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Synthesis and structure–activity relationship (SAR) study of 4-azabenzoxazole analogues as H_3 antagonists

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ABSTRACT

The synthesis and SAR of a novel series of 4-azabenzoxazole histamine H_3 antagonists is described. Introduction of substituted phenyl, pyridyl and fused heterocyclic groups to the 6-position of the 4-azabenzoxazole core gave a series of compounds with good H_3 antagonist activity in both ex vivo and in vivo assays.

Histamine plays very important roles in physiological processes. H_1 and H_2 receptors have been successfully targeted, and therapeutic agents have been developed to treat conditions such as allergies (H_1 receptor antagonist) and gastric ulcers (H_2 receptor antagonist). The newly discovered H_4 receptor is found in immune cells, suggesting its role in regulating inflammatory responses.[1] The H_3 receptor, widely distributed in the central nervous system, modulates the release of histamine and other neurotransmitters such as acetylcholine, dopamine, norepinephrine and serotonin.[2] Central administration of a histamine H_3 antagonist leads to increased central histamine levels and hence may be useful for the treatment of a variety of CNS disorders,[3] such as attention deficit and hyperactivity disorder (ADHD),[4] sleep disorders,[5] schizophrenia[6] or obesity.[7] Early generations of H_3 antagonists, based on structures containing the imidazole moiety found in histamine,[8] suffered from CYP450 inhibition and drug–drug interactions.[9] For this reason, recent efforts have been directed to the non-imidazole H_3 antagonists, such as ABT-239,[10] GSK-189254,[11] UCL-2190,[12] A-331440 and JNJ-5207852.[13,14]

Walczyński et al. identified a structurally novel non-imidazole histamine H_3 receptor antagonist **I** (Fig. 1), which has 5-azabenzothiazole moiety as the core structure.[14] Compound **I** showed $pA_2 = 7.25 \pm 0.07$ (electric field stimulation assay on guinea-pig jejunum). In our own medicinal chemistry effort, we became interested in structurally similar cores **II** and **III**. The development of a series of derivatives of **II** as potent H_3 antagonists has been published recently.[15] Herein we describe our effort in developing 4-azabenzoxazole derivatives **III** as non-imidazole antagonists.

Synthesis of the key intermediate began with the commercially available 2-aminopyridin-3-ol **1** (Scheme 1). Condensation of **1** with carbon disulfide gave compound **2**. Methylation and substitution with piperidinopiperidine, which was identified in the previous study as a privileged scaffold, afforded compound **4**. Regioselective bromination at the 6-position gave compound **5**, which served as the key intermediate for the subsequent analogue synthesis. Suzuki coupling or Buchwald–Hartwig couplings gave a series of derivatives, and their activity was evaluated.[16]

The binding affinities of the analogues were determined as K_i values in the human H_3 recombinant assay.[17] Compound **4** (human $K_i = 1200$ nM) and **4a** (human $K_i = 4000$ nM) showed weak binding affinity, but putting substitutions groups onto the 6-position of benzoxazole core increased the binding activity (Table 1). We first added smaller groups such as vinyl (compound **5**), acetamido (compound **6**), and small cyclic amino (compound **7**) groups, and the binding affinities improved to as potent as 150 nM (compound **7**). Further extension of the substituent either by fusing a phenyl ring (compound **8**) or attaching a heterocycle (compound **9**) reduced binding affinities. Aromatic rings with different substitution groups were then directly attached to the core. Polar substituents (compounds **10, 11, 12, 14**) resulted in compounds which have binding affinities at around 300 nM, with an exception (compound **13**) that the *ortho* acetamido substitution has a fivefold drop in binding affinity. Presumably the appended phenyl group is twisted out of

