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Synthesis and structure–activity relationships of 2-(1,4′-bipiperidin-1′-yl) thiazolopyridine as H_3 receptor antagonists

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ABSTRACT

A series of 2-(1,4′-bipiperidine-1′-yl)thiazolopyridines was synthesized and evaluated as a new lead of non-imidazole histamine H_3 receptor antagonists. Introduction of diversity at the 6-position of the pyridine ring was designed to enhance in vitro potency and decrease hERG activity. The structure–activity relationships for these new thiazolopyridine antagonists are discussed.

Histamine plays a variety of physiological roles in the central nervous system (CNS) and peripheral tissues through the four known G protein-coupled receptors, H_1, H_2, H_3, and H_4.[1] The histamine H_3 receptor located on the presynaptic nerve terminals inhibits release of the neurotransmitters histamine, dopamine, norepinephrine, acetylcholine, glutamate, and serotonin.[2] Histamine H_3 receptor antagonist-enhanced neurotransmitter release offers a promising approach to the treatment of several CNS disorders,[3,4] including attention deficit hyperactivity disorder,[5] sleep disorders,[6] epilepsy,[7] and schizophrenia.[8] Additionally, given the role of histamine in regulating appetite, H_3 receptor ligands are also reported to be active in various preclinical models of obesity.[9]

The H_3 receptor was first pharmacologically identified in 1983[2a] by Arrang et al. and later cloned in 1999 by Lovenberg et al.[10] This breakthrough sparked a significant synthetic interest in identifying structurally novel H_3 receptor antagonists in academia and industry alike.[11,12] Imidazole based H_3 antagonists were among the earliest structures investigated.[13] Two drawbacks to this class of compounds are the potential issue for drug–drug interactions through inhibition of hepatic cytochrome P_{450} enzymes and also relatively poor CNS penetration.[14]

More recently, attention in the field has turned to non-imidazole class of H_3 antagonists as these compounds offer improvements in binding affinity, CNS penetration, and reduced potential for CYP inhibition.[15] Most of the reported non-imidazole H_3 antagonists possess an aromatic ring-linker-basic amine motif. Notable examples include ABT-239,[16] GSK-189254,[17] UCL-2190,[18] A-331440,[19] and JNJ-5207852.[20]

Walczyński and co-workers identified a structurally new non-imidazole histamine H_3 receptor antagonist **I** (Fig. 1). Compound **I** showed $pA_2 = 7.25 \pm 0.07$ (electric field stimulation assay on guinea-pig jejunum).[21] Relative to compound **I**, we envisioned that substitution on the pyridine motif might enhance the H_3 activity with a belief that higher lipophilicity would increase drug distribution to the brain, potentially resulting in improved access to the H_3 receptors in the CNS. In this work, we report on synthesis and SAR of 2-(1,4′-bipiperidine-1′-yl)thiazolopyridines **II** as non-imidazole histamine H_3 receptor antagonists having substitution (R) at the C-6 position of the pyridine ring bearing the 1,4′-bipiperidine side chain. The results are described herein.

The synthesis of key intermediate 2-(1,4′-bipiperidine-1′-yl)-6-bromothiazolo[4,5-*b*]pyridine **5** began with commercially available 2-amino-3-chloropyridine **1**, which was cyclized to the thiazolo[4,5-*b*]pyridine-2(3*H*)-thione **2** using potassium ethyl xanthate in refluxing NMP (Scheme 1). Subsequent treatment of **2** with excess sulfuryl chloride provided 2-chlorothiazolo[4,5-*b*]pyridine **3**.[22] 2-(1,4′-Bipiperidine-1′-yl)thiazolo[4,5-*b*]pyridine **4** was prepared through nucleophilic displacement of the chlorine by 1,4′-bipiperidine in DMF in the presence of K_2CO_3. Bromination of **4** provided **5** in 50% yield.[23] The analogous 2-(1,4′-bipiperidine-1′-yl)-6-chlorothiazolo[4,5-*c*]pyridine **7** was similarly obtained from the readily available 5-amino-2,4-dichloropyridine **6**.

