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Dibasic non-imidazole histamine H_3 receptor antagonists with a rigid biphenyl scaffold

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Abstract—A class of rigid, dibasic, non-imidazole H_3 antagonists was developed, starting from a series of previously described flexible compounds. The original polymethylene chain between two tertiary amine groups was replaced by a rigid scaffold, composed by a phenyl ring or a biphenyl fragment. Modulation of the distance between the two amine groups, and of their alkyl substituents, was driven by superposition of molecular models and docking into a receptor model, resulting in the identification of 1,1′-[biphenyl-4,4′-diylbis(methylene)]bis-piperidine (**5**) as a subtype-selective H_3 antagonist with high binding affinity ($pK_i = 9.47$) at human H_3 histamine receptor.
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The biogenic amine histamine activates different G protein-coupled receptors (H_1, H_2, H_3, and H_4)[1] and exerts multiple pharmacological effects, both in the central nervous system (CNS) and in peripheral tissues. The histamine H_3 receptor was initially described as an autoreceptor,[2] mainly expressed in CNS,[3] modulating the biosynthesis and release of histamine from histaminergic neurons. Indeed, the H_3 receptor is also located presynaptically on non-histaminergic neurons, modulating the release of other neurotransmitters, such as dopamine, serotonin, norepinephrine, acetylcholine, and neuropeptides.[4–7] Different potential therapeutic applications have been proposed for H_3 receptor antagonists, mainly for the treatment of CNS disorders. Strong indications, including active clinical trials, support their use for cognitive and neurological disorders, such as epilepsy, Alzheimer's disease, memory and learning deficits, sleep and wakefulness disorders, narcolepsy, and obesity.[8–10]

Recently, the attention in the field of H_3 antagonists turned toward compounds lacking the imidazole ring that characterized classical antagonists.[11] In a previous work, we described a series of non-imidazole H_3 antagonists characterized by a basic piperidine ring connected through an alkyl spacer of variable length to another piperidine group (symmetrical compounds) or to heterocyclic rings (asymmetrical compounds).[12,13] These compounds showed interesting H_3 binding affinity and antagonistic activity, as well as good selectivity for the H_3 receptor. Compounds carrying two piperidine rings were the most potent ones, with an optimal length of the alkylene spacer comprised between 7 and 10 methylene groups. Despite the good biological data, polymethylene chain flexibility hampered the interpretation of structure–activity relationships (SARs) in terms of spatial requirements. To further investigate these requirements, we modulated the structure of the most potent derivative 1-1′-nonamethylenedi-piperidine synthesizing a new series of compounds (Table 1) in which both the basic centers and the spacer were varied. The piperidine moiety was replaced by primary, secondary or tertiary amines or by a non-basic fragment. The central portion of the polymethylene chain was substituted by a rigid biphenyl scaffold, linked to the two basic groups through one methylene spacer

Keywords: Histamine; H_3 receptor antagonists; Non-imidazole; Biphenyl; Dibasic.
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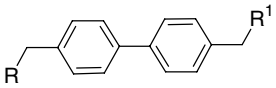
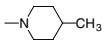
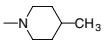
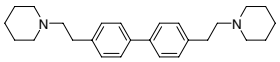
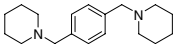
Table 1. Histamine H_3 receptor affinity (pK_i) for human and rat H_3 receptor



2-11, 13-15

Compound	R	R^1	Human H_3[a] (pK_i)	Rat H_3[b] (pK_i)
2[17]	$-NH_2$	$-NH_2$	5.35 (±0.01)	5.06 (±0.17)
3	$-N(CH_3)_2$	$-N(CH_3)_2$	8.73 (±0.08)	8.18 (±0.06)
4	$-NH-C_5H_{11}$	$-NH-C_5H_{11}$	6.12 (±0.06)	5.48 (±0.08)
5[24]			9.47 (±0.14)	8.92 (±0.03)
6			8.21 (±0.03)	7.82 (±0.05)
7			6.40 (±0.09)	6.17 (±0.05)
8[15]			7.91 (±0.09)	7.09 (±0.05)
9[15]			5.85 (±0.20)	5.41 (±0.01)
10[15]			6.25 (±0.08)	5.68 (±0.06)
11			7.43 (±0.01)	6.67 (±0.07)
13			7.33 (±0.11)	6.94 (±0.08)
14			8.20 (±0.03)	7.33 (±0.07)
15			8.77 (±0.08)	8.04 (±0.15)
17			8.93 (±0.17)	8.40 (±0.08)
18[18]			5.91 (±0.09)	5.39 (±0.09)
	Thioperamide		8.59 (±0.05)[c]	7.28 (±0.15)[c]

