

Synthesis and Evaluation of Structurally Constrained Quinazolinone Derivatives as Potent and Selective Histamine H₃ Receptor Inverse Agonists

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A series of structurally constrained derivatives of the potent H₃ inverse agonist **1** was designed, synthesized, and evaluated as histamine H₃ 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: H₁, H₂, H₃, and H₄.² 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, H₃ is predominantly expressed in the CNS, while H₁ and H₂ are expressed in both central and peripheral tissues.^{3,4} The H₄ receptor is predominantly expressed in inflammatory cells, suggesting its critical role in the regulation of inflammatory and immune responses.⁵

The H₃ receptor was discovered pharmacologically in 1983⁶ and genetically identified in 1999.³ The genetic identification of the H₃ 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 H₃.⁷ Signaling through the H₃ receptor activates G proteins that inhibit adenylate cyclase activity and reduce intracellular cAMP levels.^{3,8} In the CNS, the H₃ receptor is localized on the presynaptic membrane as an autoreceptor and negatively regulates the release and synthesis of histamine.⁶ In addition, the H₃ receptor is known to modulate the release of other neurotransmitters such as norepinephrine, dopamine, acetylcholine, serotonin, and GABA.⁹ The H₃ 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, H₃ receptor inverse agonists have been demonstrated to decrease constitutive H₃ signaling, thus blocking the tonic inhibition of histamine release and further potentiating histaminergic effects. Because

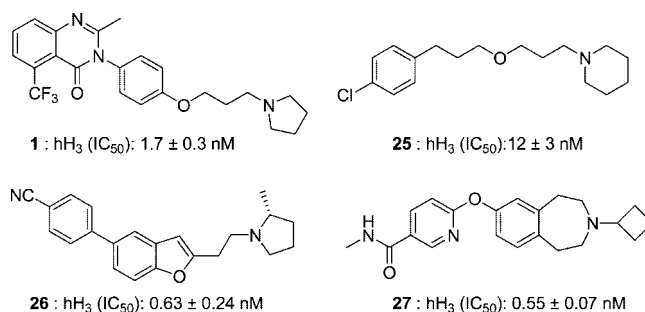


Figure 1. Structures and antagonistic activities of nonimidazole H₃ antagonists and inverse agonists. Antagonistic activities were determined by inhibition of *R*- α -methylhistamine-induced binding of [³⁵S]GTP γ S at human H₃ receptor. The values represent the mean \pm SE for $n \geq 3$.

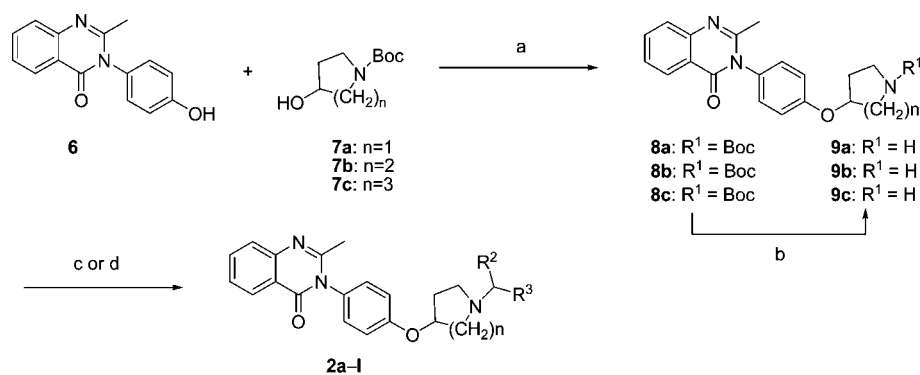
of the effects of H₃ signaling on multiple neuronal transmitters, it has been suggested that H₃ antagonists/inverse agonists could be effective therapeutics for several CNS-related disorders.¹¹ In animal models, H₃ receptor antagonists/inverse agonists have been shown to enhance wakefulness and attentive 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 H₃ receptor inverse agonist, suppressed the excessive daytime sleep of narcoleptic patients.¹⁴

First-generation imidazole-based H₃ antagonists have inhibitory activity on cytochrome P₄₅₀ enzymes, which may result in drug–drug interactions with coadministered drugs by reducing hepatic clearance.¹⁵ Current efforts have focused on nonimidazole classes of H₃ receptor antagonists/inverse agonists with the potential to reduce these liabilities. Since the identification of the H₃ receptor genes, various classes of nonimidazole H₃ receptor antagonists have been developed to target H₃ 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.

