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Structure–activity relationships of non-imidazole H_3 receptor ligands. Part 3: 5-Substituted 3-phenyl-1,2,4-oxadiazoles as potent antagonists

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Abstract—Further SAR studies on novel histamine H_3 receptor antagonists are presented. Compound **14bb** is a potent antagonist of both the rat cortical and human clone receptors, and is demonstrated to act functionally as an antagonist in an in vivo mouse dipsogenia model.

The histamine H_3 receptor has been described as a presynaptic autoreceptor in the brain, where it regulates the synthesis and release of histamine, as well as the presynaptic release of acetylcholine, γ-aminobutyric acid, dopamine, noradrenaline, and serotonin.[1] H_3 antagonists have been studied in cognitive models and they may yet prove useful for the treatment of a wide range of central nervous system disorders, including attention-deficit disorder, Alzheimer's disease, and schizophrenia.[2]

Our research began with the identification of the high throughput screening lead **1** (Fig. 1) and soon progressed to the identification of **2**.[3] This amino acid derivative bound tightly ($K_i = 1.0$ nM) to the rat cortical histamine H_3 receptor (rH_3), but once the human histamine H_3 receptor (hH_3) was cloned[1d] our assays for **2** and its analogues demonstrated a binding difference of over two orders of magnitude between the receptors (hH_3 $K_i = 760$ nM).[4] Converting the free amine to the amide of a furyl or 4-substituted phenyl ring improved binding to hH_3, and addition of a fluorine atom to provide compounds such as **3** increased binding at the hH_3 clone further (rH_3 $K_i = 1.3$ nM; hH_3 $K_i = 43$ nM).[4]

Oxadiazoles have been shown to serve as bioisosteres of carbonyls and thioguanidines in imidazole-containing H_3 antagonists.[5] We sought to apply this approach to non-imidazole H_3 antagonists. Initial results from binding assays with 1,2,4-oxadiazole analogues of **2**, **4a** and **4b** (Table 1), revealed that these were as potent as the parent molecule at rH_3 and encouraged development of the series. Oxadiazole analogues of amide **3**, **5a** and **5b**, were also comparably potent, though it is worth noting that the (L)-enantiomer of **5b** was considerably less potent (rH_3 $K_i = 110$ nM; hH_3 $K_i \geq 1000$ nM). Oxadiazole **5a** was further tested in two in vitro functional assays in order to determine whether the antagonist properties of the parent molecules remained intact. It effectively blocked (*R*)-α-methylhistamine-induced inhibition of forskolin-stimulated cAMP levels in a dose-dependent manner, giving a pK_b value of 7.22 ± 0.17 ($n = 3$) in a clonal C6 cell line expressing the long form of hH_3.[4b] In addition, **5a** effectively blocked (*R*)-α-methylhistamine-induced increases in intracellular calcium ($[Ca^{2+}]_i$) in a dose-dependent manner, giving a pK_b value of 6.81 ± 0.20 ($n = 3$) using a Fluorescence Imaging Plate Reader method and a clonal cell line co-expressing hH_3 and the G-protein chimera, Gqi_5.[6] These results showed that **5a** behaved as a functional antagonist at hH_3 receptors coupled to G_i-mediated inhibition of adenylate cyclase and Gqi_5-mediated stimulation of $[Ca^{2+}]_i$.

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The aim of these studies was to increase affinity for hH_3, while maintaining potency at rH_3, by modifying the substituents on both the oxadiazole and the terminal amide. The syntheses were accomplished by a straight forward route (Scheme 1), and resulted in a variety of compounds potent at both receptors. Commercially available 2-fluoro-4-hydroxybenzonitrile reacted with 1-bromo-3-chloropropane under basic conditions to afford alkyl chloride **6**. Displacement of the chloride with either Boc-protected piperazine or homopiperazine provided intermediate **7**, at which point the nitrile was converted to an oxadiazole by a three-step procedure. Treatment of **7** with hydroxylamine formed *N*-hydroxybenzamidine **8**, and slow addition of acyl chloride followed by heating provided **9**. After the protecting group was removed, the free amine was subjected to peptide coupling conditions to give **10** and ultimately the target series **11**.

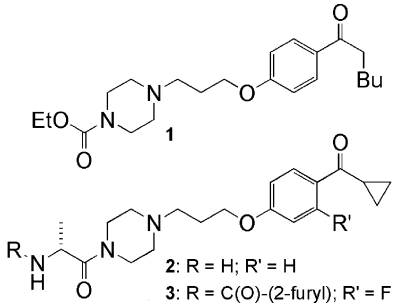
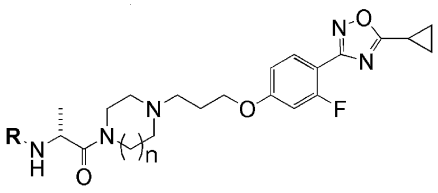


Figure 1. Structural leads.

