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Synthesis, structure–activity relationships, and biological profiles of a dihydrobenzoxathiin class of histamine H_3 receptor inverse agonists

Takahide Sasaki, Toshiyuki Takahashi, Tsuyoshi Nagase, Takashi Mizutani, Sayaka Ito, Yuko Mitobe, Yasuhisa Miyamoto, Maki Kanesaka, Ryo Yoshimoto, Takeshi Tanaka, Norihiro Takenaga, Shigeru Tokita, Nagaaki Sato *

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

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ABSTRACT

A series of novel dihydrobenzoxathiin derivatives was synthesized and evaluated as potent human histamine H_3 receptor inverse agonists. After systematic modification of lead **1a**, the potent and selective histamine H_3 inverse agonist 1-(3-{4-[(2*S*,3*S*)-8-methoxy-3-methyl-4,4-dioxido-2,3-dihydro-1,4-benzoxathiin-2-yl]phenoxy}propyl)pyrrolidine (**5k**) was identified. Compound **5k** showed good pharmacokinetic profiles and brain penetrability in laboratory animals. After 3 mg/kg oral administration of **5k**, significant elevation of brain histamine levels was observed in rats where the brain H_3 receptor was fully occupied.

Despite the pharmacological discovery of the histamine H_3 receptor in 1983,[1] it was not until 1999, with its genetic identification,[2] that the H_3 receptor gained widespread attention and redirected the detailed pharmacological characterization of the receptor and drug discovery efforts from academia and pharmaceutical industries.[3,4] Studies have shown that signaling through the H_3 receptor activates G-proteins that inhibit adenylate cyclase activity and reduce intracellular cAMP levels.[2,5] The H_3 receptors, which are predominantly expressed in the CNS, are localized on the presynaptic membrane as autoreceptors, and negatively regulate the release and synthesis of histamine.[1] The H_3 receptor is also known to modulate the release of other neurotransmitters such as norepinephrine, dopamine, acetylcholine, serotonin, and GABA,[6,7] and accordingly, it has been suggested that H_3 antagonists/inverse agonists could serve as effective therapeutics for several CNS-related disorders.[8] In animal models, H_3 receptor antagonists/inverse agonists have been shown to enhance wakefulness, improve attentive and cognitive behaviors, and reduce feeding and body weight.[9,10] It has recently been reported that BF2.649 (Chart 1), a potent and selective H_3 receptor inverse agonist, can suppress excessive daytime sleep among narcoleptic patients.[11]

First-generation imidazole-based H_3 antagonists, which possess inhibitory actions on cytochrome P_{450} activity, may cause drug–drug interactions against co-administered drugs by inhibiting hepatic clearance.[12–14] Because of these liabilities, current efforts have focused on non-imidazole classes of H_3 receptor antagonists/inverse agonists. 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 receptors.[15–17] Among them, BF2.649,[11,18,19] ABT-239,[20–22] and GSK189254,[23] as shown in Chart 1, target CNS disorders such as excessive daytime sleepiness, schizophrenia, and cognitive dysfunctions, and have successfully entered clinical trials.

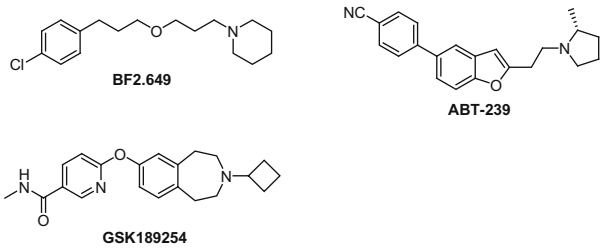

Chart 1. Structures of non-imidazole H_3 antagonists and inverse agonists.

We previously reported a series of non-imidazole H_3 inverse agonists, quinazolinone,[24,25] regioisomeric quinazolinone[26] and spiro-isobenzofuranone classes.[27] In our continuing efforts to identify structurally diverse H_3 antagonists or inverse agonists, high-throughput screening of Merck sample collections against human H_3 receptor led to the identification of the novel dihydrobenzoxathiin lead **1a,** which has an IC_{50} value of 6.7 nM (Table 1). Although **1a** is an attractive potent lead, it suffered from potent hERG K^+ channel inhibition with an IC_{50} value of 5.0 nM. In this pa-

* 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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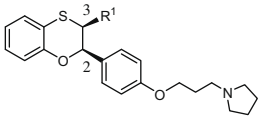
Table 1
SAR of Dihydrobenzoxathiin Derivatives; Substitution Effect at the C_3 position[a]



Compd	R^1	Human H_3[b] (IC_{50}, nM)	hERG[c] (IC_{50}, nM)
1a	Me	6.7 ± 0.6	5.0 ± 0.4
1b	Et	5.9 ± 0.8	11 ± 1
1c	Ph	3.0 ± 0.8	12 ± 2

[a] The values represent the mean ± SE for $n \geqslant 3$.
[b] Inhibition of *R*-α-methylhistamine-induced binding of [^{35}S]GTPγS at human H_3 receptor.
[c] Inhibition of [^{35}S]MK-499 binding to hERG in HEK293 cells.