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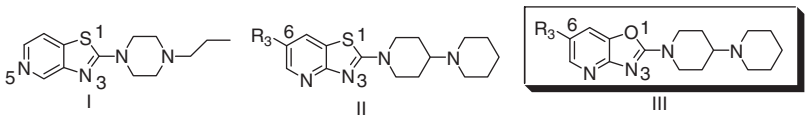
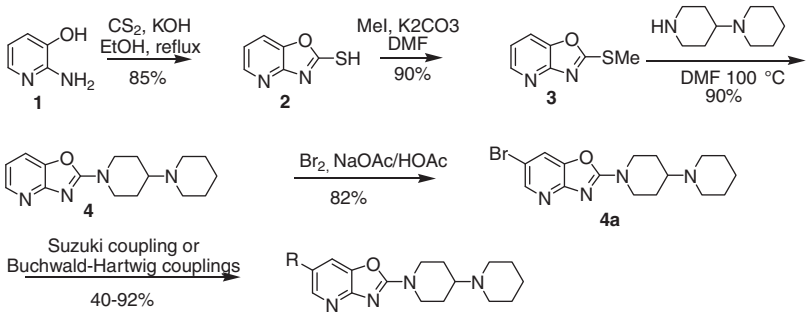
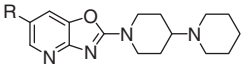


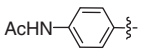
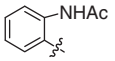
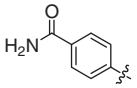
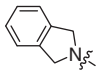
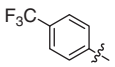
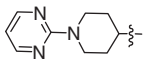
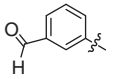
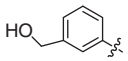
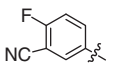
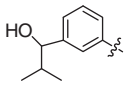
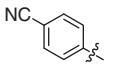
Figure 1. Structures of target molecules of study.



Scheme 1. Synthesis of the 4-azabenzoxazole derivatives.

Table 1



Compound	R	K_i (H_3, nM)	Compound	R	K_i (H_3, nM)
5		370	**12**		233
6		657	**13**		1526
7		150	**14**		288
8		260	**15**		238
9		357	**16**		58
10		249	**17**		36
11		379	**18**		37

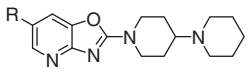
plane with the azabenzoxazole ring due to the presence of the *ortho* acetamido group, thus reducing binding affinity for the H3 receptor. Smaller substituents on the phenyl ring resulted in compounds (**16**–**18**) with 10-fold increased binding activity.

The active compounds were further tested in the ex vivo occupancy assay in the ICR mouse model.[18] Four hours following the oral administration of compounds at 10 mg/kg, ex vivo receptor displacement of control in mouse brain slices was determined. Compound **18** exhibited good receptor occupancy (59%) in mouse at the 4 h time point. It also has favourable rat p*K*, with an AUC_{0-6h} of 706 ng·h/mL (10 mg/kg) and a brain/plasma ratio of 11.3 at the 6 h time point. This is very important since H_3 activity is centrally mediated. However, compounds **17** and **18** showed significant inhibition of the hERG channel (96% and 92% at 10 μM), a classic problem associated with historic H_3 antagonists.[19a] The less active compound **13** displayed no hERG inhibition at 10 μM (−2.0%), probably due to its polar substituent group.[19b]

In order to retain activity and reduce the hERG inhibition (Table 2) we next evaluated replacing the phenyl group with heteroclyes at the 6-position. This may also be beneficial to the binding activity since it is well documented that introducing a second amine into the molecule normally improves the binding activity as potent H_3 antagonist.[20] However, one drawback of those dibasic amine compounds is that it may result in phospholipidolysis, a type of toxicity that occurs when molecules partition into lipid bilayer and impair normal phospholipid turn over.[21] We envisioned that heterocycles containing a less basic nitrogen would retain the similar activity-boosting effect, and in addition, should also have a better hERG profile due to the its reduced lipophilicity.

Pyridine was first evaluated as the phenyl replacement with 2-, 3- and 4-pyridyl substituents attached to the 6 position of the core structure (**19**, **20**, **21**). All three compounds retained good binding affinity (95, 37, 47 nM), with the 3-pyridyl slightly less active than the 2- and 4-pyridyl substituents. Then we appended

Table 2



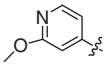
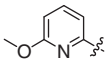
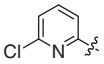
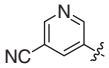
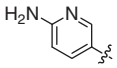
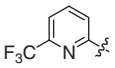
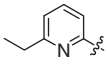
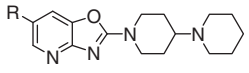
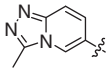
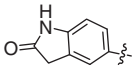
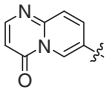
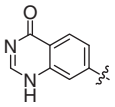
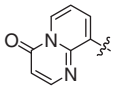
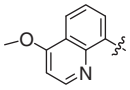
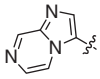
Compound	R	K_i (H_3, nM)	Compound	R	K_i (H_3, nM)
19		95	**26**		43
20		37	**27**		23
21		47	**28**		37
22		253	**29**		15
23		278	**30**		15
24		70	**31**		143
25		196	**32**		79