Table 1. Binding affinities (K_i)[a] of aryloxadiazole leads

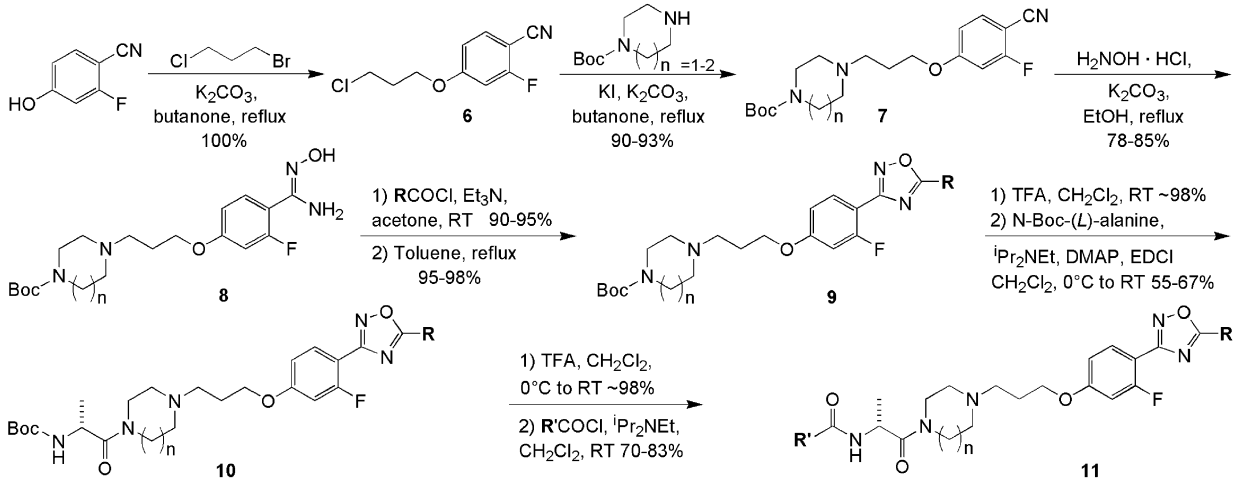


	R	*n*	rH_3	hH_3
4a	H	1	3.1	560
4b	H	2	3.0	240
5a	C(O)-(2-furyl)	1	1.1	64
5b	C(O)-(2-furyl)	2	1.5	42

[a] Values are reported as nanomolar potencies; $n \geq 3$ (SEM typically <0.2).

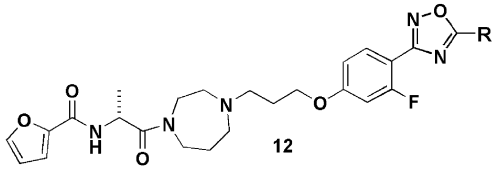
The first set of compounds derived from **5b** retained the furyl amide while the oxadiazole substituent was varied (Table 2). In vitro data consistently demonstrated that hydrophobic substituents gave the greatest potency, especially at the hH_3 receptor. Replacing one methylene unit in **12g** (rH_3 $K_i = 0.56$ nM; hH_3 $K_i = 8.6$ nM) with an oxygen atom to provide **12h** (rH_3 $K_i = 3.7$ nM; hH_3 $K_i = 110$ nM) significantly dropped potency at both the rat cortical and human cloned receptors. Similarly, compounds **12z** and **12aa** bearing a pyridyl group were much less potent at hH_3 (rH_3 $K_i = 6.9$–11 nM; hH_3 $K_i = 190$–520 nM) than **12t**, which possessed a simple phenyl moiety (rH_3 $K_i = 4.1$ nM; hH_3 $K_i = 8.6$ nM). Carbamates **12o–q** (rH_3 $K_i = 8.2$–28 nM; hH_3 $K_i = 140$–520 nM) each had poor activity, again particularly at hH_3. The greatest activity was demonstrated by those compounds bearing moderately large hydrophobic substituents. In the alkyl series, **12a** bearing a small ethyl group had the lowest activity (rH_3 $K_i = 4.1$ nM; hH_3 $K_i = 120$ nM), while compounds with larger groups, such as **12g**, **12k**, and **12r**, possessed the best potencies (rH_3 $K_i = 0.56$–0.94 nM; hH_3 $K_i = 7.5$–9.3 nM). However, a plateau appeared to be reached upon the accumulation of five or six carbons.