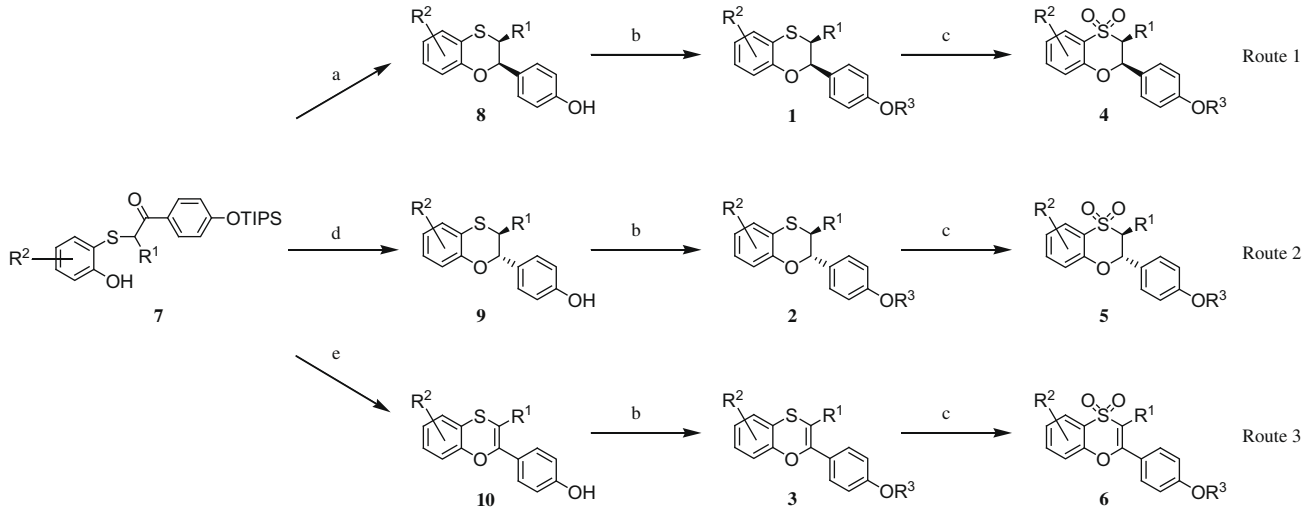
per, our modifications of **1a** to enhance H_3 activity while minimizing hERG liabilities will be described. The structure–activity relationships and biological activities of the dihydrobenzoxathiin class of histamine H_3 inverse agonists are also reported.

The synthesis of dihydrobenzoxathiin derivatives reported herein is outlined in Scheme 1. The derivatives were prepared according to the reported procedures with minor modifications,[28] and starting ketosulfides **7** were prepared according to the literature.[29] *cis*-Dihydrobenzoxathiins **1** and **4** were synthesized following Route 1. Ketosulfide **7** was reductively cyclized by Et_3SiH/TFA, followed by desilylation with TBAF in the presence of acetic acid to result in selective formation of *cis*-dihydrobenzoxathiin **8**. The phenoxy group of **8** was coupled with the desired alcohol under the Mitsunobu conditions to provide sulfide **1**. Concomitant oxidation of the sulfide and pyrrolidine groups of **1** with excess *m*-CPBA followed by selective reduction of the resulting pyrrolidine *N*-oxide using $NaHSO_3$ furnished *cis*-dihydrobenzoxathiin dioxide **4**. *trans*-Dihydrobenzoxathiins **2** and **5** were synthesized following Route 2. Reduction of ketosulfide **7** using sodium borohydride followed by acid catalyzed cyclization and subsequent desilylation with TBAF afforded *trans*-dihydrobenzoxathiin **9** in 40–49% yield.[28] The Mitsunobu alkylation of the phenol **9** with the desired aminoalcohol provided compound **2**, which was converted to *trans*-dihydrobenzoxathiin dioxide **5** following the procedure described for the preparation of **4**. Route 3 illustrates the preparation of the benzoxathiin derivatives **3** and **6**. Dehydrative cyclization of the ketosulfide **7** in the presence of *p*-toluenesulfonic acid gave benzoxathiin **10**.[28] The Mitsunobu alkylation of the phenoxy group of **10** gave benzoxathiin **3**, which was oxidized to benzoxathiin dioxide **6**. The representative potent and in vivo active H_3 inverse agonist **5k** and its enantiomer **5l** were initially prepared by chiral resolution of racemate **5e** by HPLC, using CHIRALCEL AD-H, eluting with diethylamine/isopropyl alcohol/hexane (0.5/45/54.5). The absolute stereochemistry of **5k** was determined to be (2*S*,3*S*) by a single crystal X-ray crystallography.[30]

