multiple time points up to 24 h after administration. Blood samples were centrifuged to separate the plasma, and the plasma samples were deproteinized with ethanol containing an internal standard. Compounds **5a**, **5r**, and **5s** and the internal standard were detected by LC-MS/MS in a positive ionization mode using the electrospray ionization probe, and their precursor to product ion combinations were monitored in Multiple Reaction Monitoring mode.

Acknowledgment. We thank Hirokazu Ohsawa for collecting the high-resolution mass spectral data, Hiroaki Suwa for HPLC purity analyses, and Dr. Takahiro Fukuroda for cardiovascular studies in anesthetized dogs. We also thank Drs. Nobuyoshi Yasuda, Robert J. DeVita, and Peter T. Meinke (Merck Research Laboratories, Rahway, NJ) for the editing of this manuscript.

Supporting Information Available: Preparation of benzoxazinones **24e** and **24g**−**i** and procedures for safety-related studies, HPLC retention times and purity for the target compounds, and HPLC traces for **2f**, **5a**, **5r**, and **5s**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Brown, R. E.; Stevens, D. R.; Haas, H. L. The physiology of brain histamine. *Prog. Neurobiol.* **2001**, *63*, 637–672.

(2) (a) Arrang, J. M. Pharmacological properties of histamine receptor subtypes. *Cell. Mol. Biol. (Paris)* **1994**, *40*, 275–281. (b) Parsons, M. E.; Ganellin, C. R. Histamine and its receptors. *Br. J. Pharmacol.* **2006**, *147*, S127−S135.

(3) Lovenberg, T. W.; Roland, B. L.; Wilson, S. J.; Jiang, X.; Pyati, J.; Huvar, A.; Jackson, M. R.; Erlander, M. G. Cloning and functional expression of the human histamine H_3 receptor. *Mol. Pharmacol.* **1999**, *55*, 1101–1107.

(4) Heron, A.; Rouleau, A.; Cochois, V.; Pillot, C.; Schwartz, J. C.; Arrang, J. M. Expression analysis of the histamine H_3 receptor in developing rat tissues. *Mech. Dev.* **2001**, *105*, 167–173.

(5) Oda, T.; Morikawa, N.; Saito, Y.; Masuho, Y.; Matsumoto, S. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J. Biol. Chem.* **2000**, *275*, 36781–36786.

(6) Arrang, J. M.; Garbarg, M.; Schwartz, J. C. Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature* **1983**, *302*, 832–837.

(7) (a) Celanire, S.; Wijtmans, M.; Talaga, P.; Leurs, R.; de Esch, I. J. P. Keynote review: Histamine H_3 receptor antagonists reach out for the clinic. *Drug Discovery Today* **2005**, *10*, 1613–1627. (b) Leurs, R.; Bakker, R. A.; Timmerman, H.; de Esch, I. J. P. The histamine H_3 receptor: from gene cloning to H3 receptor drugs. *Nat. Rev. Drug Discovery* **2005**, *4*, 107–120.

(8) Wulff, B. S.; Hastrup, S.; Rimvall, K. Characteristics of recombinantly expressed rat and human histamine H_3 receptors. *Eur. J. Pharmacol.* **2002**, *453*, 33–41.

(9) (a) Schlicker, E.; Malinowska, B.; Kathmann, M.; Gothert, M. Modulation of neurotransmitter release via histamine H_3 heteroreceptors. *Fundam. Clin. Pharmacol.* **1994**, *8*, 128–137. (b) Clapham, J.; Kilpatrick, G. J. Histamine H_3 receptors modulate the release of [^{3}H]-acetylcholine from slices of rat entorhinal cortex: evidence for the possible existence of H_3 receptor subtypes. *Br. J. Pharmacol.* **1992**, *107*, 919–923.

(10) (a) Morisset, S.; Rouleau, A.; Ligneau, X.; Gbahou, F.; Tardivel-Lacombe, J.; Stark, H.; Schunack, W.; Ganellin, C. R.; Schwartz, J. C.; Arrang, J-.M. High constitutive activity of native H_3 receptors regulates histamine neurons in brain. *Nature* **2000**, *408*, 860–864. (b) Arrang, J-.M.; Morisset, S.; Gbahou, F. Constitutive activity of the histamine H_3 receptor. *Trends Pharmacol. Sci.* **2007**, *28*, 350–357.

(11) Witkin, J. M.; Nelson, D. L. Selective histamine H_3 receptor antagonists for treatment of cognitive deficiencies and other disorders of the central nervous system. *Pharmacol. Ther.* **2004**, *103*, 1–20.