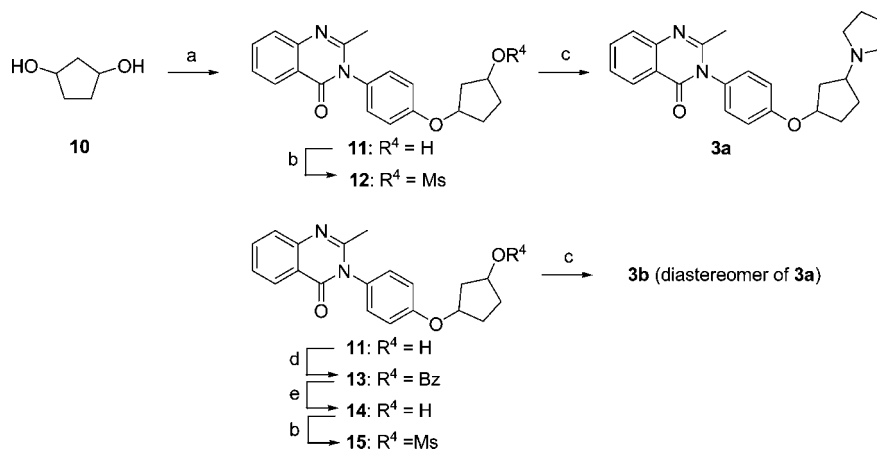
Starting from the previously reported potent H₃ inverse agonist **1** (Figure 1),²⁰ a series of structurally constrained analogues were prepared and evaluated, aimed at improving its potency. This approach was previously demonstrated by the

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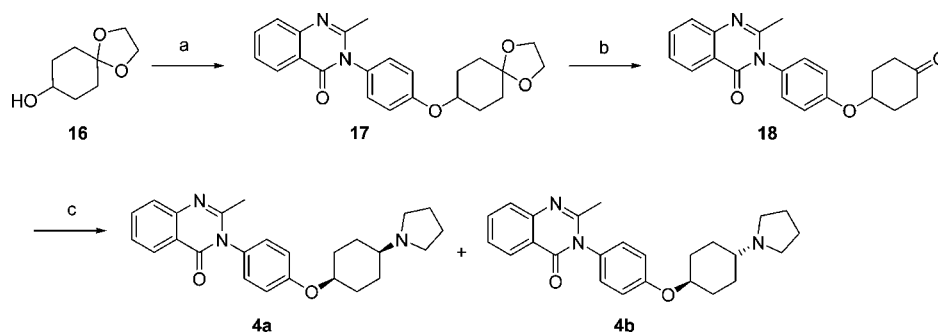
^a Abbreviations: P-gp, P-glycoprotein; SD rat, Sprague–Dawley rat; SAR, structure–activity relationship; hERG, human ether-a-go-related gene; HEK, human embryonic kidney; CNS, central nervous system; Ts, 4-toluenesulfonyl; Ms, methanesulfonyl; DIAD, diisopropyl azodicarboxylate; DEAD, diethyl azodicarboxylate.

Scheme 1^a

^a Reagents: (a) DEAD, PPh₃, THF; (b) trifluoroacetic acid, CHCl₃; (c) R²R³CHI, K₂CO₃, DMF, 60 °C; (d) R²R³CO, ZnCl₂, NaBH₃CN, MeOH.

Scheme 2^a

^a Reagents: (a) **6**, DIAD, PPh₃, THF; (b) MsCl, Et₃N, CH₂Cl₂; (c) pyrrolidine, K₂CO₃, DMF, 80 °C; (d) DIAD, PPh₃, PhCO₂H, THF; (e) K₂CO₃, MeOH.