The compounds reported herein were first subjected to the [^{35}S]GTPγS binding assay in membranes isolated from cells transfected with cloned human H_3 receptors. All the derivatives reported herein reduced basal GTPγS binding, indicating that they are inverse agonists. Then, compounds were evaluated for affinity for the hERG K^+ channel 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 to assess potential QTc prolongation liability.[31]

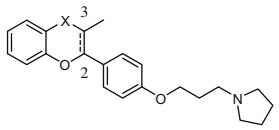
Our initial objectives were (i) further enhancement of H_3 activity and (ii) reduction of hERG inhibitory activity of the lead **1a**. In order to achieve these objectives, we initiated SAR investigations of the lead **1a**. At first, the effect of the substitution at C3 of dihydrobenzoxathiin was investigated (Table 1). Substitution with more lipophilic substituents such as ethyl and phenyl groups as in **1b** and **1c** led to slight increase in H_3 activity; however, no improvement of hERG inhibitory activity was observed.

Secondly, the C2 and C3 portions of the dihydrobenzoxathiin ring were investigated (Table 2). *trans*-Isomer **2a** showed a 3.7-fold increase in H_3 activity and a fivefold decrease in hERG inhibitory activity relative to **1a**. The benzoxathiin derivative **3a** was slightly less potent than **1a**. Next, replacement of the sulfide moiety of **1a**, **2a**, and **3a** with a sulfone group was attempted. In addition to improved H_3 potency, the sulfone derivatives **4a**, **5a** and **6a** displayed markedly decreased hERG inhibitory activity, more than 50-fold improvement over the corresponding sulfide derivatives **1a**, **2a** and **3a**. Of these, the most potent compound **5a** was selected as a benchmark compound for further modification.



Scheme 1. Reagents and conditions: (a) (i) Et_3SiH, TFA, CH_2Cl_2, −20 to 0 °C, 3.5–12 h, (ii) TBAF, AcOH, THF, 0 °C, 2 h, 51–69% from **7**; (b) R^3OH, ADDP, *n*-Bu_3P, THF, rt, 12–22 h, 58–87%; (c) (i) *m*-CPBA, $CHCl_3$, rt, 1–15 h, (ii) saturated aq $NaHSO_3$, saturated aq $NaHCO_3$, rt, 3–17 h, 13–58% for two steps; (d) (i) $NaBH_4$, MeOH, rt, 1–2 h; (ii) Amberlyst-15, toluene, rt–45 °C, 14–19 h; (iii) TBAF, AcOH, THF, 0 °C, 1–2 h; (iv) crystallized from EtOAc/hexane, 40–49% from **7**; (e) (i) TsOH, toluene, reflux, 5 h; (ii) TBAF, AcOH, THF, 0 °C, 3 h, 51–69% from **7**.

Table 2
SAR of dihydrobenzoxathiin derivatives, modification of the C_2 and C_3 portions of the dihydrobenzoxathiin ring[a]



Compd	X	$C_{2,3}$	Human H_3[b] (IC_{50}, nM)	hERG[c] (IC_{50}, nM)
1a	S	*cis*	6.7 ± 0.6	5.0 ± 0.4
2a	S	*trans*	1.8 ± 0.1	25 ± 5
3a	S	Olefin	8.6 ± 0.8	4.4 ± 0.5
4a	SO_2	*cis*	3.9 ± 1.1	440 ± 60
5a	SO_2	*trans*	0.60 ± 0.10	1300 ± 100
6a	SO_2	Olefin	0.84 ± 0.15	470 ± 60

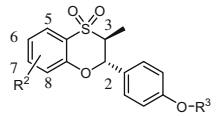
[a] The values represent the mean ± SE for $n \geqslant 3$.
[b] Inhibition of *R*-α-methylhistamine-induced binding of [^{35}S]GTPγS at human H_3 receptor.
[c] Inhibition of [^{35}S]MK-499 binding to hERG in HEK293 cells.