(12) Esbenshade, T. A.; Fox, G. B.; Cowart, M. D. Histamine H_3 Receptor Antagonists: Preclinical Promise for Treating Obesity and Cognitive Disorders. *Mol. Interv.* **2006**, *6*, 77–88.

(13) Tokita, S.; Takahashi, K.; Kotani, H. Recent advances in molecular pharmacology of the histamine systems: physiology and pharmacology of histamine H_3 receptor: roles in feeding regulation and therapeutic potential for metabolic disorders. *J. Pharmacol. Sci.* **2006**, *101*, 12–18.

(14) Lin, J. S.; Dauvilliers, Y.; Arnulf, I.; Bastuji, H.; Anaclet, C.; Parmentier, R.; Kocher, L.; Yanagisawa, M.; Lehert, P.; Ligneau, X.; Perrin, D.; Robert, P.; Roux, M.; Lecomte, J. M.; Schwartz, J. C. An inverse agonist of the histamine H_3-receptor improves wakefulness in narcolepsy: studies in orexin−/−mice and patients. *Neurobiol. Dis.* **2008**, *30*, 74–83.

(15) (a) LaBella, F. S.; Queen, G.; Glavin, G.; Durant, G.; Stein, D.; Brandes, L. J. H_3 receptor antagonist, thioperamide, inhibits adrenal steroidogenesis and histamine binding to adrenocortical microsomes and binds to cytochrome P450. *Br. J. Pharmacol.* **1992**, *107*, 161–164. (b) Alves-Rodrigues, A.; Leurs, R.; Wu, T. S.; Prell, G. D.; Foged, C.; Timmerman, H. [^{3}H]-thioperamide as a radioligand for the histamine H_3 receptor in rat cerebral cortex. *Br. J. Pharmacol.* **1996**, *118*, 2045–2052. (c) Yang, R.; Hey, J. A.; Aslanian, R.; Rizzo, C. A. Coordination of histamine H_3 receptor antagonists with human adrenal cytochrome P450 enzymes. *Pharmacology* **2002**, *66*, 128–135.

(16) (a) Berlin, M.; Boyce, C. W. Recent advances in the development of histamine H_3 antagonists. *Expert Opin. Ther. Patents* **2007**, *17*, 675–687. (b) Wijtmans, M.; Leurs, R.; de Esch, I. Histamine H_3 receptor ligands break ground in a remarkable plethora of therapeutic areas. *Expert Opin. Investig. Drugs* **2007**, *16*, 967–985. (c) Letavic, M.; Barbier, A. J.; Dvorak, C. A.; Carruthers, N. I. Recent medicinal chemistry of the histamine H_3 receptor. *Prog. Med. Chem.* **2006**, *44*, 181–206.

(17) (a) Ligneau, X.; Perrin, D.; Landais, L.; Camelin, J.-C.; Calmels, T. P.G.; Berrebi-Bertrand, I.; Lecomte, J.-M.; Parmentier, R.; Anaclet, C.; Lin, J.-S.; Bertaina-Anglade, V.; la Rochelle, C. D.; d'Aniello, F.; Rouleau, A.; Gbahou, F.; Arrang, J.-M.; Ganellin, C. R.; Stark, H.; Schunack, W.; Schwartz, J.-C. BF2.649 [1-(3-[3-(4-Chlorophenyl)propoxy]propyl)piperidine, hydrochloride], a nonimidazole inverse agonist/antagonist at the human histamine H_3 receptor: preclinical pharmacology. *J. Pharmacol. Exp. Ther* **2007**, *320*, 365–375. (b) Ligneau, X.; Landais, L.; Perrin, D.; Piriou, J.; Uguen, M.; Denis, E.; Robert, P.; Parmentier, R.; Anaclet, C.; Lin, J. -S.; Burban, A.; Arrang, J. -M.; Schwartz, J. C. Brain histamine and schizophrenia: potential therapeutic applications of H_3-receptor inverse agonists studied with BF2.649. *Biochem. Pharmacol.* **2007**, *73*, 1215–1224.

