Structure - activity studies with histamine H_3-receptor ligands

Estudios sobre estructura-actividad con ligandos del receptor histamina H_3

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RESUMEN

Se han sintetizado análogos de tioperamida. Los compuestos han sido ensayados in vitro para explorar los factores que permitan diseñar compuestos derivados de la tioperamida sin grupo tiourea que mejoren la penetración cerebral. Los compuestos más activos como H_3-antagonistas contienen un átomo de nitrógeno aromático hetorocíclico sobre la cadena lateral. Estos compuestos se han empleado como cabeza de serie para obtener potentes H_3-antagonistas de histamina con estructura de ariloxietil y ariloxipropilimidazoles.

Las relaciones estructura actividad de agonistas se han revisado brevemente. Se han estudiado un grupo de análogos de (S-[2-imidazol-4-il)etil]isotiourea (imetit) con el objeto de explorar la transición entre agonistas y antagonistas. N,N'-dibutil-[S-[3-(imidazol-4-il)propil]isotiourea es un muy potente antagonistas que tiene Ki=1.5 nM.

Palabras clave: Histamina. Ariloxialquilimidazoles. H_3-agonistas. H_3-antagonistas.

ABSTRACT

Analogues of thioperamide have been synthesised and tested in vitro on rat cerebral cortex to explore structure-activity relationships with the intention of designing compounds which do not possess the thiourea group of thioperamide and which may have improved brain penetration. Compounds derived from histamine and having an aromatic nitrogen-containing heterocycle on the side-chain amino group have been found to act as H_3-antagonists. These have served as leads to provide aryloxyethyl- and aryloxypropylimidazoles which are potent H_3 antagonists of histamine.

Structure-activity relationships for agonists are briefly reviewed. Analogues of the very potent and selective agonist, imetit (S-[2-imidazol-4-yl)ethyl]isothiourea) have been studied to explore the transition between agonist, partial agonist and antagonist. The isosteric isourea is also a potent agonist. N,N'-Dibutyl-[S-[3-(imidazol-4-yl)propyl]isothiourea is a very potent antagonist having K_i=1.5 nM.

Key words: Histamine. Aryloxyalkyl-imidazoles. H_3-agonists. H_3-antagonistas.

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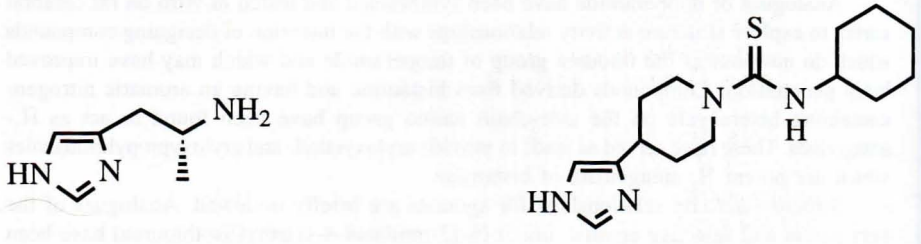
INTRODUCTION

The actions of histamine have been characterised pharmacologically as being mediated by three subtypes of receptor, designated H_1, H_2 and H_3 (1-3). The H_3 receptor was first characterised pharmacologically in 1983. It has been shown to function as a presynaptic autoreceptor inhibiting histamine synthesis and histamine release from histaminergic neurones in the central nervous system (3, 4) where it modulates the release of histamine into the synaptic cleft. Thus activation of the H_3 receptor by histamine leads to a decrease in the concentration released of neurotransmitter histamine. The H_3 receptor also appears to function as a heteroreceptor on non-histaminergic axon terminals, modulating the release of other important neurotransmitters both in the CNS and the periphery, e.g., acetylcholine, noradrenaline, dopamine, 5-hydroxytryptamine and neuropeptides (5).

The first substances used to characterise H_3 receptors in 1983 were drawn from available compounds and in particular it was shown (3) that burimamide (the first compound described as a selective H_2-receptor antagonist (2)) was actually active at 100 fold lower concentrations in antagonising histamine at the putative H_3 receptor. More potent H_2-antagonists were, however, much less active. Thus, there was no correlation between the antagonist potencies of these compounds for the established H_2 receptor and the putative H_3 receptor.