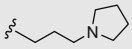
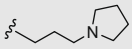
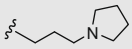
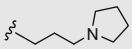
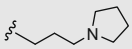
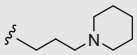
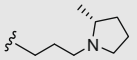
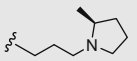
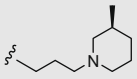
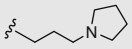
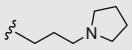
In our previous study on a quinazolinone class of H_3 inverse agonists, introduction of a methoxy group to the core quinazolinone ring was found to be effective to reduce hERG activity; hence, we applied the same strategy to the current dihydrobenzoxathiin lead (Table 3).[24,26] The introduction of a methoxy group was tolerated as in the derivatives **5b**–**e**. In this instance, the 5-and 8-methoxy derivatives **5b** and **5e** were found to exhibit reduced hERG inhibitory activities (IC_{50} = 6.3 and 7.0 μM for **5b** and **5e**, respectively). Finally, SAR around the 3-(pyrrolidin-1-yl)propoxy portion was investigated by incorporating appropriate structural modifications based on knowledge acquired from prior SAR studies.[24,25] The piperidine and 2-(*R*)-methyl pyrrolidine derivative **5f** and **5g** were equipotent to **5e**, and the antipode **5h** still had appreciable potency (IC_{50} = 0.82 nM). The 3-(*S*)-methyl piperidine derivative **5i** was 2.4-fold more potent than **5e**. The structurally rigid cyclobutyl piperidine derivative **5j** displayed not only enhanced H_3 activity (IC_{50} = 0.16 nM) but also significantly improved hERG activity (IC_{50} = 14 μM). Compounds **5e** and **5j** were further evaluated for rat hepatic clearance using the in vitro serum incubation method previously reported by our laboratory.[32] The predicted hepatic clearance of **5j** (CL_h = 43 mL/min/kg) was twice as large as that of **5e** (CL_h = 21 mL/min/kg). Evaluation of optically active isomers of the racemate **5e** revealed that the *2S,3S*-isomer **5k** is slightly more active than the *2R,3R*-isomer **5l**. *2S,3S*-isomer **5k** showed potent H_3 activity (IC_{50} = 0.43 nM) and good selectivity over hERG (IC_{50} = 4.9 μM). Given these excellent in vitro profiles, compound **5k** was selected for further evaluation.

In vitro characterization of **5k** was carried out by radioligand binding assays with [^{3}H]*R*-α-methylhistamine using human, rat and mouse histamine H_3 receptors expressed in CHO or HEK293 cell membranes. Compound **5k** displayed potent binding affinity to human, rat and mouse H_3 receptors with K_i values of 1.0 ± 0.1 nM, 2.8 ± 0.1 nM, and 5.9 ± 1.2 nM, respectively (n = 3). Compound **5k** is selective against other histamine receptor subtypes (hH_1, hH_2, hH_4; IC_{50} >10 μM).

An efficient asymmetric synthesis of **5k** was established for bulk preparation for further in vivo evaluation (Scheme 2). Compound **11** was protected as its triisopropylsilyl ether to give **12**. Ketone **12** was converted to (*E*)-alkene **14** following the literature procedure.[33] The osmium-catalyzed asymmetric dihydroxylation of alkene **14** gave (*1R,2R*)-1,2-diol **15**,[34] which was selectively dehydrated to afford epoxide **16**.[35] The epoxide ring of **16** was opened by 2-mercapto-6-methoxyphenol to give sulfide **17**. Subsequent exposure of **17** to acidic conditions afforded optically active *trans*-dihydrobenzoxathiin **19**, presumably via a rearrangement involving episulfonium ion **18**, as reported previously.[36] Desilylation of **19** with TBAF in the presence of acetic acid yielded phenol **20**. The Mitsunobu alkylation of the phenol **20** with 3-(pyrrolidin-1-yl) propan-1-ol followed by oxidation of the sulfide group afforded **5k** with high enantiopurity (95.1% ee).

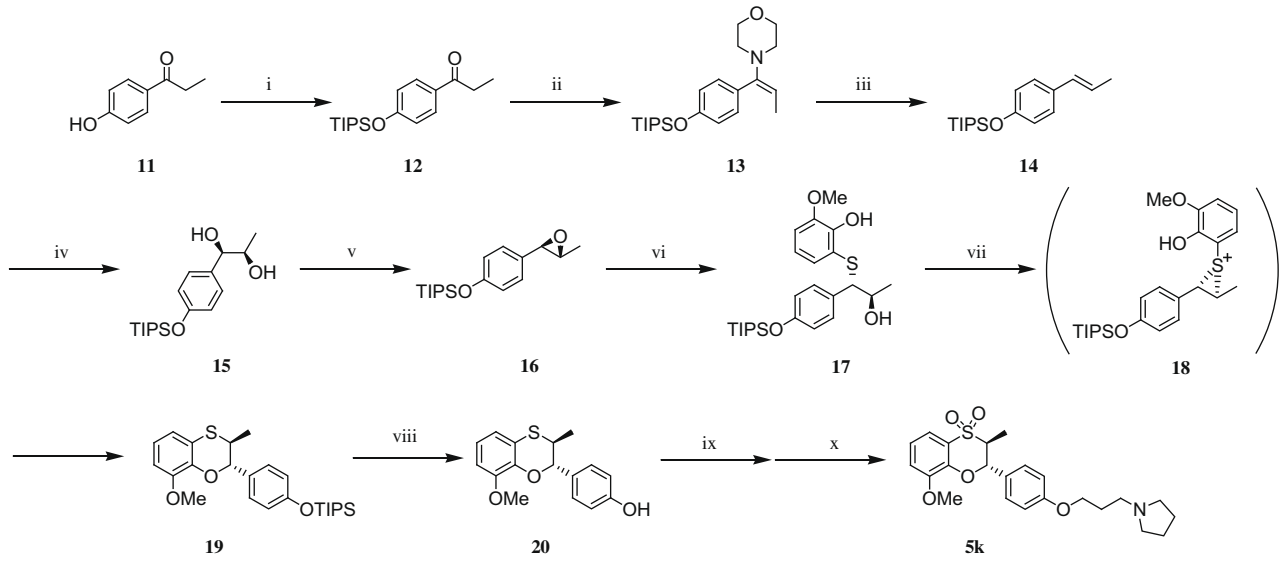
Table 3
SAR of dihydrobenzoxathiin derivatives; substitution of the dihydrobenzoxathiin ring and variation of the amine moiety[a]