(18) (a) Cowart, M.; Faghih, R.; Curtis, M. P.; Gfesser, G. A.; Bennani, Y. L.; Black, L. A.; Pan, L.; Marsh, K. C.; Sullivan, J. P.; Esbenshade, T. A.; Fox, G. B.; Hancock, A. A. 4-(2-[2-(2(*R*)-Methylpyrrolidin-1-yl)ethyl]benzofuran-5-yl)benzonitrile and Related 2-Aminoethylbenzofuran H_3 Receptor Antagonists Potently Enhance Cognition and Attention. *J. Med. Chem.* **2005**, *48*, 38–55. (b) Esbenshade, T. A.; Fox, G. B.; Krueger, K. M.; Miller, T. R.; Kang, C. H.; Denny, L. I.; Witte, D. G.; Yao, B. B.; Pan, L.; Wetter, J.; Marsh, K.; Bennani, Y. L.; Cowart, M. D.; Sullivan, J. P.; Hancock, A. A. Pharmacological properties of ABT-239 [4-(2-(2-[(2*R*)-2-methylpyrrolidinyl]ethyl)-benzofuran-5-yl)benzonitrile]: I. Potent and selective histamine H_3 receptor antagonist with drug-like properties. *J. Pharmacol. Exp. Ther* **2005**, *313*, 165–175. (c) Fox, G. B.; Esbenshade, T. A.; Pan, J. B.; Radek, R. J.; Krueger, K. M.; Yao, B. B.; Browman, K. E.; Buckley, M. J.; Ballard, M. E.; Komater, V. A.; Miner, H.; Zhang, M.; Faghih, R.; Rueter, L. E.; Bitner, R. S.; Drescher, K. U.; Wetter, J.; Marsh, K.; Lemaire, M.; Porsolt, R. D.; Bennani, Y. L.; Sullivan, J. P.; Cowart, M. D.; Decker, M. W.; Hancock, A. A. Pharmacological properties of ABT-239 [4-(2-(2-[(2*R*)-2-methylpyrrolidinyl]ethyl)-benzofuran-5-yl)benzonitrile]: II. Neurophysiological characterization and broad preclinical efficacy in cognition and schizophrenia of a potent and selective histamine H_3 receptor antagonist. *J. Pharmacol. Exp. Ther* **2005**, *313*, 176–190.

(19) Medhurst, A. D.; Atkins, A. R.; Beresford, I. J.; Brackenborough, K.; Briggs, M. A.; Calver, A. R.; Cilia, J.; Cluderay, J. E.; Crook, B.; Davis, J. B.; Davis, R. K.; Davis, R. P.; Dawson, L. A.; Foley, A. G.; Gartlon, J.; Gonzalez, M. I.; Heslop, T.; Hirst, W. D.; Jennings, C.; Jones, D. N. C.; Lacroix, L. P.; Martyn, A.; Ociepka, S.; Ray, A.; Regan, C. M.; Roberts, J. C.; Schogger, J.; Southam, E.; Stean, T. O.; Trail, B. K.; Upton, N.; Wadsworth, G.; Wald, J. A.; White, T.; Witherington, J.; Woolley, M. L.; Worby, A.; Wilson, D. M. GSK189254, a novel H_3 receptor antagonist that binds to histamine H_3 receptors in Alzheimer's disease brain and improves cognitive performance in preclinical models. *J. Pharmacol. Exp. Ther.* **2007**, *321*, 1032–1045.

(20) Nagase, T.; Mizutani, T.; Ishikawa, S.; Sekino, E.; Sasaki, T.; Fujimura, T.; Ito, S.; Mitobe, Y.; Miyamoto, Y.; Yoshimoto, R.; Tanaka, T.; Ishihara, A.; Takenaga, N.; Tokita, S.; Fukami, T.; Sato, N. Synthesis, structure−activity relationships, and biological profiles of a quinazolinone class of histamine H_3 receptor inverse agonists. *J. Med. Chem.* **2008**, *51*, 4780–4789.

(21) Dvorak, C. A.; Apodaca, R.; Barbier, A. J.; Berridge, C. W.; Wilson, S. J.; Boggs, J. D.; Xiao, W.; Lovenberg, T. W.; Carruthers, N. I. 4-Phenoxypiperidines: potent, conformationally restricted, non-imidazole histamine H_3 antagonists. *J. Med. Chem.* **2005**, *48*, 2229–2238.

(22) The *N*-cyclobutylpiperidine-4-oxy group was recently utilized by GlaxoSmithKline group. (a) Procopiou, P. A.; Ancliff, R. A.; Bamford, M. J.; Browning, C.; Connor, H.; Davies, S.; Fogden, Y. C.; Hodgson, S. T.; Holmes, D. S.; Looker, B. E.; Morriss, K. M. L.; Parr, C. A.; Pickup, E. A.; Sehmi, S. S.; White, G. V.; Watts, C. J.; Wilson, D. M.; Woodrow, M. D. 4-Acyl-1-(4-aminoalkoxyphenyl)-2-ketopiperazines as a novel class of non-brain-penetrant histamine H_3 receptor antagonists. *J. Med. Chem.* **2007**, *50*, 6706–6717.