Confirmation of the H_3 classification came in 1987 with the discovery of two very potent and selective compounds, namely a chiral agonist (R)α-methylhistamine, and a competitive antagonist, thioperamide (Fig. 1) (6). (R)α-Methylhistamine was some 15 times more potent than histamine as an H_3



(R) α-methylhistamine thioperamide

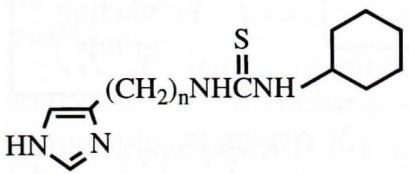
Fig. 1.—The two selective and potent compounds used to characterise histamine H_3 receptors (4). (R)α-Methylhistamine (agonist) and thioperamide (antagonist).

agonist and 100 times more potent than its S enantiomer in inhibiting histamine release in vitro from rat cerebral cortex slices. These results indicated that a stereochemically dependent interaction takes place and hence provided excellent evidence for a new (H_3) receptor.

STRUCTURE-ACTIVITY EXPLORATION OF ANTAGONISTS

Although thioperamide is a very potent antagonist in vitro (K_i = 4.3 nM) relatively high doses are required in vivo to inhibit histamine release from the brain (in the rat). This could be due to the pharmacokinetic properties of thioperamide and might also include poor penetration of the blood-brain-barrier. Unfortunately, thioperamide cannot be used for human studies because of potential toxicity and another H_3-antagonist is required to explore potential therapeutic applications.

It appears that the imidazole ring is an essential structural feature of compounds acting at H_3 receptors, but in order to use synthetically more accessible starting materials for a structure-activity exploration we investigated (7) whether we could replace the piperidine substructure of thioperamide by an open chain. H_3-Antagonist potency was found to be dependent on the chain length (Fig. 2, n=2-4), the most potent compound (n=3) which had approximately one third of the potency of thioperamide corresponds to the "ring-opened" analogue of thioperamide.



UCL No	n	$K_i \pm$ SEM (nM)
1108	2	200
1053	3	13 ± 3
1088	4	20 ± 7
thioperamide		4

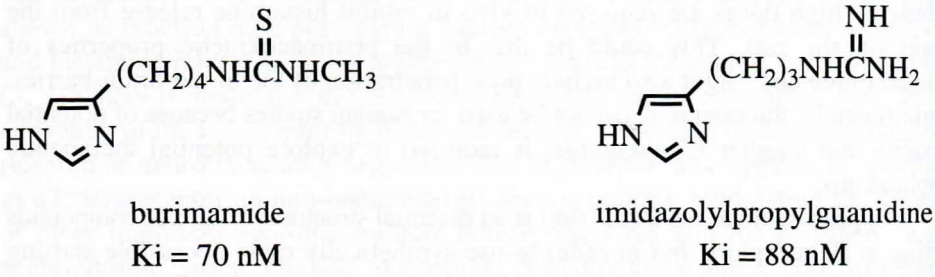
Fig. 2.—H_3-Antagonist potency depends on the chain length n for N-imidazolylalkyl-N'-cyclohexyl-thioureas, tested on rat cerebral cortex (7).

The higher homologue (n=4), is the cyclohexyl analogue of burimamide (the first described H_2-receptor histamine antagonist) and is approximately 5 times less potent than thioperamide as an H_3 antagonist, whereas burimamide is about 60 times less potent than thioperamide. Comparing these structures one may infer that the cyclohexyl group contributes additional affinity at H_3 receptors through hydrophobic interaction with lipophilic regions of the receptor and that the piperidine ring contributes selectivity by reducing the affinity for H_2 receptors.

HETEROARYLAMINES AND AROMATIC ETHERS AS ANTAGONISTS

We were interested in replacing the thiourea group of thioperamide since some thiourea compounds have been associated with toxic side effects.

The strong similarities in the structures of these H_3 antagonists with H_2 antagonists such as burimamide and imidazolylpropylguanidine (Fig. 3) led us to explore whether the polar hydrogen-bonding "urea equivalent" groups in the structures of H_2 antagonists (Fig. 4) could be used to provide H_3 antagonists.



burimamide
Ki = 70 nM

imidazolylpropylguanidine
Ki = 88 nM

Fig. 3.—H_2-Receptor antagonists, burimamide and imidazolylpropylguanidine (SK8F 91486) which were found in 1983 to be much more potent as H_3-receptor antagonists (3).

