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Development of novel 2-[4-(aminoalkoxy)phenyl]-4(3*H*)-quinazolinone derivatives as potent and selective histamine H_3 receptor inverse agonists

Takashi Mizutani, Tsuyoshi Nagase, Sayaka Ito, Yasuhisa Miyamoto, Takeshi Tanaka, Norihiro Takenaga, Shigeru Tokita, Nagaaki Sato *

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

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ABSTRACT

Novel 2-[4-(aminoalkoxy)phenyl]-4(3*H*)-quinazolinone derivatives were identified as potent human H_3 receptor inverse agonists. After systematic modification of lead **5a**, the potent and selective analog **5r** was identified. Elimination of hERG K^+ channel and human α_{1A}-adrenoceptor activities is the main focus of the present study.

The histamine H_3 receptor was pharmacologically discovered in 1983,[1] and genetically identified in 1999.[2] The genetic identification of the H_3 receptor generated significant interest, and shifted both the detailed pharmacological characterization of the receptor and associated drug discovery efforts from academia and pharmaceutical industries.[3] Signaling through the H_3 receptor activates G-proteins that inhibit adenylate cyclase activity and reduce intracellular cAMP level.[2,4] The H_3 receptor, which is predominantly expressed in the CNS, is localized on the presynaptic membrane as an autoreceptor, and negatively regulates the release and synthesis of histamine.[1] In addition, the H_3 receptor is known to modulate the release of other neurotransmitters such as norepinephrine, dopamine, acetylcholine, serotonin, and GABA.[5] Due to the effects of H_3 signaling on multiple neuronal transmitters, it has been suggested that H_3 antagonists/inverse agonists could be effective therapeutic agents for several CNS-related disorders.[6] In animal models, H_3 receptor antagonists/inverse agonists have been shown to enhance wakefulness, attentive and cognitive behaviors, and to reduce feeding and body weight.[7,8] Since the identification of the H_3 receptor genes, various classes of non-imidazole H_3 receptor antagonists have been developed to target the CNS H_3 receptor.[3,7,10] Among them, **1** (BF2.649),[9,11] **2** (ABT-239)[12] and **3** (GSK189254)[13] have entered clinical trials for treatment of CNS disorders such as excessive daytime sleepiness, schizophrenia, and cognitive dysfunctions (Fig. 1).

We previously reported a series of novel quinazolinone H_3 inverse agonists.[14] Representative quinazolinone lead **4** is shown in Figure 2. Based on structure–activity relationships (SAR) developed by modification of lead **4**, we designed and synthesized regioisomeric quinazolinone **5a** in order to further extend structural diversity. The regioisomeric quinazolinone derivative **5a** was found to have 2-fold more potent human H_3 (hH_3) activity than the original quinazolinone **4**; however, **5a** displayed relatively potent affinity for both the human ether-a-go-go-related gene (hERG) K^+ channel and human α_{1A}-adrenoceptor ($h\alpha_{1A}$) (Fig. 2). The main focus of this communication is SAR development aimed at eliminating these off-target activities while potentiating the hH3 activity.

The synthetic route for these quinazolinone derivatives reported herein is illustrated in Scheme 1. Commercially available anthranilic acids **6** or 3-amino-2-methoxyisonicotinic acid (**13**) were converted to the corresponding amide **7**. The amide was thermally condensed with 4-aminoalkoxy benzaldehyde **8** or **9** in the presence of a catalytic amount of *p*-toluenesulfonic acid, followed by oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone to furnish the desired product **5a**–**r**. Compound **13** was prepared by lithiation and carboxylation of the corresponding *tert*-butoxycarbonyl (Boc)-protected aminopyridine, as shown in Scheme 2. Commercially available aminopyridine **10** was protected with a Boc group to give **11**, which was treated with *n*-BuLi in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, followed by addition of solid CO_2 to give carboxylic acid **12**. Removal of the Boc group was effected by trifluoroacetic acid to give the desired 3-amino-2-methoxyisonicotinic acid (**13**).

* Corresponding author. Tel.: +81 29 877 2004; fax: +81 29 877 2029.
E-mail address: nagaaki_sato@merck.com (N. Sato).