(23) Botros, S.; Saad, S. F. Synthesis, antihypertensive and β-adrenoreceptor antagonist activities of 3-[4-[3-(4-aryl-1-piperazinyl)-isopropanoloxy]phenyl]-4(3*H*)-quinazolones. *Eur. J. Med. Chem.* **1989**, *24*, 585–590.

(24) (a) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, 1–28. (b) Hughes, D. L. Progress in the Mitsunobu reaction. A review. *Org. Prep. Proc. Int.* **1996**, *28*, 127–164.

(25) For the preparation of aza-benzoxazinoes **24n**−**q**, see: (a) Littell, R.; Allen, D. S., Jr. 5-Aryl-1,3-dihydro-2*H*-pyrido-1,4-diazepin-2-ones. *J. Med. Chem.* **1965**, *8*, 722–724. (b) For the preparation of benzoxazinone intermediates **24e** and **24g**−**i** that were not reprted in ref 20, see Supporting Information and refereces cited therein.

(26) For hERG binding assay protocol, see: Butcher, J. W.; Claremon, D. A.; Connolly, T. M.; Dean, D. C.; Karczewski, J.; Koblan, K. S.; Kostura, M. J.; Liverton, N. J.; Melillo, D. G. Radioligand and binding assay. PCT Int. Appl. WO20020058602002.

(27) See Supporting Information for experimental details.

(28) For P-glycoprotein assay protocols, see: (a) Yamazaki, M.; Neway, W. E.; Ohe, T.; Chen, I-W.; Rowe, J. F.; Hochman, J. H.; Chiba, M.; Lin, J. H. In vitro substrate identification studies for P-glycoprotein-mediated transport: species difference and predictability of in vivo results. *J. Pharmacol. Exp. Ther* **2001**, *296*, 723–735. (b) Ohe, T.; Sato, M.; Tanaka, S.; Fujino, N.; Hata, M.; Shibata, Y.; Kanatani, A.; Fukami, T.; Yamazaki, M.; Chiba, M.; Ishii, Y. Effect of P-glycoprotein-mediated efflux on cerebrospinal fluid/plasma concentration ratio. *Drug Metab. Dispos.* **2003**, *31*, 1251–1254.

(29) Miyamoto, Y.; Yoshimoto, R.; Yumoto, M.; Ishihara, A.; Takahashi, K.; Kotani, H.; Kanatani, A.; Tokita, S. Simultaneous fluorometric measurement of histamine and *tele*-methylhistamine levels in rodent brain by high-performance liquid chromatography. *Anal. Biochem.* **2004**, *334*, 89–96.

(30) The Cephalon group reported that high H_3 receptor occupancy is required for H_3 antagonists to produce robust waking activity. Le, S.; Gruner, J. A.; Mathiasen, J. R.; Marino, M. J.; Schaffhauser, H. Correlation between ex vivo receptor occupancy and wake-promoting activity of selective H_3 receptor antagonists. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 902–909.

(31) (a) Lankas, G. R.; Cartwright, M. E.; Umbenhauer, D. P-glycoprotein deficiency in a subpopulation of CF-1 mice enhances avermectin-induced neurotoxicity. *Toxicol. Appl. Pharmacol.* **1997**, *143*, 357–365. (b) Umbenhauer, D. R.; Lankas, G. R.; Pippert, T. R.; Wise, L. D.; Cartwright, M. E.; Hall, S. J.; Beare, C. M. Identification of a P-glycoprotein-deficient subpopulation in the CF-1 mouse strain using a restriction fragment length polymorphism. *Toxicol. Appl. Pharmacol.* **1997**, *146*, 88–94.

(32) Itadani, H.; Takimura, T.; Nakamura, T.; Ohta, M. Cloning of cDNA sequences encoding mammalian G protein-coupled receptor BG2. PCT Int. Appl. WO9933978, 1999.

(33) Niwa, H.; Yamamura, K.; Miyazaki, J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* **1991**, *108*, 193–199.

(34) Ito, S.; Yoshimoto, R.; Miyamoto, Y.; Mitobe, Y.; Nakamura, T.; Ishihara, A.; MacNeil, D. J.; Kanatani, A.; Tokita, S. Detailed pharmacological characterization of GT-2331 for the rat histamine H_3 receptor. *Eur. J. Pharmacol.* **2006**, *529*, 40–46.

JM800569W