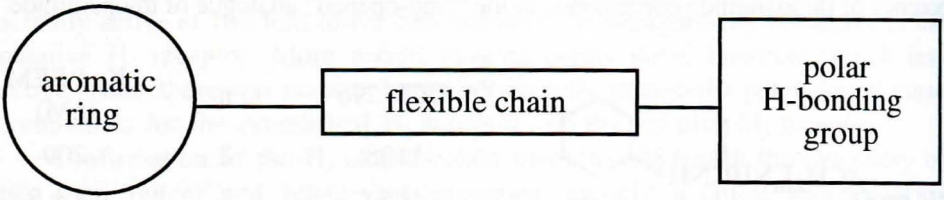


Fig. 4.—Generalised structure which encompasses most of the H_3-receptor antagonists (12b).

Groups such as NH-Het (where Het = an aromatic nitrogen heterocycle) are much weaker hydrogen-bonding groups than amides or thioamides (9) and, since hydrogen-bond strength appears to reduce brain penetration, these groups had been investigated in the design of the brain-penetrating H_2 - antagonist, zolantidine (in which Het = 2-benzothiazolyl) (Fig. 5).

As shown in Table 1 the 2-benzothiazolyl derivative of histamine (UCL 1029) had K_i = 330 nM as an H_3 antagonist thus indicating that heterocyclic groups can be used in structures for antagonist activity at H_3 as well as H_2 receptors (11). The 2-pyridyl analogue (UCL 1038) was similarly active and introducing a nitro substituent markedly increased the potency (UCL 1040 had K_i = 29 nM). Other aminopyridines substituted by electronegative groups in the 5-position, namely CF_3 (UCL 1235) and CO_2Me (UCL 1249) were also markedly

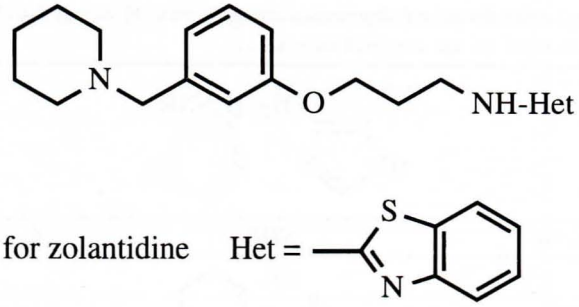


Fig. 5.—Zolantidine, a brain-penetrating H_2-receptor agonist which was designed through reducing H-bonding capability (10).

more potent. On the other hand, a 5-NH_2 group (UCL 1334) did not increase potency. We also investigated the replacement of NH by S and this structural modification led to a further increase in potency, providing UCL 1199 (Table 2) having K_i = 5 nM ie equipotent with thioperamide.

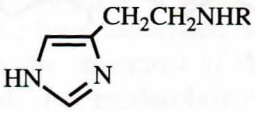
Since the 2-amino-5-trifluoromethylpyridyl group (UCL 1235) increased potency by 10 fold relative to the corresponding cyclohexylthiourea (UCL 1108), the same replacement was made in thioperamide to give compound UCL 1283 (Table 1). In this case however, the compound was not more potent but actually some 10 fold *less* potent than thioperamide. This suggests that the thioureas and aminoheterocycles do not bind in the same manner to the H_3 receptor.

Since the nitro group makes the pyridine ring much less basic it seemed worthwhile to check whether a ring nitrogen atom was needed at all and so the corresponding nitroaniline (UCL 1205) was synthesised; this compound (UCL 1205) was found to have similar potency to that of the nitropyridine analogue (UCL 1040) (Table 2).

To remove the hydrogen-bond donor character of the nitroaniline, the corresponding ether (UCL 1291) was made. This, too, was found to be active and therefore it was followed up by making a series of phenoxyethylimidazoles. At first, it seemed that the presence of a mesomeric electron-withdrawing group enhanced potency. However this did not have to be in the *para* position and *meta* substituted compounds were also found to be active (e.g., UCL 1340). The most potent was UCL 1306, K_i = 5 nM which has a p-carbomethoxy substituent. Furthermore, the compound UCL 1344 with a non-polar lipophilic electron-releasing propyl substituent was also active.

Higher homologues were also investigated and here, some compounds were more potent than the phenoxyethyl analogues eg for p-NO_2 and p-CF_3 but others were not significantly different (Table 3).