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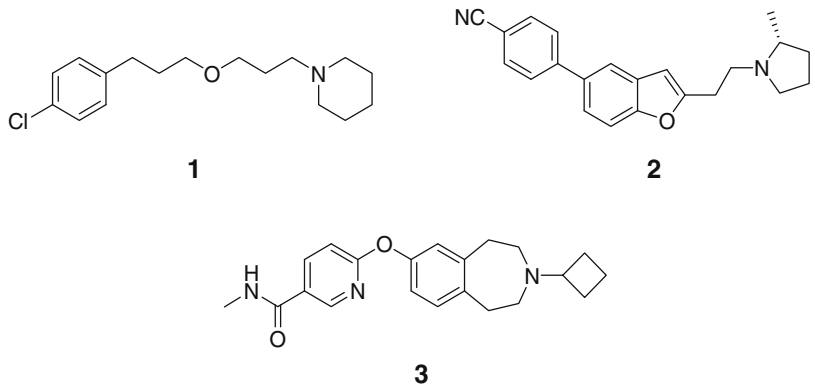
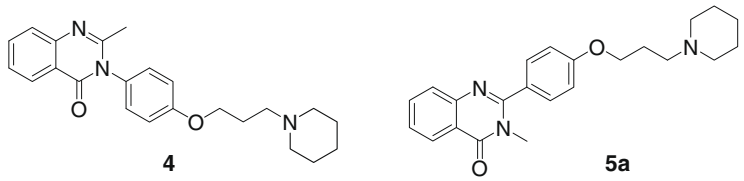


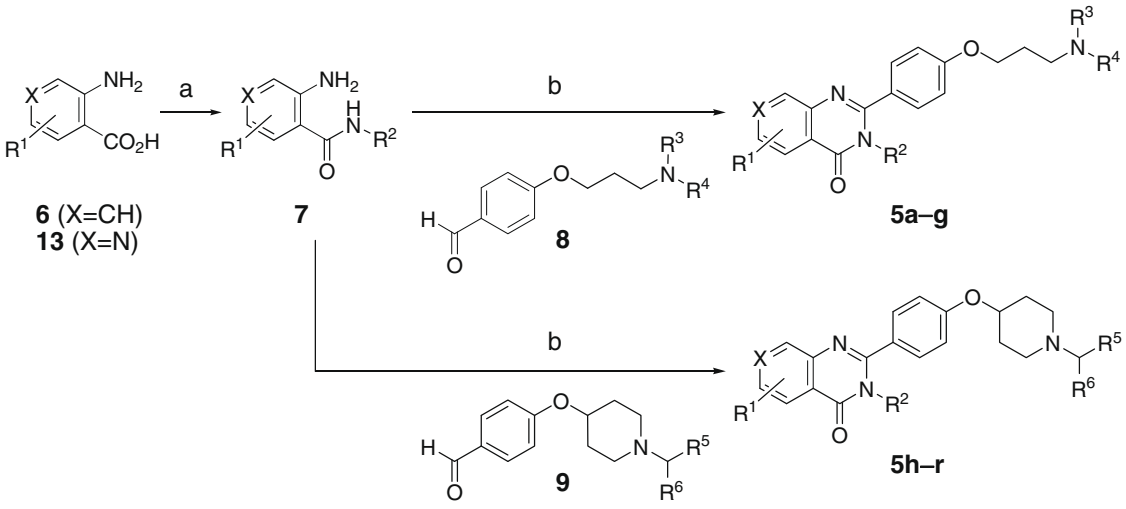
Figure 1. Histamine H_3 antagonists/inverse agonists in clinical trials.



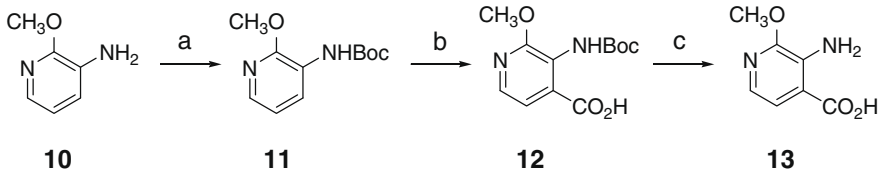
4
hH_3 IC_{50} = 3.1 nM
hERG IC_{50} = 7.9 μM
$h\alpha_{1A}$ IC_{50} = 6.5 μM

5a
hH_3 IC_{50} = 1.2 nM
hERG IC_{50} = 2.8 μM
$h\alpha_{1A}$ IC_{50} = 2.9 μM

Figure 2. Structures of quinazolinone **4** and regioisomer **5a.**

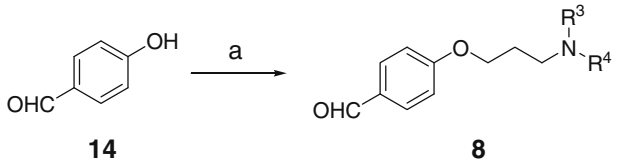


Scheme 1. Reagents and conditions: (a) 1,1-carbonyldiimidazole, DMF, 40 °C, 1–24 h, then R^2NH_2, rt, 0.5–12 h, 68–99%; (b) 4-aminoalkoxy benzaldehyde **8** or **9**, *p*-toluenesulfonic acid, 1,4-dioxane, 120 °C, 3–15 h, then 2,3-dichloro-5,6-dicyanobenzoquinone, rt, 8–42 h, 24–72%.



Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, 1,4-dioxane, reflux, 24 h, 100%; (b) *n*-BuLi, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, Et_2O, −78 °C, 1 h, then CO_2, 30 min, 70%; (c) trifluoroacetic acid, MeOH, $CHCl_3$, rt, 8 h, 88%.

Synthesis of the right hand aldehyde units **8** and **9** is illustrated in Schemes 3 and 4. Compound **8** was prepared from 4-hydroxybenzaldehyde (**14**) by alkylation with 3-bromochloropropane, followed by displacement with the desired amine (Scheme 3). Synthesis of aldehyde **9** was started from the Mitsunobu condensation[15] of methyl 4-hydroxybenzoate (**15**) and *tert*-butyl 4-hydroxy-



Scheme 3. Reagents and conditions: (a) 3-bromochloropropane, K_2CO_3, DMF, 60 °C, 4 h, then R^3R^4NH, KI, DMF, 60 °C, 10–20 h, 54–60%.

1-piperidinecarboxylate. Removal of the Boc group from **16** followed by reductive alkylation with the desired ketone afforded **17**. Ester **17** was reduced with DIBAL to give the corresponding alcohol, which was oxidized with MnO_2 to give aldehyde **9** (Scheme 4).

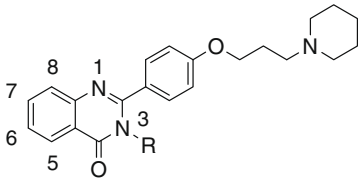
A series of regioisomeric quinazolinone derivatives were tested in the [^{35}S]GTPγS binding assay.[16] All the compounds reduced basal GTPγS binding, indicating that they are inverse agonists. Inhibitory activity for hERG K^+ channel was evaluated using the [^{35}S]*N*-[(4*R*)-1′-[(2*R*)-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxyspiro[2*H*-1-benzopyran-2,4′-piperidin]-6-yl]methanesulfonamide ([^{35}S]MK-499) binding assay[17] to assess cardiac QTc prolongation liability. Binding activity for hα_{1A} was evaluated using the [^{3}H]prazosin binding assay[18] to assess the potential risks of hypertension and agitation.

Variation of the 3-substituent on the quinazolinone ring of **5** was investigated first (Table 1). Substituted methyl derivatives **5b**–**d** displayed improved hH_3 activities compared to the parent, **5a**. The benzyl derivative **5e** was 4-fold more potent (IC_{50} = 0.31 nM), whereas the phenyl derivative **5f** was less potent than **5a**. Potent hERG inhibitory activities of the benzyl and phenyl derivatives, **5e** and **5f**, were observed, and no improvements were observed for the alkyl derivatives **5b**–**d**. A reduced hα_{1A} activity was observed in **5e**. Although the potent hH_3 activities of **5c** and **5e** were attractive, 3-methyl derivative **5a** was selected as a template for further SAR studies based on its hERG and hα_{1A} activities.

Next, the right hand piperidinopropoxy portion was optimized (Table 2). Pyrrolidine derivative **5g** showed enhanced hH_3 activity, but its off-target activities were not improved. The structurally rigid cycloalkyl piperidine derivatives **5h** and **5i** were found to possess potent hH_3 activities. The activity of cyclobutyl derivative **5h** was noticeable (IC_{50} = 0.22 nM), 5-fold improvement over the parent **5a**. In addition to its enhanced potency, **5h** exhibited reduced hERG (IC_{50} = 5.6 μM) and hα_{1A} (IC_{50} = 6.3 μM) activities.

Further optimization of **5h** by modification of the quinazolinone part is summarized in Table 3. The effect of introducing a methoxy group was examined first. Although substitution with a methoxy group had no influence on hH_3 activity, the 5- and 7-methoxy derivatives **5j** and 5**l** showed improved off-target selectivity.

Table 1
SAR of compounds **5a**–**f**.