[a] Inhibition of [^{3}H]RAMHA binding to GPCR97-transfected SK-N-MC cells stably expressing the human histamine H_3 receptor.
[b] Inhibition of [^{3}H]RAMHA binding to rat brain membranes. Values are means of three experiments, standard error is given in parentheses.
[c] Ref. 12.

at each position. This structure was selected on the basis of its similarity to that of 1-1′-nonamethylenedi-piperidine in its fully extended conformation, as can be seen in Figure 1, where the protonated piperidine NH groups of this flexible compound were superposed

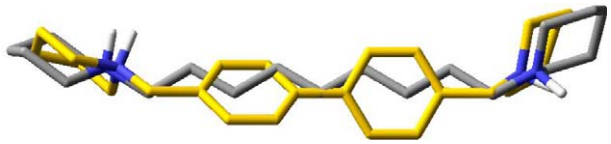
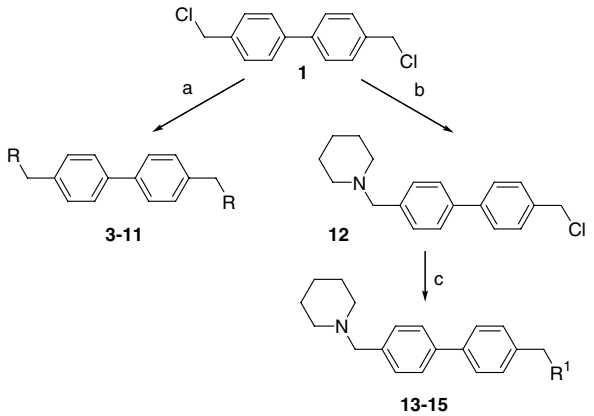


Figure 1. Superposition of **5** (yellow carbons) and 1-1′-nonamethylenedi-piperidine (gray carbons). Only polar hydrogens are depicted.

to those of the rigidified analogue **5**. Similar structures having a biphenyl scaffold have also been recently reported in a patent on a series of H_3 antagonists.[14] To evaluate the influence of spacer length, we tested the homologous derivative, having two methylene groups each in 4 and 4′ positions (**17** in Table 1), and the shorter analogue with only one phenyl ring (**18** in Table 1).

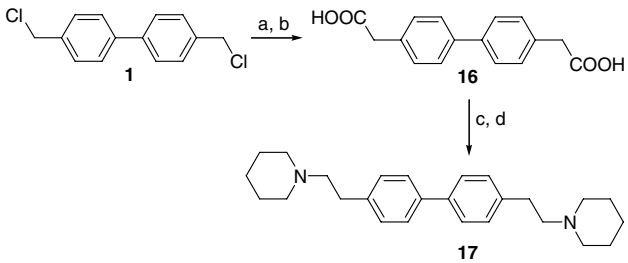
Compounds **3**–**15** were synthesized as outlined in Scheme 1. The symmetrical compounds **3**–**11** were obtained by reacting the commercially available 4,4′-bis(chloromethyl)-biphenyl (**1**) with the appropriate amine.[15] The asymmetrical compounds **13**–**15** were



Scheme 1. Reagents and conditions: (a) primary or secondary amine 2 equiv, anhydrous toluene, reflux, overnight, 60–80%; (b) piperidine 1 equiv, anhydrous toluene, reflux overnight, 55%; (c) 2-piperidone (sodium salt), 1-acetylpiperazine or cyclohexylamine 2 equiv, anhydrous toluene, reflux, overnight, 60–70%.

prepared reacting **1** with one equivalent of piperidine to obtain the monosubstituted intermediate **12**. After isolation and purification, **12** was reacted with 2-piperidone (sodium salt), 1-acetylpiperazine, and cyclohexylamine to obtain the desired products. Compound **17** was synthesized as described in Scheme 2. 4,4′-Bis (chloromethyl)-biphenyl (**1**) was reacted with NaCN to obtain the dicyano derivative, that was hydrolyzed in ethanolic KOH. After acidification, the symmetrical dicarboxylic acid (**16**) was obtained. Intermediate **16** was activated with CDI and reacted with piperidine to give the corresponding tertiary amide that was finally reduced with RedAl®[16] to the target diamino compound **17**. Compound **2** was prepared according to Rosa et al.[17] and compound **18** was purchased from Labo-Test.[18] ^{1}H NMR and CHN data confirmed the structures of all tested compounds.