Table 1.—Heteroaryl derivatives of histamine as antagonists at H_3-receptors (heteroaryl replacing thioureido) tested in vitro on rat cerebral cortex (11).



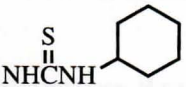
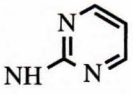
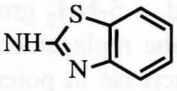
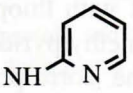
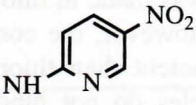
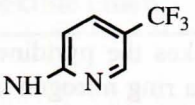
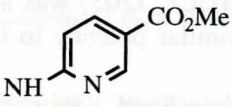
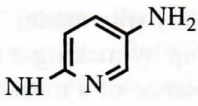
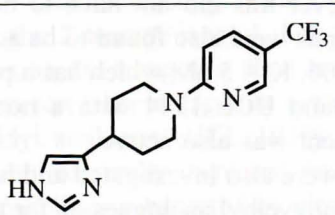
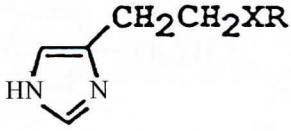
UCL No	NHR	K_i (nM)
1108		200
1017		2100
1029		330
1038		200
1040		29
1235		17
1249		42
1334		186
1283		42

Table 2.—Aryloxethylimidazoles and related compounds as H_3-receptor antagonists tested in vitro on rat cerebral cortex (11).



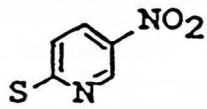
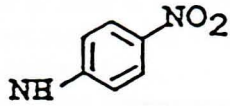
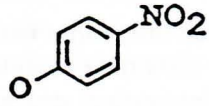
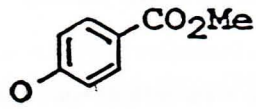
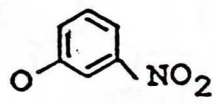
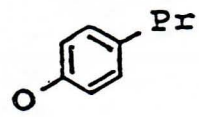
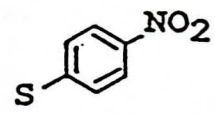
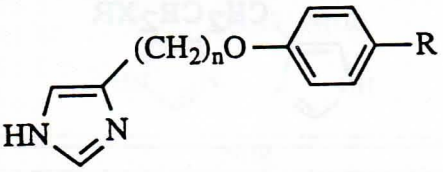
UCL N.º	XR	Ki ± SEM nM
1199		5 ± 1
1205		23 ± 9
1291		35 ± 6
1306		5 ± 2
1340		12 ± 2
1344		19 ± 9
1384		9 ± 3

Table 3.—p-Substituted phenoxyethylimidazoles and phenoxypropyl homologues as H_3-receptor antagonists tested in vitro on rat cerebral cortex.

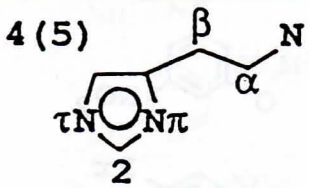


	Ki ± SEM (nM)	
R	n = 2	n = 3
pNO_2	35 ± 6	6 ± 1
pCN	9 ± 5	12 ± 3
pCF_3	68 ± 18	14 ± 6

METHYLHISTAMINES AS AGONISTS

All the monomethyl-substituted histamines have been synthesised and tested for agonist activities at all three histamine receptor subtypes (Table 4). These studies have led to some very interesting structure-activity relationships and excellent receptor selectivities (see refs. 12-13).

Table 4.—Agonist activities in vitro of methylhistamines at all three histamine receptors, H_1, H_2 and H_3, determined in vitro (6, 13). Potencies given relative to histamine = 100.



	H_1 guinea-pig ileum	*H_2 guinea-pig atrium*	*H_3 rat brain*
Histamine	100	100	100
N^{π}-Me	<0.01	<0.1	<4
2-Me	17	4.4	<0.1
N^{τ}-Me	0.42	<0.1	<4
β-Me	0.83 (rac)	0.89 (rac)	280 (rac)
α-Me	0.36 (rac)	0.74 (rac)	1550 (R)
			13 (S)
N-Me	72	74	270

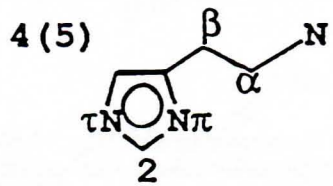
N-Methylation in the imidazole ring effectively removes agonist activity at all three receptor subtypes, but N-methylation at the side-chain amino group is well tolerated, and this also holds for side-chain N, N-dimethylation.