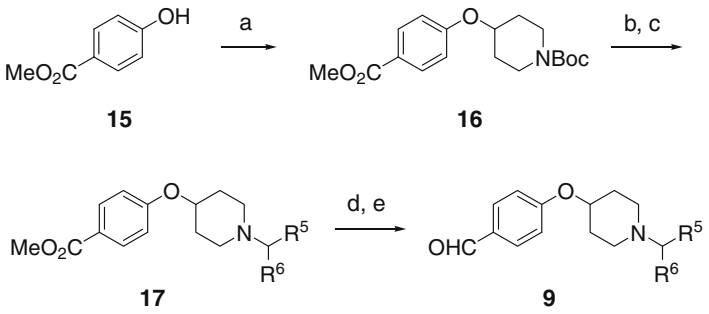
Compound	R	hH_3[a] IC_{50} (nM)	hERG[b] IC_{50} (μM)	hα_{1A}[c] IC_{50} (μM)
5a	Methyl	1.2	2.8	2.9
5b	Ethyl	0.87	1.7	1.3
5c	*n*-Propyl	0.47	1.8	1.8
5d	*i*-Propyl	0.87	3.0	1.1
5e	Benzyl	0.31	0.17	7.2
5f	Phenyl	1.6	0.46	2.4

[a] Inhibition of *R*-α-methylhistamine-induced binding of [^{35}S]GTPγS to human H_3 receptor. Values are means of at least two independent experiments. The maximum variability of each assay in this series was ±3-fold.
[b] Inhibition of [^{35}S]MK-499 binding to hERG K^+ channel in HEK293 cells.
[c] Inhibition of [^{3}H]Prazosin binding to human α_{1A}-adrenoceptor in LMtk$^-$ cells.

Importantly, 8-methoxy derivative **5m** was found to be devoid of both hERG and hα1A activity. Encouraged by the binding profile of **5m**, we introduced several substituents at the 8-position of the quinazolinone ring (**5n**–**q**). The 8-methyl and 8-chloro derivatives **5n** and **5o** were slightly more potent than **5h,** yet their hERG inhibitory activities were greater than desired. The 8-trifluoromethyl derivative **5p** showed reduced hH_3 activity with very potent hERG inhibitory activity. The binding profile of the 8-fluoro derivative **5q** was similar to that of the parent **5h**. Incorporation of a nitrogen atom into the 7-position of **5m** led to the more potent derivative **5r**, which retained good selectivity against hERG and hα_{1A}. In addition, compound **5r** was selective over other histamine receptor subtypes (hH_1, hH_2, and hH_4; IC_{50} > 10 μM).

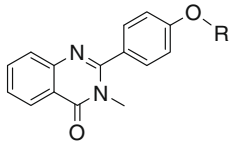
Compound **5r** was assessed in in vivo studies. Brain exposure and pharmacokinetics of **5r** were evaluated in Sprague–Dawley (SD) rats. Brain and plasma levels 2 h after 10 mg/kg oral dosing with **5r** were 2.96 nmol/g and 2.08 μM, respectively. Compound **5r** exhibited a suitable pharmacokinetic profile, as summarized in Table 4.

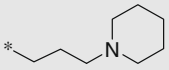
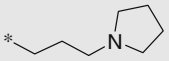
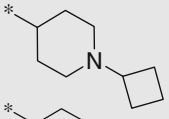
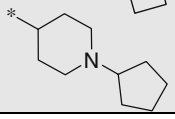
Having demonstrated excellent potency, selectivity, pharmacokinetic profile and brain penetration, **5r** was tested for brain histamine release activity in SD rats.[19] In our histamine release assay,[20] an inverse agonist (po), pargyline and a monoamine oxidase inhibitor (ip), were co-dosed in SD rats. After 2 h the whole brain was rapidly removed, and the concentration of *tele*-methylhistamine, a major extracellular metabolite of histamine, was measured.



Scheme 4. Reagents and conditions: (a) *tert*-butyl 4-hydroxy-1-piperidinecarboxylate, diisopropyl azodicarboxylate, PPh_3, THF, rt, 24 h, 62%; (b) trifluoroacetic acid, rt, 3 h; (c) $R^5R^6C{=}O$, $NaBH_3CN$, $ZnCl_2$, MeOH, rt, 16–20 h; (d) DIBAL, toluene, 0 °C, 1–3 h; (e) MnO_2, CH_2Cl_2, rt, 40–50 h, 45–49% over 4 steps from **16**.