Scheme 3^a

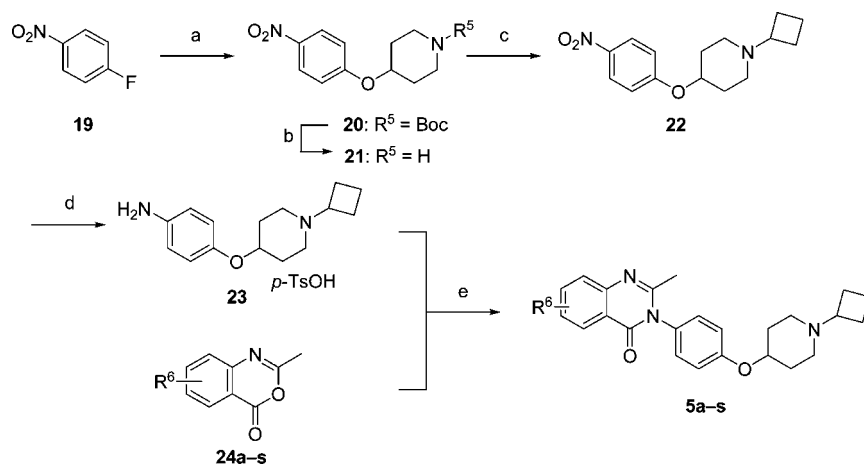
^a Reagents: (a) **6**, DIAD, PPh₃, THF; (b) 10% HCl aq, THF; (c) pyrrolidine, ZnCl₂, NaBH₃CN, MeOH.

Johnson & Johnson group, in which an *N*-alkylpiperidin-4-yloxy group was systematically investigated as a structurally constrained replacement for the aminopropoxy 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 group²² 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 quinazoli-

none class of H₃ 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–1**. Known compounds **6**²³ and **7** were coupled by the Mitsunobu reaction²⁴ 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–1**. Preparation of

Scheme 4^a

^a Reagents: (a) **7b**, NaH, DMF; (b) trifluoroacetic acid, CHCl₃; (c) cyclobutanone, ZnCl₂, NaBH₃CN, MeOH; (d) (i) H₂, 10% Pd/C, MeOH, (ii) TsOH·H₂O; (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-ethylenedioxcyclohexanol (**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 [³⁵S]GTPγS binding assay in membranes isolated from cells transfected with cloned H₃ 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 [³⁵S]*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 H₃ 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

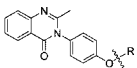
of compound **1** (hH₃, IC₅₀ = 1.7 nM) (Figure 1) might be further improved by imparting structural constraints to the flexible propoxy moiety. In addition to the previously studied *N*-alkylpiperidin-4-yloxy group,²¹ novel structurally constrained tethers were designed to have a cyclic structure in 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 (IC₅₀ = 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** (IC₅₀ = 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-1**), 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 H₃ activity (IC₅₀ = 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.

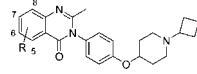
Further optimization of **2f** by the modification of the quinazolinone group is summarized in Table 2. The fluorine-substituted derivatives **5a-e** displayed improved activities

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	<i>d</i>
2a		740 ± 68	<i>d</i>
2b		49 ± 9	<i>d</i>
2c		60 ± 12	<i>d</i>
2d		91 ± 11	<i>d</i>
2e		14 ± 2	<i>d</i>
2f		1.5 ± 0.3	>10
2g		3.2 ± 0.3	>10
2h		43 ± 11	<i>d</i>
2i		103 ± 29	<i>d</i>
2j		37 ± 9	<i>d</i>
2k		10 ± 1	7.3 ± 0.9
2l		7.0 ± 1.5	6.8 ± 0.8
3a		3.4 ± 0.4	>10
3b (diastereomer of 3a)		8.6 ± 0.1	10 ± 0.7
4a		10 ± 1	8.0 ± 0.5
4b		813 ± 99	<i>d</i>