We then chose compounds with one of the best aryl (phenyl) and alkyl (cyclopentylmethyl) substituents in the above series and used these in our studies of the terminal amide (Table 3) among both piperazine- and homopiperazine-containing compounds. Compounds **13** possessing a phenyl group on the oxadiazole showed no strong trend when the amide was varied. Aromatic moieties appeared to be somewhat superior to alkyl groups, but in our hands two were inexplicably poor. Molecules **14** and **15** possessing a cyclopentylmethyl



Scheme 1. Preparation of target molecules.

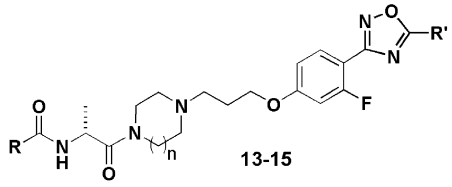
Table 2. Binding affinities[a] (K_i) of substituted oxadiazoles at rat cortical H_3 receptors and cloned human H_3 receptors



	R	rH_3	hH_3		*R*	rH_3	hH_3		*R*	rH_3	hH_3
12a	Et	4.1	120	**12j**	Cyclopentyl	0.86	15	**12s**	CH_2Ph	1.6	40
12b	$CHMe_2$	2.1	46	**12k**	Cyclopentylmethyl	0.83	9.3	**12t**	Ph	4.1	8.6
12c	CH_2CHMe_2	1.7	33	**12l**	Cyclohexyl	2.4	19	**12u**	3-Thienyl	2.6	49
12d	CMe_3	4.1	85	**12m**	Cyclohexylmethyl	2.6	21	**12v**	2-Thienyl	6.2	160
12e	CH_2CMe_3	2.4	40	**12n**	Cycloheptyl	3.3	15	**12w**	3-Furyl	1.7	85
12f	$CHEt_2$	2.3	34	**12o**	(*S*)-CH(Me)(NHBoc)	8.2	140	**12x**	2-Furyl	5.0	170
12g	$(CH_2)_2CHMe_2$	0.56	8.6	**12p**	(*R*)-CH(Me)(NHBoc)	28	520	**12y**	2-Thiazolyl	6.2	240
12h	CH_2OCHMe_2	3.7	110	**12q**	*rac*-CH(^{i}Pr)(NHBoc)	9.8	170	**12z**	3-Pyridinyl	6.9	190
12i	*rac*-2-Tetrahydrofuryl	7.7	210	**12r**	$(CH_2)_2Ph$	0.94	7.5	**12aa**	4-Pyridinyl	11	520

[a] Values (nM) were estimated from at least three separate competition experiments (SEM < 0.3).

Table 3. Binding affinities[a] (K_i) of substituted amides at rat cortical H_3 receptors and cloned human H_3 receptors



R	rH_3	HH_3	*R*	rH_3	hH_3	*R*	rH_3	hH_3	*R*	rH_3	HH_3
13a–t: $n = 1$; $R' = Ph$											
$CHMe_2$	14	110	Cyclopentylmethyl	21	130	Morpholinoethyl	6.4	88	2-Furyl	2.4	43
CH_2CHMe_2	11	56	CH_2OEt	10	100	Ph	4.6	39	2-Furyl	13	83
$(CH_2)_2CHMe_2$	14	68	(*S*)-Tetrahydrofuranyl	8.4	80	4-NC–C_6H_4	2.6	20	5-Isooxazolyl	13	86
Cyclopentyl	7.0	70	(*R*)-Tetrahydrofuranyl	4.2	92	2-Thienyl	5.0	40	3-Pyridyl	2.3	27
Cyclohexyl	6.9	49	Morpholinomethyl	8.2	92	3-Thienyl	4.2	28	2-Pyrazinyl	1.7	29
14a–ff: $n = 1$; $R' =$ Cyclopentylmethyl											
Me	8.9	200	Morpholinoethyl	1.1	37	3-F_3C–C_6H_4	10	87	3,4-diCl–C_6H_3	1.8	15
Et	7.2	170	2-Me–C_6H_4	2.4	13	3-F_3CO–C_6H_4	24	65	4-Br–C_6H_4	6.2	18
$CHMe_2$	8.0	230	3-Me–C_6H_4	4.7	31	2-F–C_6H_4	2.9	16	3-Me_2N–C_6H_4	2.6	47
CH_2CHMe_2	5.0	110	2,5-diMe–C_6H_3	6.6	75	3-F–C_6H_4	3.8	18	4-Me_2N–C_6H_4	2.4	15
Bu	2.6	79	2-MeO–C_6H_4	7.0	54	4-F–C_6H_4	1.5	17	3-NC–C_6H_4	1.5	35
CMe_3	7.0	130	3-MeO–C_6H_4	3.6	34	2-Cl–C_6H_4	2.8	8.6	4-NC–C_6H_4	1.2	9.5
CH_2CMe_3	6.4	100	4-MeO–C_6H_4	1.9	11	3-Cl–C_6H_4	6.7	36	3-Pyridyl	1.7	20
$(CH_2)_2CHMe_2$	6.1	98	3,4-diMeO–C_6H_3	2.5	24	4-Cl–C_6H_4	2.8	24	4-HO–C_6H_4	1.3	16
15a–o: $n = 2$; $R' =$ Cyclopentylmethyl											
Me	2.0	40	Bu	1.4	18	Morpholinoethyl	0.58	13	4-F–C_6H_4	1.2	14
Et	1.1	26	CMe_3	2.0	36	4-Me–C_6H_4	1.4	17	4-Cl–C_6H_4	5.2	31
$CHMe_2$	1.3	14	CH_2CMe_3	4.9	74	4-MeO–C_6H_4	1.1	9.8	3,4-diCl–C_6H_3	31	140
CH_2CHMe_2	1.1	13	$(CH_2)_2CHMe_2$	2.8	23	4-NC–C_6H_4	1.5	12			