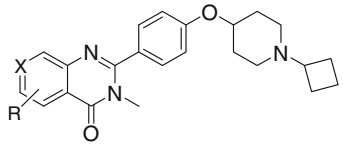
Table 2
SAR of compounds **5g–i**; variation of the basic amine unit.



Compound	R	hH_3[a] IC_{50} (nM)	hERG[b] IC_{50} (μM)	$h\alpha_{1A}$[c] IC_{50} (μM)
5a		1.2	2.8	2.9
5g		0.68	2.9	2.2
5h		0.22	5.6	6.3
5i		0.74	2.9	6.2

[a] Inhibition of *R*-α-methylhistamine-induced binding of [^{35}S]GTPγS to human H_3 receptor. Values are means of at least two independent experiments. The maximum variability of each assay in this series was ±3-fold.
[b] Inhibition of [^{35}S]MK-499 binding to hERG K^+ channel in HEK293 cells.
[c] Inhibition of [^{3}H]Prazosin binding to human α_{1A}-adrenoceptor in LMtk$^-$ cells.

Table 3
SAR of compounds **5j**–**r**; variation of the quinazolinone core.



Compound	R	X	hH_3[a] IC_{50} (nM)	hERG[b] IC_{50} (μM)	$h\alpha_{1A}$[c] IC_{50} (μM)
5h	H	CH	0.22	5.6	6.3
5j	5-OMe	CH	0.54	>10	4.2
5k	6-OMe	CH	0.31	3.8	5.9
5l	7-OMe	CH	0.34	4.3	>10
5m	8-OMe	CH	0.48	>10	>10
5n	8-Me	CH	0.10	2.0	10
5o	8-Cl	CH	0.18	1.1	6.5
5p	8-CF_3	CH	0.70	0.35	>10
5q	8-F	CH	0.25	7.9	4.5
5r	8-OMe	N	0.31	>10	>10

[a] Inhibition of *R*-α-methylhistamine-induced binding of [^{35}S]GTPγS to human H_3 receptor. Values are means of at least two independent experiments. The maximum variability of each assay in this series was ±3-fold.
[b] Inhibition of [^{35}S]MK-499 binding to hERG K^+ channel in HEK293 cells.
[c] Inhibition of [^{3}H]Prazosin binding to human α_{1A}-adrenoceptor in LMtk$^-$ cells.

Significant and dose-proportional elevation of *tele*-methylhistamine was observed in rat brain after oral administration of **5r** (Fig. 3).

In conclusion, we have designed a series of novel quinazolinone derivatives which had potent H_3 receptor inverse agonist activity. The major focus of this study was to eliminate the hERG and $h\alpha_{1A}$ activities of lead compound **5a**. A potent and selective derivative **5r** was identified which demonstrated suitable brain exposure along with an appropriate pharmacokinetic profile. Significant and dose-proportional elevation of *tele*-methylhistamine was observed in rat brain after oral administration of **5r**. Further evaluation of this class of compounds is currently underway.

Table 4
Pharmacokinetic parameters of **5r** in SD rats.[a]

Compound	Iv (1 mg/kg)			Po (3 mg/kg)		
	CL_p (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	C_{max} (μM)	$AUC_{0-\infty\ h}$ (μM h)	F[b] (%)
5r	29	4.8	2.0	0.57	2.1	48

[a] Values represent the means, $n = 3$.
[b] Based on $AUC_{0-\infty\ h}$ values after iv and po dosing.

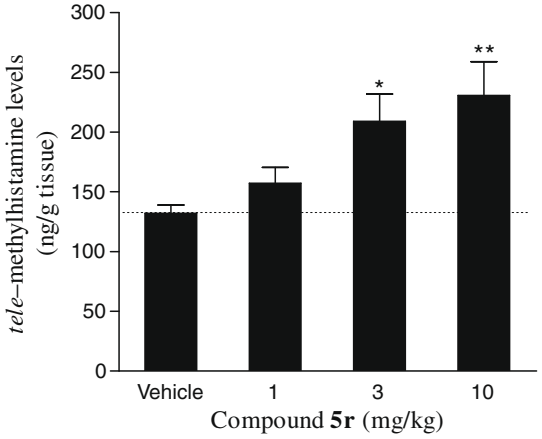


Figure 3. Brain *tele*-methylhistamine levels in SD rats after oral administration of compound **5r**. Values are means ± SE, determined from five experiments. $^*P < 0.05$, $^{**}P < 0.01$ (ANOVA Dunnett) compared with the vehicle control.

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