Compd	Stereochemistry	R^2	R^3	Human H_3[b] (IC_{50}, nM)	hERG[c] (IC_{50}, μM)
5a	Racemate	H		0.60 ± 0.10	1.3 ± 0.1
5b	Racemate	5-MeO		0.44 ± 0.06	6.3 ± 0.3
5c	Racemate	6-MeO		0.40 ± 0.06	1.0 ± 0.1
5d	Racemate	7-MeO		0.32 ± 0.04	1.7 ± 0.1
5e	Racemate	8-MeO		0.39 ± 0.11	7.0 ± 0.5
5f	Racemate	8-MeO		0.34 ± 0.07	4.8 ± 0.3
5g	Racemate	8-MeO		0.21 ± 0.03	2.9 ± 0.1
5h	Racemate	8-MeO		0.82 ± 0.09	3.8 ± 0.4
5i	Racemate	8-MeO		0.16 ± 0.03	2.5 ± 0.1
5j	Racemate	8-MeO		0.16 ± 0.01	14 ± 1
5k	(*2S,3S*)	8-MeO		0.43 ± 0.03	4.9 ± 0.3
5l	(*2R,3R*)	8-MeO		0.607 ± 0.003	6.2 ± 0.3

[a] The values represent the mean ± SE for $n \geqslant 3$.
[b] Inhibition of *R*-α-methylhistamine-induced binding of [^{35}S]GTPγS at human H_3 receptor.
[c] Inhibition of [^{35}S]MK-499 binding to hERG in HEK293 cells.

The pharmacokinetic parameters of **5k** were evaluated in rats and dogs. The results are summarized in Table 4. Compound **5k** displayed good pharmacokinetic profiles with excellent oral bioavailability in rats and dogs. The brain penetrability of **5k** was assessed in Sprague-Dawley (SD) rats (Table 5). Compound **5k** showed good brain penetrability 2 h after 10 mg/kg oral dosing (brain = 1.38 nmol/g, plasma = 0.56 μM, brain-to-plasma ratio = 2.4). With respect to P-glycoprotein (P-gp) transporter susceptibility, the transcellular transport ratio ((B-to-A)/(A-to-B)) for **5k** in human and mouse P-gp were less than 3.0 (Table 5), indicating that the effects of P-gp mediated efflux in human and mouse brain is negligible.[37,38] Compound **5k** displayed no significant competitive inhibitory activity against CYP 3A4, 2D6, and 2C9, (IC_{50} >10 μM), and no time dependent inhibition of CYP3A4.



Scheme 2. Reagents and conditions: (i) TIPSCl, NaH, THF, 0 °C–rt, 14 h, quant.; (ii) morpholine, $TiCl_4$, toluene, reflux, 14 h; (iii) (a) BH_3–dimethylsulfide, THF, 0 °C, 5 h; (b)MeOH, rt, 12 h; (c) H_2O_2, THF, rt, 2 h, 64% from **12**; (iv) AD-mix-β, $MeSO_2NH_2$, *t*-BuOH, H_2O, 1–5 °C, 10 h, (v) (a) TMSCl, $MeC(OMe)_3$, $CHCl_3$, rt, 1 h, (b) K_2CO_3, MeOH, rt, 4.5 h, 70% from **14**; (vi), 2-methoxy-6-sulfanylphenol, DIEA, MeOH, rt, 14 h, 92%; (vii) Amberlyst-15, toluene, 45 °C, 21 h; (viii) (a) TBAF, AcOH, THF, rt, 1 h; (b) crystallized from EtOAc/hexane, 49% from **17**; (ix) 3-(pyrrolidin-1-yl)propan-1-ol, ADDP, *n*-Bu_3P, THF, rt, 13 h, 87%; (x) (a) *m*-CPBA, $CHCl_3$, rt, 2.5 h; (b) saturated aq $NaHSO_3$, saturated aq $NaHCO_3$, rt, 13.5 h, 28%.

Table 4
Pharmacokinetic parameters of **5k** in rats and dogs

	iv (Rat: 1 mg/kg, Dog: 0.3 mg/kg)			po (Rat: 3 mg/kg, Dog: 1 mg/kg)		
	CL_p (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	C_{max} (μM)	$AUC_{0-\infty}$ (μM h)	F^c (%)
Rat[a]	44	21.5	6.6	0.28	2.41	86
Dog[b]	25	10	5.7	0.16	1.21	78

[a] The values represent the mean, $n = 3$ animals.
[b] The values represent the mean, $n = 2$ animals.
[c] Based on $AUC_{0-\infty}$ values after iv and po dosings.