H_3 receptor affinity was measured by displacement of [^{3}H](*R*)-α-methylhistamine (RAMHA) from GPCR97-transfected SK-N-MC cells stably expressing the human histamine H_3 receptor,[19] and from rat cerebral cortex membranes.[20,21] The H_3 antagonistic potency of the compounds was studied on RAMHA-induced inhibition in electrically stimulated guinea pig isolated ileum.[22] Selectivity against H_1 and H_2 histamine receptor subtypes was studied on isolated ileal and atrial guinea pig preparations; inhibition of cerebral rat AChE was determined following Ellman's method.[23]



Scheme 2. Reagents and conditions: (a) NaCN 2 equiv, acetonitrile, microwave irradiation (150 W, 150 °C, 10 bar, 5 min), 97%; (b) i—KOH 2 equiv, ethanol, microwave irradiation (150 W, 150 °C, 10 bar, 5 min), ii—HCl 2 N, 92%; (c) i—CDI 2.2 equiv, THF, microwave irradiation (250 W, 130 °C, 10 bar, 5 min); ii—piperidine 2 equiv, THF, microwave irradiation (250 W, 130 °C, 10 bar, 5 min), 77%; (d) RedAl® 4 equiv, anhydrous toluene, 1 h rt, 3 h reflux, 85%.

The compounds revealed an interesting binding profile, with some derivatives characterized by remarkable H_3 affinity (Table 1). All compounds showed a moderate preference for human H_3 receptor compared to rat receptor, with the highest pK_i difference (about one log unity) observed for compound **14**. Compound **5**, carrying two piperidines on the central 4,4′-bis-methyl-biphenyl moiety, was the most potent derivative at the receptors of both species, showing subnanomolar affinity for human H_3 receptor. The 4,4′-bis-methyl-biphenyl scaffold provided the optimum distance between the basic centers, as evidenced by the moderate, but significant affinity reduction observed for the longest derivative **17** and by the huge affinity drop of the shortest derivative **18**. Within this limited exploration the piperidine ring resulted in optimal receptor binding. Further steric hindrance on this ring was detrimental for binding affinity: 4-methyl-, 3,5-dimethyl-piperidine and 1-acetylpiperazine fragments (compounds **6, 7**, and **9**) led to a loss of binding affinity in proportion to their increased bulkiness. The most potent compounds of the present series are characterized by tertiary amine groups, with only a modest loss in binding affinity when a dimethylamine (**3**) replaced the piperidine ring. In contrast, the primary amine derivative **2** showed a dramatic decrease of potency, and secondary amines (**4**, **10**) were only moderately active. The slightly higher potency of **11** compared to **10** is consistent with this observation, as well as the binding affinity observed for the asymmetric derivative **15**, being intermediate between those of the symmetric compounds **5** and **10**. Comparison of binding data for compounds **3** and **11** suggests that the cyclohexyl substituent is hardly tolerated by the H_3 receptor. The decreased basicity and higher hydrophilicity of **14**, **8**, and **13** led to reduced potency.

The compounds were tested for their histamine receptor subtype selectivity, showing no interaction with H_1 and H_2 receptors up to a concentration of 10 μM, and all of them behaved as competitive H_3 antagonists on electrically stimulated guinea pig isolated ileum (data not shown).

Moreover, they showed no significant inhibition of AChE activity up to a concentration of 10 μM, with the exception of compounds **6** and **15** which caused about 50% inhibition of rat brain AChE at 10 μM concentration. The most potent compound **5** was tested in a functional assay performed on SK-N-MC cells expressing human H_3 receptors.[25] It showed an antagonist behavior, with a pK_B value of 8.77 ± 0.17.

The potent and rigid compound **5** was employed for molecular modeling studies to investigate the putative binding mode for this class of dibasic compounds. Compound **5** was docked within a previously developed rat

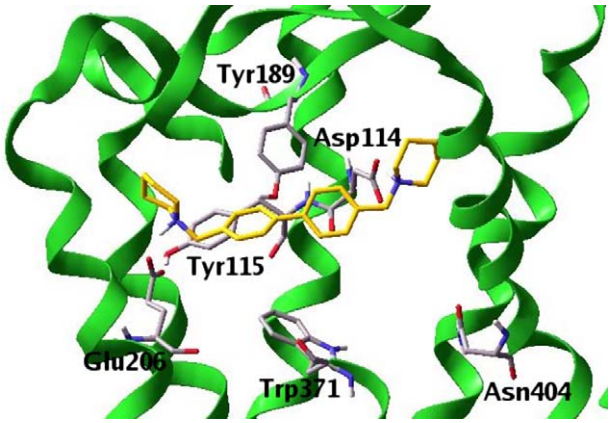


Figure 2. Compound **5** (yellow carbons) within the H_3 receptor model. Only selected amino acids are represented, with gray carbons, and only polar hydrogens are depicted. The protein backbone is represented by a ribbon; for clarity, a portion of TM6 is not shown.