[a] Values (nM) were estimated from at least three separate competition experiments (SEM < 0.3).

group on the oxadiazole showed a greater difference between alkyl and aryl substituents, though only in the piperazine series; in the homopiperazine series the difference was negligible. A variety of aryl groups with small substituents conferred slightly better activity than other aromatic groups, and the morpholinoethyl group was the most potent of non-aromatic substituents.

A possible trend was also seen between the three series. Just as the phenyl derivative **12k** was more potent at rH_3 than cyclopentylmethyl **12t**, compounds **14** and **15** seemed to show more potency than their analogues **13**. Furthermore, among those compounds bearing alkyl substituents (R = alkyl), compounds **15** bound with the greatest affinity for the receptors.

Compound **14bb** was selective against hH_1 ($K_i = 890$ nM), hH_2 ($K_i > 10$ μM), and hH_4 ($K_i > 10$ μM), and FLIPR and cAMP assays showed it bound to hH_3 as a competitive antagonist (data not shown). Recent cloning and pharmacological analysis of the mouse histamine H_3 receptor show a high degree of similarity to rH_3,[7] and previous studies of neurotransmitter release have demonstrated analogous functional roles across species.[8] With this idea in mind, **14bb** was then utilized in vivo in a previously characterized mouse dipsogenia

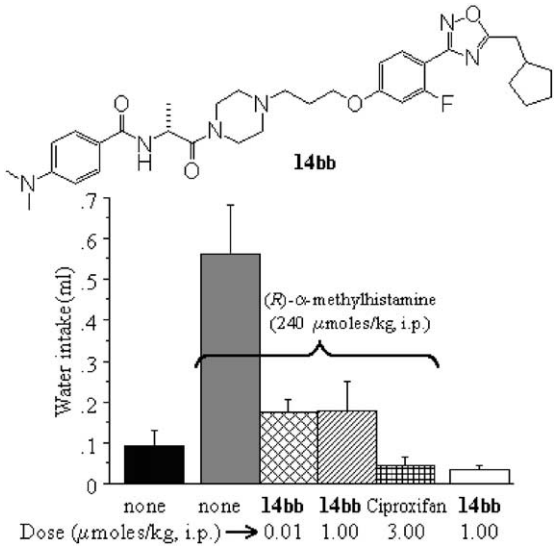


Figure 2. Dipsogenia results for **14bb**.

model[6] (Fig. 2). Peripheral or central administration of the H_3 receptor agonist (*R*)-α-methylhistamine induced a rapid and pronounced increase (up to 10 times basal levels) in water consumption that could be assessed over discrete time periods in mice. Prior peripheral or central administration of H_3 antagonists blocked this response, which is believed to be mediated by H_3 receptors in the brain. Compound **14bb** potently blocked agonist-induced dipsogenia at 0.01 μmol/kg, ip without affecting basal water intake when given alone. These results suggest that **14bb** acts functionally as an antagonist at H_3 receptors in the CNS.

In summary, we have developed a new series of non-imidazole H_3 ligands, which expands on previous examples.[9] Compounds previously reported from our laboratories[3] had first been tested only against the rat cortical receptor and later proved substantially less potent against the human cortical receptor, and subsequently at the cloned human receptor. Our new compounds have proven to be potent antagonists at both the rat cortical and cloned human receptors, and possess the balance between the receptors which we had sought. Furthermore, we have demonstrated that **14bb** acts effectively at low dose in an in vivo dipsogenia model. Additional in vivo data on these or related series will be reported elsewhere.

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