^a The values represent the mean ± SE for $n \geq 3$. ^b Inhibition of *R*- α -methylhistamine-induced binding of [³⁵S]GTP γ S at human H₃ receptor. ^c Inhibition of [³⁵S]*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** (IC₅₀ = 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 [³⁵S]GTP γ S at human H₃ receptor. ^c Inhibition of [³⁵S]*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–∞} (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 GTP γ S binding with EC₅₀ 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–∞} = 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**

compd	brain penetration in SD rats ^a					P-gp susceptibility ^b	
	plasma (μ M)	brain (nmol/g)	CSF (μ M)	ratio		transcellular transport ratio (B-to-A)/(A-to-B)	
				brain/plasma	CSF/brain	mouse <i>mdr1a</i>	human <i>MDR1</i>
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 H₃ Receptors^a

compd	H ₃ binding affinity (K_i , nM) ^b		
	human	rat	mouse
5a	6.8 \pm 1.9	6.0 \pm 0.7	8.4 \pm 1.0
5r	1.7 \pm 0.4	1.8 \pm 0.1	3.9 \pm 0.6
5s	3.6 \pm 0.7	2.3 \pm 0.1	3.9 \pm 0.5

^a The values represent the mean \pm SE for $n \geq 3$. ^b Displacement of [³H]N- α -methylhistamine binding to cell membranes expressing recombinant H₃ 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 evaluated²⁸ 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 ($IC_{50} > 10$ μ M for hH₁, hH₂, and hH₄) and to a panel of 115 diverse, unrelated binding sites ($IC_{50} > 1$ μ M for all the binding sites tested). The binding affinities of compounds **5a**, **5r**, and **5r** for the human, rat, and mouse H₃ 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 H₃ receptor occupancy was evaluated by an ex vivo receptor occupancy method for correlation with the observed histamine elevation. The brain H₃ 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 H₃ 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 mice³¹ 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 H₃ inverse agonist **1** were designed, synthesized, and evaluated as histamine H₃ 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

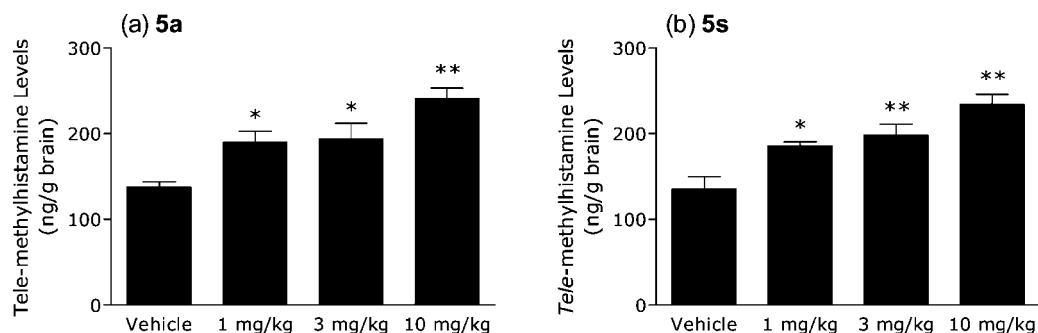


Figure 2. Brain *tele*-methylhistamine levels in SD rats after oral administration of **5a** and **5s**. Values are means \pm SE, determined from five experiments. * $P < 0.05$ and ** $P < 0.01$ (ANOVA Dunnett) compared with the vehicle control.

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 (μ M)	brain ^b (nmol/g)	brain/ plasma ^c	plasma Occ90 ^d (nM)
5a	<i>mdr1a</i> ($+/+$)	0.032 \pm 0.002	0.062 \pm 0.002	1.9	144
	<i>mdr1a</i> ($-/-$)	0.028 \pm 0.001	0.260 \pm 0.039	9.4	30
5s	<i>mdr1a</i> ($+/+$)	0.012 \pm 0.001	0.031 \pm 0.002	2.6	58
	<i>mdr1a</i> ($-/-$)	0.010 \pm 0.002	0.137 \pm 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 \pm 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).