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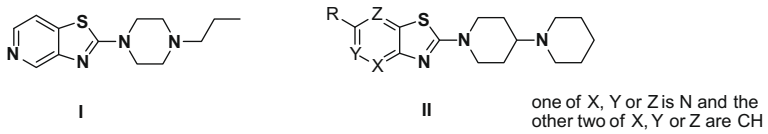
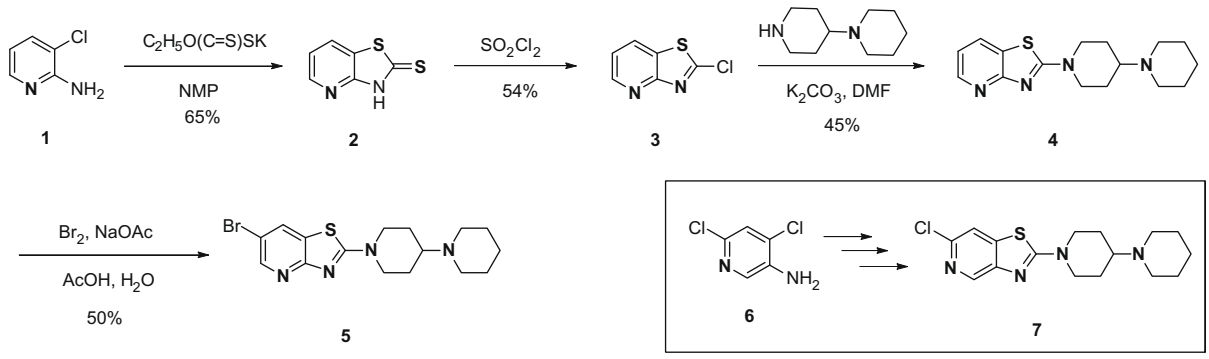


Figure 1. Structure of 1-(2-thiazolo[4,5-*c*]pyridine)-4-*n*-propylpiperazine and the target molecules of study.



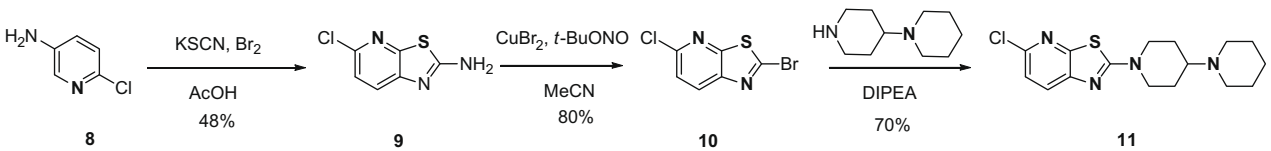
Scheme 1. Synthesis of 2-(1,4′-bipiperidine-1′-yl)-6-halothiazolo[4,5-*b*]pyridine.

Isomeric 2-(1,4′-bipiperidine-1′-yl)-5-chlorothiazolo[5,4-*b*]pyridine **11** (Scheme 2) was obtained by treatment of 3-amino-6-chloropyridine **8** with potassium thiocyanate and bromine to give 5-chlorothiazolo[5,4-*b*]pyridine-2-amine derivative **9** that was then converted to the 2-bromo derivative **10** with $CuBr_2$ and *t*-butylnitrite.[24] Microwave assisted nucleophilic displacement of the bromine by 1,4′-bipiperidine in $PhCF_3$-1,4-dioxane mixture (1:4, v/v) afforded 2-(1,4′-bipiperidine-1′-yl)-5-chlorothiazolo[5,4-*b*]pyridine **11**.

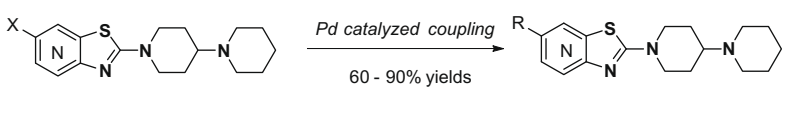
The binding affinities of the isomeric halogenated thiazolopyridines **5**, **7**, and **11** were determined as K_i values in a human H_3 recombinant assay.[25] Compound **11** displayed weak H_3 receptor affinity (K_i = 1.7 μM) when compared to compound **5** (K_i = 141 nM) and compound **7** (K_i = 10 nM) and so was not pursued further. Therefore compounds **5** and **7** provided an excellent entry into new classes of H_3 antagonists with more elaborate functionalizations of the pyridine core. The halo groups in **5** and **7** were exchanged for sterically less demanding groups as well as aryl and heteroaryl groups by means of boronic acid or Buchwald–Hartwig couplings in moderate to excellent yields (Scheme 3).[26,27]