C-Methylation in the imidazole ring renders the compounds inactive as H_3 agonists but introduces selectivity towards H_1 or H_2 receptors, 4(5)-methylhistamine being a highly selective H_2 agonist.

C-Methylation in the side chain introduces selectivity towards H_3 receptors. Thus α and β methylhistamines have less than 1% of the potency of histamine as agonists at H_1 and H_2 receptors, but, remarkably, are more potent than histamine as H_3 agonists. Furthermore, the introduction of C-methyl also induces chirality in the molecule and, although histamine is achiral, it has been shown that H_3-agonist activity mainly resides in the R enantiomer, (R)α-methylhistamine being some 15 times more potent than histamine on rat cerebral cortex slices. The eudismic ratio is approximately 100. This compound has also been into human volunteer studies (16). (R)α-Methylhistamine, when tritiated is especially useful as a radioligand (6).

Dimethylhistamines have also been investigated as agonists. Thus it has been shown that for α,β-dimethylhistamine (Table 5), which has two chiral centres, the (R, S) isomer is the most potent having 18 times the potency of histamine in vitro on rate cerebral cortex slices (14) and, again, the eudismic ratio is approximately 100.

Table 5.—Agonist activities of some side-chain substituted dimethylhistamines at histamine H_3 receptors tested in vitro on rat cerebral cortex slices (14, 15). Potencies given relative to histamine = 100.



Histamine		100
N^{α},N^{α}		170
a,N^{α}	(R)	4.1
	(S)	0.13
α,α		270
α,β erythro (±)		1000
	(R,S)	1800
	(S,R)	18
α,β threo (±)		33
β,β		3.6

Some studies have been made at joining the alkyl groups into an aliphatic ring system (17) and recently a homologous piperidine derivative immepip (*imi*dazolyl*meth*yl*pip*eridine) (Fig. 6) has been shown to be equiactive with (R)α-methylhistamine (17, 18).

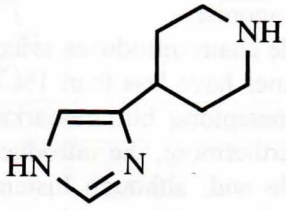
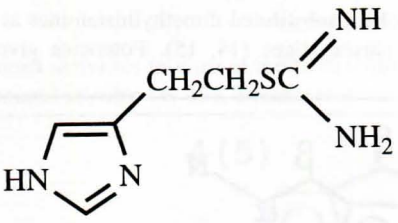


Fig. 6.—Immepip, a potent H_3-receptor agonist which incorporates a piperidine ring.

IMETIT, A VERY POTENT ISOTHIOUREA AGONIST

Several laboratories identified imetit (S-[2-(*imi*dazol-4-yl)*e*thyl]*iso*thiourea, Fig. 7) as a highly potent and selective H_3-receptor agonist (19-22) which is 4 times more potent than (R)α-methylhistamine and approximately 60 times more potent than histamine itself.



UCL 1095

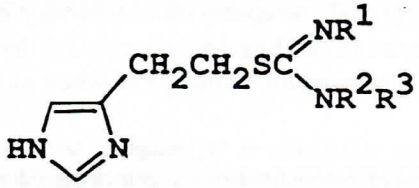
SK&F 91105

VUF 8325

Fig. 7.—Imetit, a highly potent H_3-receptor agonist. EC_{50} = 1 nM (i.e., 60 times the potency of histamine) as an inhibitor of [^{3}H]histamine release from rat cerebral cortex (19).

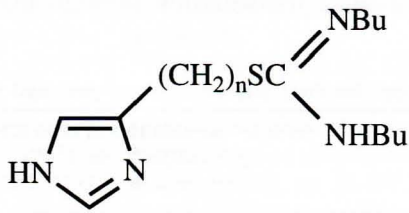
It was of interest to us to explore the structural specificity of imetit for agonist action at the H_3 receptor. It was found (23) that a single methyl group (Table 6, UCL 1123, R_1=CH_3, R_2=R_3=H) was tolerated for agonism but the compound was less active than imetit although still 4 times more potent than histamine. Increasing the size of the substituent to ethyl afforded a partial agonist and increasing the size still further to propyl or cyclohexyl gave antagonists.