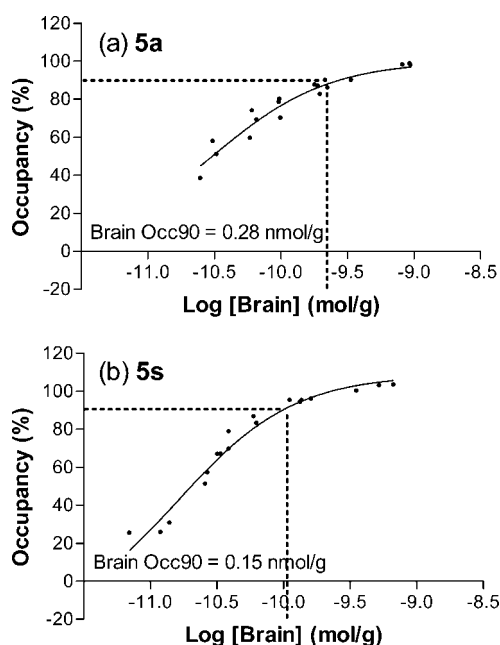


Figure 3. The brain concentration–receptor occupancy relationship of (a) **5a** and (b) **5s** in CF-1 mice. Receptor occupancy and exposure were determined 2 h following oral administration of vehicle or compound **5a** or **5s** (0.1, 0.3, 1, and 3 mg/kg). See Supporting Information for experimental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

was observed, indicating that the brain H₃ 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 ($C_{max} = 3.2 \mu$ M), no adverse treatment-related cardiovascular effects were observed. Regarding gross behavior in mice, oral admin-

istration 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 ¹H 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 pre-packed 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 \times 30 mm id), eluting with a gradient of CH₃CN/0.1% aqueous CF₃CO₂H 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 \times 150 mm id), eluting with a gradient of (A) 0.1% H₃PO₄/CH₃CN = 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)/CH₃CN = 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-piperidinyloxy]phenyl)-2-methyl-4(3H)-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% CHCl₃ in methanol to give **2b** as a colorless solid (25 mg, 46%); HPLC purity (99.2%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₂H₂₆N₃O₂, 364.2025; found, 364.2020.

2-Methyl-3-(4-[(1-methyl-4-piperidinyl)oxy]phenyl)-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₁H₂₄N₃O₂, 350.1869; found, 350.1865.

2-Methyl-3-(4-[(1-propyl-4-piperidinyl)oxy]phenyl)-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₃H₂₈N₃O₂, 378.2168; found, 378.2182.

3-(4-[(1-Butyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₃₀N₃O₂, 392.2338; found, 392.2337.

3-(4-[(1-Isopropyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₃H₂₈N₃O₂, 378.2182; found, 378.2186.

Representative Procedure for the Preparation of 2f–I via Reductive Amination. **3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₈N₃O₂, 390.2182; found, 390.2185.

3-(4-[(1-Cyclopentyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₆H₃₂N₃O₂, 418.2495; found, 418.2505.

3-(4-[(1-Cyclohexyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₆H₃₂N₃O₂, 418.2495; found, 418.2505.

3-(4-[(1-Cyclobutyl-3-pyrrolidinyl)oxy]phenyl)-2-methyl-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₃H₂₆N₃O₂, 376.2025; found, 376.2019.

3-(4-[(1-Cyclopentyl-3-pyrrolidinyl)oxy]phenyl)-2-methyl-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₈N₃O₂, 390.2182; found, 390.2185.

3-(4-[(1-Cyclobutyl-4-azepanyl)oxy]phenyl)-2-methyl-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₃₀N₃O₂, 404.2338; found, 404.2346.

3-(4-[(1-Cyclopentyl-4-azepanyl)oxy]phenyl)-2-methyl-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₆H₃₂N₃O₂, 418.2495; found, 418.2491.