Table 1 summarizes the human H_3 binding SAR of sterically less demanding substitution at the C-6 position of the 4- and 5-pyridyl analogs. In general, electron donating substituents (NH_2) as in **12b**, and **13b** showed less H_3 receptor binding affinity when compared to electron withdrawing substituents (CN, Cl, and NHCOMe) in **12c**, **13c**, **7**, and **12e**. The best affinity was shown by **7** (K_i = 10 nM). Owing to concern over potential reactivity of chloride in **7**, it was evaluated for glutathione adducts by incubating in human and rat liver microsomes. However, no glutathione adducts were observed at the C-6 position.

Next, we investigated the effect of aryl substitution on the 6-position to introduce lipophilic groups and the receptor binding data are summarized in Table 2. The aryl motif showed potency similar to the smaller substituents (Table 1) with the thiazolo[4,5-*b*]pyridine **12** showing slightly better affinities versus the

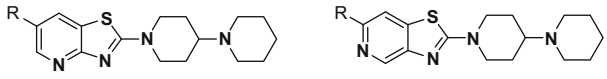


Scheme 2. Synthesis of 2-(1,4′-bipiperidine-1′-yl)-5-chlorothiazolo[5,4-*b*]pyridine.



Scheme 3. Pd catalyzed coupling of 2-(1,4′-bipiperidine-1′-yl)-6-halothiazolopyridine.

Table 1
Binding affinities of 2-(1,4′-bipiperidine-1′-yl)thiazolopyridines **12** and **13**

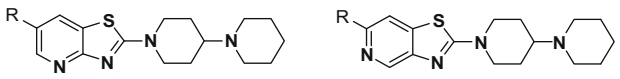


12 **13**

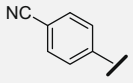
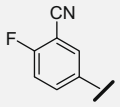
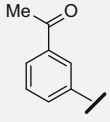
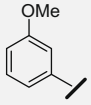
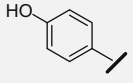
R	Compd	Human H_3[a] (K_i, nM)	Compd	Human H_3[a] (K_i, nM)
H	**4**	139	**13a**	128
NH_2	**12b**	79	**13b**	73
CN	**12c**	36	**13c**	35
Cl	**12d**	125	**7**	10
NHCOMe	**12e**	31	**13e**	53

[a] Inhibition of [^{3}H]-*N*-α-methylhistamine binding to human brain receptor. H_3 binding K_i values are the average of at least two independent determinations. The assay-to-assay variation was generally ±2-fold.[25]

Table 2
Binding affinities of aryl substituted 2-(1,4′-bipiperidine-1′-yl)thiazolopyridine



12 **13**

R	Compd	Human H_3[a] (K_i, nM)	Compd	Human H_3[a] (K_i, nM)
	**12f**	28	**13f**	126
	**12g**	23	**13g**	46
	**12h**	33	**13h**	80
	**12i**	140	**13i**	230
	**12j**	59	**13j**	213

[a] Inhibition of [^{3}H]-*N*-α-methylhistamine binding to human brain receptor. H_3 binding K_i values are the average of at least two independent determinations. The assay-to-assay variation was generally ±2-fold.[25]

thiazolo[4,5-*c*]pyridine **13**. As seen earlier electron withdrawing substituents on the phenyl ring (**12f**, **12g**, and **12h**) seem to be critical in improved binding affinities over electron donating substituents (**12i**, **13i**, and **13j**). The 3-cyano-4-fluoro phenyl derivative **12g** was the most potent derivative (K_i = 23 nM) among the aryl substituted derivatives.

The hERG inhibitory activity and rat AUC data of three potent derivatives were subsequently assessed, the results of which are summarized in Table 3.[28] All of these compounds

Table 3
hERG inhibitory activity and rat exposure data for selected analogs

Compd	hERG[a] (10 μM) (% inh)	Rat $AUC_{0-6\ h}$ (10 mpk, po) (h ng/mL)
7	59	56
12f	90	697
12g	100	339

[a] hERG IonWorks Quattro assay.[28]

exhibited high hERG inhibitory activities. The most potent derivative **7** displayed moderate hERG inhibitory activity and poor oral exposure in rat. While aryl substituted analogs **12f** and **12g** showed a slightly improved pharmacokinetic (PK) profile, high hERG inhibitory activity precluded further progression of these compounds.