Table 6.—Imetit analogues. From agonist, through partial agonist to antagonists at H_3 receptors. Tested in vitro on rat brain cerebral cortex, agonist (or partial agonist) activities are given as EC_{50}'s, antagonists as K_i ($\pm$SEM) (23).



UCL N.°	*R1*	*R2*	*R3*	*Action*	*EC_{50} or K_i (nM)*
Imetit	H	H	H	agonist	1.0 ± 0.3
1532	Me	H	H	agonist	15 ± 3
1538	Et	H	H	partial agonist	160 ± 60
1538	Pr	H	H	antagonist	22 ± 8
1209	C_6H_{11}	H	H	antagonist	18 ± 8
1124	Me	Me	H	antagonist	51 ± 22
1428	H	Me	Me	antagonist	ca 500
1140	Me	Me	Me	antagonist	> 500

A second methyl group, on the same or different isothiourea nitrogen atoms converted the compound into an antagonist. Higher alkyl groups (one on each of the two isothiourea nitrogen atoms) enhanced the antagonist potency, affinity increasing in proportion to the number of methylene groups up to an optimum where $R_1 = R_2$ = butyl, which had K_i = 5.4 nM. The higher homologue of this compound is the imidazolylpropyl-N,N[1] - dibutylisothiourea (UCL 1524) had a K_i = 1.5 nM ie three times more potent than thioperamide (Fig. 8). S-

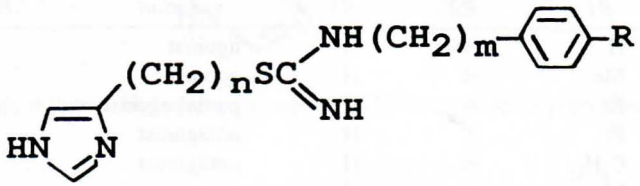


UCL No	n	Ki ± SEM (n M)
1176	2	5.4 ± 0.9
1524	3	1.5 ± 0.3
1649	4	11 ± 3

Fig. 8.—Imidazolylalkyl-N,N'-dibutylisothioureas are potent H_3 antagonists on rat cerebral cortex.

Imidazolylpropylisothioureas substituted by aralkyl have been shown to be very potent antagonists by van der Goot *et al.* (22) (Table 7). The p-chlorobenzyl derivative, clobenpropit is the most potent H_3-antagonist published so far (K_i =0.12 nM) for antagonising H_3 receptors on electrically evoked contractions of the isolated guinea-pig ileum (22). The p-iodophenylethyl derivative, iodophenpropit has been proposed (24) as a useful radioligand when substituted by ^{125}I.

Table 7.—Potent isothiourea antagonists at H_3 receptors tested in vitro against histamine inhibition of electrically evoked contractions of the guinea-pig ileum (22).



n	m	R	K_B(nM)	
2	1-4	H	10-25	
3	1-4	H	1.6-5	
3	1	Cl	0.12	Clobenpropit
3	2	I	0.50	Iodophenpropit

H_3-RECEPTOR FUNCTIONS AND POTENTIAL DRUG TARGETS

No H_3 antagonist is yet available for investigation of the role of H_3 receptors in humans to verify the potential therapeutic applications for the H_3-receptor histamine antagonists. At present one may only extrapolate from animal data and speculate (Table 8). For example, an H_3 antagonist entering the brain would permit an increase in histamine transmission through histaminergic pathways

Table 8.— H_3 Receptors as sites for drug intervention and potential therapies.

The physiological consequences of H_3 receptor stimulation in man are not proven but possible effects are:

In the brain:	reduce alertness?	Increase food intake?
In the lung:	reduce severity of asthma?	
In the stomach:	reduce gastric acid secretion?	
In the gut:	reduce motility?	

Hence, useful drugs would most likely be:

Brain-penetrating antagonist
Peripherally-acting or brain-penetrating agonists.

and therefore potentiate the role of histamine in controlling the waking state (25) and so act as a stimulant. Histamine H_3 receptor antagonists could also increase locomotor activity (26) and pituitary hormone (27) secretion, act as anticonvulsants (28) and antinociceptives (29), and suppress food intake (30).

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