2-Methyl-3-[4-[(3-(1-pyrrolidinyl)cyclopentyl)oxy]phenyl]-4(3H)-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 CHCl₃ to give **3a** as a colorless solid (143 mg, 28%); HPLC purity (99.2%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₈N₃O₂, 390.2182; found, 390.2166.

2-Methyl-3-[4-[(3-(1-pyrrolidinyl)cyclopentyl)oxy]phenyl]-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₈N₃O₂, 390.2182; found, 390.2179.

cis-2-Methyl-3-(4-[(1-(1-pyrrolidinyl)cyclohexyl)oxy]phenyl)-4(3H)-quinazolinone (4a) and trans-2-Methyl-3-(4-[(1-(1-pyrrolidinyl)cyclohexyl)oxy]phenyl)-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₃₀N₃O₂, 404.2338; found, 404.2351. For *trans*-isomer (**4b**), HPLC purity (98.2%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₃₀N₃O₂, 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(3H)-quinazolinone (5s).** A mixture of **23** tosylate (20.0 g, 47.8 mmol), 2-methyl-5-(trifluoromethyl)-4H-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 CHCl₃ followed by recrystallization from ethyl acetate to afford **5s** as a colorless solid (13.7 g, 63%); HPLC purity (99.6%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₂₇N₃O₂F₃, 458.2055; found, 458.2045.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-5-fluoro-2-methyl-4(3H)-quinazolinone (5a). Compound **5a** was prepared from 5-fluoro-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₇N₃O₂F, 408.2087; found, 408.2101.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-6-fluoro-2-methyl-4(3H)-quinazolinone (5b). Compound **5b** was prepared from 6-fluoro-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₇N₃O₂F, 408.2087; found, 408.2094.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-7-fluoro-2-methyl-4(3H)-quinazolinone (5c). Compound **5c** was prepared from 7-fluoro-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₇N₃O₂F, 408.2087; found, 408.2090.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-8-fluoro-2-methyl-4(3H)-quinazolinone (5d). Compound **5d** was prepared from 8-fluoro-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₇N₃O₂F, 408.2087; found, 408.2106.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-6,7-difluoro-2-methyl-4(3H)-quinazolinone (5e). Compound **5e** was prepared from 6,7-difluoro-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₆N₃O₂F₂, 426.1993; found, 426.1998.

5-Chloro-3-(4-[(1-cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3H)-quinazolinone (5f). Compound **5f** was prepared from 5-chloro-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₇N₃O₂Cl, 424.1792; found, 424.1785.

6-Chloro-3-(4-[(1-cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3H)-quinazolinone (5g). Compound **5g** was prepared from 6-chloro-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₇N₃O₂Cl, 424.1792; found, 424.1794.

7-Chloro-3-(4-[(1-cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3H)-quinazolinone (5h). Compound **5h** was prepared from 7-chloro-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₇N₃O₂Cl, 424.1792; found, 424.1803.

8-Chloro-3-(4-[(1-cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3H)-quinazolinone (5i). Compound **5i** was prepared from 8-chloro-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₇N₃O₂Cl, 424.1792; found, 424.1786.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-5-methoxy-2-methyl-4(3H)-quinazolinone (5j). Compound **5j** was prepared from 5-methoxy-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₃₀N₃O₃, 420.2287; found, 420.2289.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-6-methoxy-2-methyl-4(3H)-quinazolinone (5k). Compound **5k** was prepared from 6-methoxy-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₃₀N₃O₃, 420.2287; found, 420.2302.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-7-methoxy-2-methyl-4(3H)-quinazolinone (5l). Compound **5l** was prepared from 7-methoxy-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₃₀N₃O₃, 420.2287; found, 420.2294.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-8-methoxy-2-methyl-4(3H)-quinazolinone (5m). Compound **5m** was prepared from 8-methoxy-2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₃₀N₃O₃, 420.2287; found, 420.2295.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methylpyrido[3,2-*d*]pyrimidin-4(3H)-one (5n). Compound **5n** was prepared from 2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₃H₂₇N₄O₂, 391.2134; found, 391.2144.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methylpyrido[4,3-*d*]pyrimidin-4(3H)-one (5o). Compound **5o** was prepared from 2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₃H₂₇N₄O₂, 391.2134; found, 391.2122.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methylpyrido[3,4-*d*]pyrimidin-4(3H)-one (5p). Compound **5p** was prepared from 2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₃H₂₇N₄O₂, 391.2134; found, 391.2134.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2-methylpyrido[2,3-*d*]pyrimidin-4(3H)-one (5q). Compound **5q** was prepared from 2-methyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₃H₂₇N₄O₂, 391.2134; found, 391.2122.