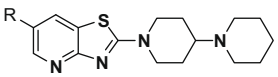
To develop a successful lead optimization strategy aiming at overcoming hERG-related safety issues, we focused our modification efforts on replacement of the aryl motif with heteroaryl substituents as summarized in Table 4.[29] Both electron withdrawing (F, CN) and electron donating (OMe) substituents as in **12l**, **12n,** and **12p**, led to a slight increase in H_3 potency but also produced increased hERG inhibitory activities. Polar functional groups, an approach that has frequently demonstrated a positive impact on hERG activity, were introduced on the pyridine moiety. To this end compounds that had hydroxy, amino, and carbamoyl groups on the pyridine moiety were synthesized. Compound **13r** (K_i = 8 nM), **12s** (K_i = 16 nM) and **12t** (K_i = 10 nM) not only showed very good binding affinity but also had the lowest hERG channel inhibitory activity. In addition to substituted pyridines, several other heteroaryl motifs were found to be tolerated albeit with slightly weaker affinity for the histamine H_3 receptor (**u**, **v**).

In an effort to further differentiate key compounds with good binding activity and lower hERG activity, several analogs were subjected to an ex vivo receptor occupancy study in the ICR mouse model.[30] Four hours following oral administration of compounds at 10 mg/kg, ex vivo receptor displacement of control in mouse brain slices was determined. The results of the correlation between potency (mouse) and H_3 receptor occupancy are shown in Table 5. Receptor occupancy was excellent (>80%) in **7** and **13k** and moderate (45%) for **12t**. There was almost no receptor occupancy for **13r**, **12s**, and **12u** thought to be a result of poor PK and/or low brain penetration. Although additional studies are needed, at least a certain level of high receptor occupancy seems necessary for H_3 antagonists to exhibit a significant increase in histamine levels in mouse brain (Table 5).

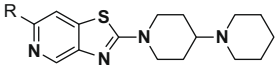
The 4-fluoropyridyl analog **13k** possessed the best overall profile in the thiazolopyridine series and was selected for further evaluation. Compound **13k** showed potent H_3 functional activity with K_b value of 0.1 nM in the human cAMP assay.[31] **13k** showed no significant competitive inhibitory activity against CYP_{450}. Furthermore, pharmacokinetic parameters of **13k** were evaluated in rats and monkeys. The results are summarized in Table 6. **13k** displayed moderate profiles in both species.

In conclusion, a new series of thiazolopyridine derivatives was evaluated as H_3 antagonists. The nature of the substituent on the pyridine ring does not seem to play a major role in the binding profile. However, the heteroaryl substituent seems critical in improving PK and hERG. Among this series, 2-fluoro pyridine **13k** showed the best overall profile. To advance our lead optimization strategy we intend to further evaluate SAR in this series to improve potency and pharmacokinetic profile. These results will be reported in due course.

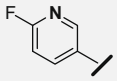
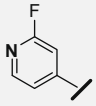
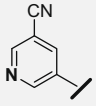
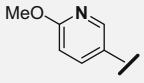
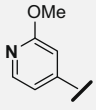
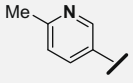
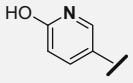
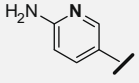
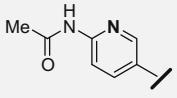
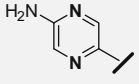
Table 4
SAR of heteroaryl substituted 2-(1,4′-bipiperidine-1′-yl)thiazolopyridine



12

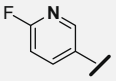
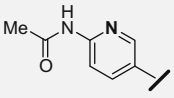
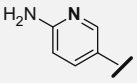
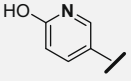