H_3 receptor model[26] by means of an automated docking procedure,[27,28] and the resulting complexes were submitted to molecular dynamics (MD) simulations[29] to evaluate their stability. The best solution is depicted in Figure 2. The basic centers in compound **5** interact with the carboxyl groups of the highly conserved Asp114 in transmembrane (TM) 3 and of Glu206 in TM5, thought to interact with the amine group and the imidazole ring of histamine, respectively. One phenyl ring of **5** is sandwiched between Tyr115 and Tyr189, and this interaction could stabilize the complex. The compound is near Trp371, belonging to the CWXP motif in TM6 involved in the process of receptor activation. This binding mode is consistent with the docking results reported for other flexible dibasic non-imidazole H_3 antagonists.[30]

However, the automated docking protocol also provided another putative accommodation of **5** within the H_3 receptor TM bundle (Fig. 3). According to this alternative binding scheme, a piperidine nitrogen interacts with Asp114 side chain, while the compound is deeply inserted in a lipophilic pocket, mainly delimited by TM helices 3, 6, and 7, and close to Trp371. The other piperidine is hydrogen bonded to the side chain carbonyl oxygen of Asn404 in TM7. A similar accommodation, parallel to the TM helices, had already been proposed for both classical and non-imidazole H_3 antagonists.[31] Further SAR studies to validate these docking hypotheses are ongoing; at the moment, due to the nature of homology-built receptor models, alternative binding mechanisms cannot be excluded.

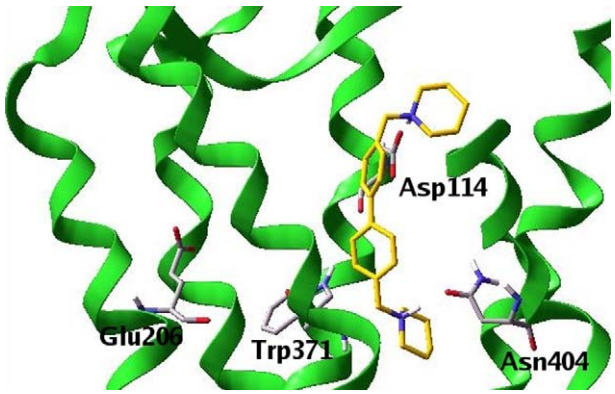


Figure 3. Alternative binding scheme for compound **5**. For clarity, a portion of TM 7 ribbon is not shown.

In conclusion, starting from a series of flexible ligands, we developed a novel class of rigid dibasic H_3 receptor antagonists. The evaluation of different basic moieties and the variation of spacer length led to compound **5** endowed with subnanomolar potency at the human H_3 receptor. The rigid scaffold also provided more information for molecular modeling studies on the putative interactions with H_3 receptor.

Acknowledgments

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21. Homogenates of SK-N-MC cells expressing human H_3 histamine receptors or rat cerebral cortex membranes were used for determining affinity values of the compounds prepared in radioligand displacement studies. Membranes were incubated for 60 min at room temperature with 0.5 nM [^{3}H](*R*)-α-methylhistamine (30.0 Ci/mmol, Amersham Bioscience) in buffer solution (pH 7.4) (50 mM Tris–HCl/5 mM EDTA for cells or 50 mM Tris–HCl/0.5 mM EDTA/50 mM NaCl for rat) with or without competing ligands. Incubation was terminated by rapid filtration over Millipore AAWPO2500 filters followed by two washes with ice-cold buffer (50 mM Tris–HCl/5 mM EDTA). Retained radioactivity was determined by liquid scintillation counting. Non-specific binding was defined using 10 μM histamine or 10 μM thioperamide as competing ligands in SK-N-MC cells or in rat cortex, respectively. IC_{50} values were estimated from the displacement curves of the tested compounds (0.01 nM–10 μM) versus [^{3}H](*R*)-α-methylhistamine and converted to K_i values according to Cheng and Prusoff's equation Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
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28. Automated docking was performed on **5** in its diprotonated state, starting from a minimum energy conformation placed in an arbitrary position, within a box centered on Asp114 and including the extracellular portion of the receptor. Docking solutions were ranked according to their Emodel values.
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