3-(4-[(1-Cyclobutyl-4-piperidinyl)oxy]phenyl)-2,5-dimethyl-4(3H)-quinazolinone (5r). Compound **5r** was prepared from 2,5-dimethyl-4H-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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₃₀N₃O₂, 404.2338; found, 404.2343.

3-(4-[(1-*tert*-Butoxycarbonyl-4-piperidinyl)oxy]phenyl)-2-methyl-4(3H)-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%). ¹H NMR (400 MHz, CDCl₃) 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(3H)-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. ¹H NMR (400 MHz, CDCl₃) 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-azepanyloxy]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. ¹H NMR (400 MHz, CDCl₃) 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 CHCl₃ (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%). ¹H NMR (400 MHz, CDCl₃) 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**). ¹H NMR (400 MHz, CDCl₃) 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**). ¹H NMR (400 MHz, CDCl₃) 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. ¹H NMR (400 MHz, CDCl₃) 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%). ¹H NMR (400 MHz, CDCl₃) 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*)-quinazolinyloxy]phenyl]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 μ L, 0.892 mmol) dropwise at 0 °C under a 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. ¹H NMR (400 MHz, CDCl₃) 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 NaHCO₃ 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**). ¹H NMR (400 MHz, CDCl₃) 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). ¹H NMR (400 MHz, CDCl₃) 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-ethylenedioxy-cyclohexanol (**16**) using a similar procedure described for **8b** as a pale-purple solid, which was used for the next reaction without further purification. ¹H NMR (400 MHz, CDCl₃) 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 CHCl₃ to give **18** as a light-orange solid (511 mg, 49% yield for 2 steps). ¹H NMR

(400 MHz, CDCl₃) 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%). ¹H NMR (400 MHz, CDCl₃) 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%). ¹H NMR (400 MHz, CDCl₃) 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. ¹H NMR (400 MHz, CDCl₃) 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%). ¹H NMR (400 MHz, CDCl₃/CD₃OD = 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 H₃ Receptor. Human histamine H₃ receptor (GenBank accession no. AB045369) was cloned as previously described.³² The DNAs were inserted into the mammalian expression vector pCAGGS³³ (pCAGGS-hH₃), and cells, stably expressing the human histamine H₃ receptors, were generated by transfecting pCAGGS-hH₃ into CHO-K1 cells. HEK293/CRE- β -lactamase cells expressing rat histamine H₃ receptor were prepared as previously described.³⁴

Ligand Binding Assay. Membrane preparations from the cells expressing histamine H₃ receptors and [³H] *N*- α -methylhistamine binding studies were performed as previously described.³⁴

[³⁵S]GTP γ S Functional Binding Assay. [³⁵S]GTP γ S functional binding assays were carried out with a minor modification to the method described previously.³⁴ In brief, membranes were incubated with 200 pM [³⁵S]GTP γ S in 50 mM Tris-HCl, 100 mM NaCl, 5 mM MgCl₂, and 10 μ M 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 proximity 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 μ m 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-([1,1,1-³H]methyl)-2-(4-([3-(1-pyrrolidinyl)propyl]oxy)phenyl)-4(3*H*)-quinazolinone (a selective histamine H₃ 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 μ M). 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 mm² 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/H₂O = 50/10/40 or orally by gavage in a vehicle of 0.5% methylcellulose 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.

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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>.

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