13

R	Compd	Human H_3[a] (K_i, nM)	hERG[b] (10 μM) (% inh)	Compd	Human H_3[a] (K_i, nM)	hERG[b] (10 μM) (% inh)
	**12k**	63	97	**13k**	35	51
	**12l**	13	98	**13l**	44	85
	**12m**	42	79	**13m**	20	88
	**12n**	16	89	**13n**	22	60
	**12o**	35	99	**13o**	93	90
	**12p**	4	98	**13p**	64	91
	**12q**	133	57	**13q**	104	55
	**12r**	42	3	**13r**	8	5
	**12s**	16	34	**13s**	25	5
	**12t**	10	20	**13t**	27	54
	**12u**	25	30	**13u**	25	61
	**12v**	66	20	**13v**	17	7

[a] Inhibition of [^{3}H]-*N*-α-methylhistamine binding to human brain receptor. H_3 binding K_i values are the average of at least two independent determinations. The assay-to-assay variation was generally ±2-fold.[25]
[b] hERG IW Quattro assay.[28]

Table 5
Ex vivo displacement by H_3 antagonists (10 mpk) and brain/plasma ratio in ICR mouse

Compd	R	Mouse H_3[a] (K_i, nM)	Ex vivo displacement of control @10 mpk (%)	Brain/plasma ratio @ 10 mpk; 4 h
13k		89	95	23.6
7	Cl	75	80[b]	4.52[c]
12t		20	45	3.0
12s		32	3	0.92
13r		20	1	0.14
12u		66	0	1.04

[a] Inhibition of [^{3}H]-*N*-α-methylhistamine binding to mouse brain receptor. H_3 binding K_i values are the average of at least two independent determinations. The assay-to-assay variation was generally ±2-fold.
[b] % Inhibition at 30 mpk.
[c] Dosed at 30 mpk.

Table 6
Pharmacokinetic profile of compound **13k**

Species	AUC[a] (h ng/mL)	C_{max} (ng/mL)
Rat	1069	234
Monkey	624	33

[a] $AUC_{0-6\ h}$, po, 10 mpk (rat) and $AUC_{0-24\ h}$, po, 3 mpk (monkey).

Acknowledgments

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25. For binding assays, membranes (P2 pellet) from rHu H_3-HEK cells (3 μg protein) were incubated in 200 μl 50 mM Tris–HCl, pH 7.4, with 1 nM [^{3}H]*N*-α-methylhistamine (82 Ci/mmol) and compounds at concentrations equivalent to half orders of magnitude over a five-order of magnitude range. Nonspecific binding was determined in the presence of 10^{-5} M thioperamide. After 30 min at 30 °C, assay mixtures were filtered through 0.3% polyethylenimine-soaked GF/B glass fiber filters, which were rinsed thrice with buffer, dried, impregnated with Meltilex wax scintillant, and counted. K_i values were determined from curves fit to the data using GraphPad Prism nonlinear, least-squares, curve-fitting program.
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28. For the hERG IW protocol, see the following: Sorota, S.; Zhang, X.-S.; Margulis, M.; Tucker, K.; Priestley, T. *Assay Drug Dev. Technol.* **2005**, *3*, 47–57.
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30. ICR, Imprinting Control Region mice (from Taconics, nomenclature IcrTacICR). Individual mouse cortexes were dissected and homogenized in ice cold assay buffer, 50 mM Na_2HPO_4–KH_2PO_4 buffer (pH 6.8). Samples were then frozen at −80 °C for at least 12 h. Protein concentration was determined by BCA Protein Assay. A 30 min room temperature incubation was performed, containing 140 μg/assay homogenized cortex sample, 0.1% BSA, 1 nM ^{3}H-*N*-α-methylhistamine and 10 μM thioperamide for non-specific binding or assay buffer for total binding. Incubations were performed in quadruplet and stopped by rapid filtration on a Brandel Harvester using Unifilter-96, GF/B plates presoaked in 0.3% PEI for 30 min. The remaining radioactivity was measured on a Packard TopCount-NTX. Specific binding was calculated by subtracting the non-specific binding from the total.
31. HEK 293 cells expressing recombinant human histamine H_3 receptor were stimulated 30 min with 3 μM forskolin and compounds were characterized for their ability to reverse the inhibition of cAMP formation caused by 10^{-5} M *N*-α-methylhistamine over this time. cAMP assays were performed with an AlphaScreen cAMP assay kit.