Table 5
Brain penetration and P-gp transport ratio of **5k**

Compd	Brain penetration in SD rats[a]			P-gp transporter assay transcellular transport ratio (B-to-A)/(A-to-B)	
	Plasma (μM)	Brain (nmol/g)	CSF (μM)	*MDR1*[b]	*mdr1a*[c]
5k	0.56	1.38	0.077	2.6	2.6

[a] The values represent the mean for $n = 3$. The concentrations were determined at 2 h after 10 mg/kg po.
[b] Transcellular transport ratio ((B-to-A)/(A-to-B)) was determined by human P-glycoprotein transporter assays with a human *MDR1*-transfected porcine renal epithelial cell line.
[c] Transcellular transport ratio ((B-to-A)/(A-to-B)) was determined by mouse P-glycoprotein transporter assays with a mouse *mdr1a*-transfected porcine renal epithelial cell line.

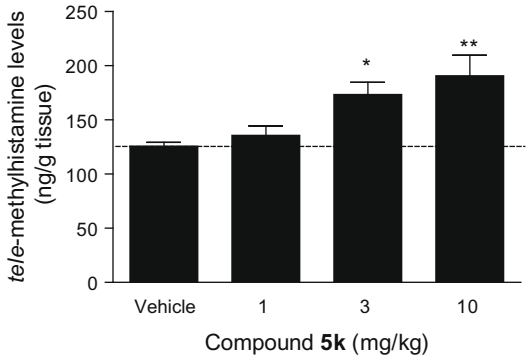


Figure 1. Brain *tele*-methylhistamine levels in SD rats after oral administration of vehicle or compound **5k** (1, 3, and 10 mg/kg). Values are means ± SE, determined from five experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ (ANOVA Dunnett) compared with the vehicle control.

Having demonstrated the excellent potency, selectivity, and pharmacokinetic profile of **5k**, this compound was tested for brain histamine release assay in SD rats.[39] As shown in Figure 1, compound **5k** showed significant and dose-proportional increase in *tele*-methylhistamine levels in SD rats following 3 mg/kg oral dosing, indicating that **5k** significantly elevated histamine levels in the rat brain. In order to investigate the correlation between efficacy in the histamine release assay and brain H_3 receptor occupancy in the rat brain, an ex vivo receptor occupancy study with **5k** was performed in SD rats. Two hours following oral administration of 3 mg/kg (the minimum effective dose in the histamine release assay) of **5k**, ex vivo receptor occupancy in rat brain slices was determined to be approximately 100%.

In conclusion, we have synthesized and evaluated a new series of dihydrobenzoxathiin derivatives as H_3 receptor inverse agonists. An initial issue was potent hERG inhibitory activity of lead compound **1a**. A number of potent H_3 active compounds with reduced hERG activity were identified. Of them, compound **5k** was shown to have excellent brain exposure, pharmacokinetic profile, and in vivo efficacy. Further evaluation of **5k** is currently underway.

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Supplementary data

Supplementary data (X-ray crystal structure of compound **5k**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.101.