Table 3



	R	K_i (H_3, nM)	hERG (10 μM) (%)	R	K_i (H_3, nM)	hERG (10 μM) (%)
33		117	95		33	5
34		109	N.D.		119	23
35		153	15		25	20
36		79	96		4	62
37		35	92		18	17

different substituents onto the pyridines. Substitution of 3-pyridine with cyano and amino groups (**22**, **23**) were detrimental to activity while the F/H replacement (**24**) did not offer much benefit either. For the 4-substituted pyridine, adding methyl or methoxyl group (compounds **25**, **26**) gave compounds with inferior/comparable binding activity. Gratifyingly, substitution of 2-pyridyl with methyl, chloro, or methoxyl groups gave compounds with improved potency (**27**–**30**, compared to **21**). Changing methyl to ethyl or a trifluoromethyl group reduced the potency (**31, 32** compared to **29**), suggesting there may be a size limit at the ortho position.

Two of the most active compounds **29** and **30** were evaluated in the hERG binding assay and they demonstrated a better profile (49% and 32% inhibition at 10 μM). Unfortunately, these compounds showed very low receptor occupancy in the ex vivo mouse study, with compound **29** showing 17% occupancy and compound **30** 18% at 4 h time point, probably due to poor p*K* and/or low brain penetration.

To improve ex vivo activity and further improve hERG profile of these H_3 antagonists, we then turned to fused heterocycles to combine the beneficial effect of pyridine basic nitrogen and the small non-polar substitution groups (Table 3). Phenyl fused heterocycles such as compounds **33**–**35** showed no dramatic improvement in binding activity, but compounds **36** and **37** showed improved binding activity probably due to the basic nitrogen at the ortho position. Pyridine-fused heterocycles (**38–41**) generally exhibited better binding activity, such as compound **41** with binding affinity of 4 nM and compound **42** with binding of 18 nM. The hERG activity of these compounds was also tested, and they showed better hERG profile compared to the previous compounds (Table 3). Compound **41** had a moderate hERG inhibition of 62% while **42** showed only 17% inhibition.

Compound **41** was further evaluated in the ex vivo study. Fortunately, it exhibited very good receptor occupancy (65%) at 4 h time point with a dose of 10 mg/kg. We then studied it in the in vivo

STZ-DIO mouse assay.[22] After a two-day feed with 30 mg/kg dose, **41** demonstrated both antidiabetic (63 mg/L, glucose level reduction) and anti-obesity effect (3.85% food inhibition, 0.64% body weight loss).

In summary, a new series of 4-azabenzoxazole derivatives was evaluated as H_3 antagonists. Appending the phenyl, pyridyl or fused heterocyclic moieties to the 6-position of the core gave a series of H_3 antagonists with good in vitro binding activity. The hERG issues within this series were also addressed by incorporation of fused pyridine derived heterocycles. Compound **41** was identified as an active H_3 antagonist, with single digit in vitro binding activity and good ex vivo binding activity. It also demonstrated good in vivo activity in the rodent biological assay for obesity.

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17. For binding assays, membranes (P2 pellet) from rHu H3-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.
18. 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.
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22. Five week old male ICR mice (Taconic, Tac:Icr:Ha (ICR)fBR) were fed high fat/cholesterol diet (45% kcal fat and 0.12% cholesterol, Research Diets D04012801) for 3 weeks, and were given streptozotocin (STZ) intraperitoneally at 80 mg/kg to induce type 2 diabetes. T2D mice were chosen for the study two weeks after STZ injection (n = 12 per group, with non-fasting glucose between 250 and 500 mg/dl) and were balance into groups. Compounds were administered by PO dosing with 0.4% MC daily before the dark onset. Non-fasting glucose was monitored daily at 4 h after the lights on. Individual body weight and food intake were monitored daily.