References and notes

1. Arrang, J. M.; Garbarg, M.; Schwartz, J. C. *Nature* **1983**, *302*, 832.
2. Lovenberg, T. W.; Roland, B. L.; Wilson, S. J.; Jiang, X. X.; Pyati, J.; Huvar, A.; Jackson, M. R.; Erlander, M. G. *Mol. Pharmacol.* **1999**, *55*, 1101.
3. Celanire, S.; Wijtmans, M.; Talaga, P.; Leurs, R.; de Esch, I. J. P. *Drug Discovery Today* **2005**, *10*, 1613.
4. Leurs, R.; Bakker, R. A.; Timmerman, H.; de Esch, I. J. P. *Nat. Rev. Drug Disc.* **2005**, *4*, 107.
5. Wulff, B. S.; Hastrup, S.; Rimvall, K. *Eur. J. Pharmacol.* **2002**, *453*, 33.
6. Clapham, J.; Kilpatrick, G. J. *Br. J. Pharmacol.* **1992**, *107*, 919.
7. Schlicker, E.; Malinowska, B.; Kathmann, M.; Gothert, M. *Fundam. Clin. Pharmacol.* **1994**, *8*, 128.
8. Witkin, J. M.; Nelson, D. L. *Pharmacol. Ther.* **2004**, *103*, 1.
9. Esbenshade, T. A.; Fox, G. B.; Cowart, M. D. *Mol. Interventions* **2006**, *6*, 77.
10. Tokita, S.; Takahashi, K.; Kotani, H. J. *Pharmacol. Sci.* **2006**, *101*, 12.
11. Lin, J. S.; Dauvilliers, Y.; Arnulf, I.; Bastuji, H.; Anaclet, C.; Parmentier, R.; Kocher, L.; Yanagisawa, M.; Lehert, P.; Ligneau, X.; Perrin, D.; Robert, P.; Roux, M.; Lecomte, J. M.; Schwartz, J. C. *Neurobiol. Dis.* **2008**, *30*, 74.
12. Labella, F. S.; Queen, G.; Glavin, G.; Durant, G.; Stein, D.; Brandes, L. J. *Br. J. Pharmacol.* **1992**, *107*, 161.
13. AlvesRodrigues, A.; Leurs, R.; Wu, T. S.; Prell, G. D.; Foged, C.; Timmerman, H. *Br. J. Pharmacol.* **1996**, *118*, 2045.
14. Yang, R.; Hey, J. A.; Aslanian, R.; Rizzo, C. A. *Pharmacology* **2002**, *66*, 128.
15. Letavic, M. A.; Barbier, A. J.; Dvorak, C. A.; Carruthers, N. I. *Prog. Med. Chem.* **2006**, *44*, 181.
16. Berlin, M.; Boyce, C. W. *Expert Opin. Ther. Patents* **2007**, *17*, 675.
17. Wijtmans, M.; Leurs, R.; de Esch, I. *Expert Opin. Invest. Drugs* **2007**, *16*, 967.
18. Ligneau, X.; Perrin, D.; Landais, L.; Camelin, J. C.; Calmels, T. P. G.; Berrebi-Bertrand, I.; Lecomte, J. M.; Parmentier, R.; Anaclet, C.; Lin, J. S.; Bertaina-Anglade, V.; la Rochelle, C. D.; d'AnielloRouleau, F.; Gbahou, F.; Arrang, J. M.; Ganellin, C. R.; Stark, H.; Schunack, W.; Gbahou, F.; Arrang, J. M.; Ganellin, C. R.; Stark, H.; Schunack, W.; Schwartz, J. C. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 365.
19. Ligneau, X.; Landais, L.; Perrin, D.; Piriou, J.; Uquen, M.; Denis, E.; Robert, P.; Parmentier, R.; Anaclet, C.; Lin, J. S.; Burban, A.; Arrang, J. M.; Schwartz, J. C. *Biochem. Pharmacol.* **2007**, *73*, 1215.
20. Cowart, M.; Faghih, R.; Curtis, M. P.; Gfesser, G. A.; Bennani, Y. L.; Black, L. A.; Pan, L. P.; Marsh, K. C.; Sullivan, J. P.; Esbenshade, T. A.; Fox, G. B.; Hancock, A. A. *J. Med. Chem.* **2005**, *48*, 38.
21. Esbenshade, T. A.; Fox, G. B.; Krueger, K. M.; Miller, T. R.; Kang, C. H.; Denny, L. I.; Witte, D. G.; Yao, B. B.; Pan, L. P.; Wetter, J.; Marsh, K.; Bennani, Y. L.; Cowart, M. D.; Sullivan, J. P.; Hancock, A. A.; Esbenshade, T. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 165.
22. Fox, G. B.; Esbenshade, T. A.; Pan, J. B.; Radek, R. J.; Krueger, K. M.; Yao, B. B.; Browman, K. E.; Buckley, M. J.; Ballard, M. E.; Komater, V. A.; Miner, H.; Zhang, M.; Faghih, R.; Rueter, L. E.; Bitner, R. S.; Drescher, K. U.; Wetter, J.; Marsh, K.; Lemaire, M.; Porsolt, R. D.; Bennani, Y. L. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 176.
23. Medhurst, A. D.; Atkins, A. R.; Beresford, I. J.; Brackenborough, K.; Briggs, M. A.; Calver, A. R.; Cilia, J.; Cluderay, J. E.; Crook, B.; Davis, J. B.; Davis, R. K.; Davis, R. P.; Dawson, L. A.; Foley, A. G.; Gartlon, J.; Gonzalez, M. I.; Heslop, T.; Hirst, W. D.; Jennings, C.; Jones, D. N. C.; Lacroix, L. P.; Martyn, A.; Ociepka, S.; Ray, A.; Regan, C. M.; Roberts, J. C.; Schogger, J.; Southam, E.; Stean, T. O.; Trail, B. K.; Upton, N.; Wadsworth, G.; Wald, J. A.; White, T.; Witherington, J.; Woolley, M. L.; Worby, A.; Wilson, D. M. *J. Pharmacol. Exp. Ther.* **2007**, *321*, 1032.
24. Nagase, T.; Mizutani, T.; Ishikawa, S.; Sekino, E.; Sasaki, T.; Fujimura, T.; Ito, S.; Mitobe, Y.; Miyamoto, Y.; Yoshimoto, R.; Tanaka, T.; Ishihara, A.; Takenaga, N.; Tokita, S.; Fukami, T.; Sato, N. *J. Med. Chem.* **2008**, *51*, 4780.
25. Nagase, T.; Mizutani, T.; Sekino, E.; Ishikawa, S.; Ito, S.; Mitobe, Y.; Miyamoto, Y.; Yoshimoto, R.; Tanaka, T.; Ishihara, A.; Takenaga, N.; Tokita, S.; Sato, N. *J. Med. Chem.* **2008**, 6889.
26. Mizutani, T.; Nagase, T.; Ito, S.; Miyamoto, Y.; Tanaka, T.; Takenaga, N.; Tokita, S.; Sato, N. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6041.
27. Jitsuoka, M.; Tsukahara, D.; Ito, S.; Tanaka, T.; Takenaga, N.; Tokita, S.; Sato, N. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5101.
28. Kim, S.; Wu, J. Y.; Birzin, E. T.; Frisch, K.; Chan, W.; Pai, L. Y.; Yang, Y. T.; Mosley, R. T.; Fitzgerald, P. M. D.; Sharma, N.; Dahllund, J.; Thorsell, A. G.; DiNinno, F.; Rohrer, S. P.; Schaeffer, J. M.; Hammond, M. L. *J. Med. Chem.* **2004**, *47*, 2171.
29. (a) Kim, S.; Wu, J. Y.; Chen, H. Y.; DiNinno, F. *Org. Lett.* **2003**, *5*, 685; The major tautmeric form of **7** would be the keto form as shown, see: (b) Shtsuka, Y.; Oishi, T. *Chem. Pharm. Bull.* **1983**, *31*, 443.
30. Colourless block-shaped crystals of compound **5k** were obtained from a 2:2:1 solution of isopropyl alcohol/hexanes/CH_2Cl_2, M_r = 431.546, orthorhombic, $P2_12_12_1$, a = 14.015(9) Å, b = 14.234(9) Å, c = 21.145(13) Å, V = 4218(5) Å^3, Z = 8, Dx = 1.359 g cm^{-3}, monochromatized radiation λ(Mo) = 0.71073 Å, μ = 0.19 mm^{-1}, F(0 0 0) = 1840, T = 100 K. Data were collected on a Bruker CCD diffractometer to a θ limit of 26.96° which yielded 47329 reflections. There are 9077 unique reflections with 8278 observed at the 2σ level. The structure was solved by direct methods (SHELXS-97, Sheldrick, G. M. *Acta Crystallogr., Sect. A*, **1990**, *46*, 467–473) and refined using full-matrix least-squares on *F2* (SHELXL-97, Sheldrick, G. M. *SHELXL-97. Program for the Refinement of Crystal Structures.* Univ. of Göttingen, Germany). The final model was refined using 541 parameters and all 9077 data. All non-hydrogen atoms were refined with anisotropic thermal displacements. The final agreement statistics are: R = 0.037 (based on 8278 reflections with $I > 2\sigma(I)$), wR = 0.086, S = 1.06 with (Δ/σ)max < 0.01. The maximum peak height in a final difference Fourier map is 0.284 e Å^{-3} and this peak is without chemical significance. The choice of the absolute configuration is confirmed by the calculated Flack parameter, 0.06(5). CCDC 726589 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. See Supplementary data for the X-ray crystal structure of **5k**.
31. For the hERG binding assay protocol, see the following: Butcher, J. W.; Claremon, D. A.; Connolly, T. M.; Dean, D. C.; Karczewski, J.; Koblan, K. S.; Kostura, M. J.; Liverton, N. J.; Melillo, D. G. Radioligand and binding assay. PCT Int. Appl. WO2002005860, 2002.
32. Shibata, Y.; Takahashi, H.; Ishii, Y. *Drug Metab. Dispos.* **2000**, *28*, 1518.
33. Singaram, B.; Rangaishenvi, M. V.; Brown, H. C.; Goralski, C. T.; Hasha, D. L. *J. Org. Chem.* **1991**, *56*, 1543.
34. Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K. S.; Kwong, H. L.; Morikawa, K.; Wang, Z. M. *J. Org. Chem.* **1992**, *57*, 2768.
35. Kolb, H. C.; Sharpless, K. B. *Tetrahedron* **1992**, *48*, 10515.
36. Arnoldi, A.; Bassoli, A.; Caputo, R.; Merlini, L.; Palumbo, G.; Pedatella, S. *J. Chem. Soc., Perkin Trans 1* **1994**, 1241.
37. Ohe, T.; Sato, M.; Tanaka, S.; Fujino, N.; Hata, M.; Shibata, Y.; Kanatani, A.; Fukami, T.; Yamazaki, M.; Chiba, M.; Ishii, Y. *Drug Metab. Dispos.* **2003**, *31*, 1251.
38. Yamazaki, M.; Neway, W. E.; Ohe, T.; Chen, I. W.; Rowe, J. F.; Hochman, J. H.; Chiba, M.; Lin, J. H. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 723.
39. Miyamoto, Y.; Yoshimoto, R.; Yumoto, M.; Ishihara, A.; Takahashi, K.; Kotani, H.; Kanatani, A.; Tokita, S. *Anal. Biochem.* **2004**, *334*, 89.