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Synthesis and structure–activity relationships for biphenyl H_3 receptor antagonists with moderate anti-cholinesterase activity

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ABSTRACT

The combination of antagonism at histamine H_3 receptors and inhibition of acetylcholinesterase has been recently proposed as an approach to devise putative new therapeutic agents for cognitive diseases. The 4,4′-biphenyl fragment has been reported by us as a rigid scaffold leading to potent and selective non-imidazole H_3-antagonists. Starting from these premises, the current work presents an expanded series of histamine H_3 receptor antagonists, characterized by a central 4,4′-biphenyl scaffold, where the structure–activity profile of both mono-basic and di-basic compounds is further explored and their ability to inhibit rat brain cholinesterase activity is determined. The steric properties and basicity of the terminal groups were modulated in symmetrical compounds, carrying identical substituents, and in asymmetrical compounds, having a piperidine ring at one end and different groups at the other. The length of the linker connecting the biphenyl scaffold to the terminal groups was also modulated. Binding studies at rat and human H_3 receptors evidenced the highest binding affinities for di-basic compounds, in the order of nM concentrations, and that the steric requirements for the two terminal groups are different. Many potent compounds showed good selectivity profiles over the other histamine receptors. Interestingly, some derivatives displayed a moderate ability to inhibit rat brain cholinesterase, for example compound **12** (1-[2-(4′-piperidinomethyl-biphenyl-4-yl)ethyl]piperidine) has a $pIC_{50} = 5.96$ for cholinesterase inhibition and high H_3 receptor binding affinity and antagonist potency ($pK_i = 8.70$; $pK_B = 9.28$). These compounds can be considered as rigid analogs of a recently reported class of dual-acting compounds and as a promising starting point for the design of new H_3-antagonists with anti-cholinesterase activity.

1. Introduction

Histamine is a biogenic amine that influences a wide range of pathophysiological processes through the activation of different G protein-coupled receptors (GPCRs). At present, four subtypes of histamine GPCRs are known. H_1 and H_2 receptors are implicated in allergic responses and gastric acid secretion, respectively. The most recently discovered H_4 receptor is mainly located on mast cells, eosinophils and lymphoid tissues and seems to be involved in inflammatory processes.[1] The histamine H_3 receptor was identified in 1983 and was initially described as an autoreceptor, mainly expressed in the central nervous system (CNS), regulating histamine biosynthesis and release from histaminergic neurons.[2] Subsequently, H_3 receptors have also been shown to act as heteroreceptors on non-histaminergic neurons, where they inhibit the release of other neurotransmitters such as acetylcholine, dopamine, norepinephrine, serotonin and various neuropeptides.[3–6] The high density of H_3 receptors in different CNS areas and their influence on the release of a large variety of neurotransmitters encouraged wide pharmacological investigation on their physiological role and the quest for potential therapeutic applications of H_3-antagonists in the treatment of various CNS diseases. Among them, the most promising ones include attention-deficit hyperactivity disorders (ADHD), Alzheimer's disease, epilepsy, schizophrenia, obesity and eating disorders.[7,8]

Since the discovery of the reference antagonist thioperamide,[9] many classes of potent and selective H_3-antagonists have been reported. The earliest generation of H_3-antagonists was derived from the endogenous neurotransmitter histamine and the compounds contained an imidazole ring in their structures.[10,11] It is now well established that the presence of the imidazole ring may lead to low CNS penetration[12,13] and potential metabolic liabilities due to the interaction with cytochrome P450.[14–18] Such liabilities seem to be avoided by new classes of non-imidazole antagonists,[19–22] comprising some interesting compounds that proved to block the H_3-receptor at nanomolar concentrations and to possess promising efficacy in several experimental models of central disorders. This

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approach led to the selection of some imidazole-free compounds for clinical studies.[21,23] Recently, some new classes of H_3-antagonists have been described, endowed with additional pharmacological properties that may synergistically potentiate their therapeutic efficacy in the treatment of disorders related to neurotransmitter deficits, such as Alzheimer's disease. Accordingly, the development of dual-acting compounds combining H_3 receptor-blocking properties with cholinesterase inhibition has been put forward (Fig. 1),[24,25] as well as dual H_3 and muscarinic M_2 antagonists.[26]

Our researches in the field of H_3-antagonists led us to the preparation of a series of non-imidazole derivatives, characterized by a basic piperidine ring connected through an alkyl spacer of variable length to another piperidine or to heterocyclic rings (e.g. **1**).[27–29] This flexible class of H_3-antagonists was subsequently rigidified by the introduction of a biphenyl central core, leading to compounds, such as **3a** with two piperidines on the 4,4′-bis-methyl-biphenyl scaffold, displaying nM binding affinities at both human and rodent H_3 receptors [30] (Chart 1).

Within this class of biphenyl H_3-antagonists, the reduction of basicity of one or both the terminal fragments or the introduction of primary or secondary amines led to a huge drop in binding affinity.[30] The most interesting derivative **3a** was further characterized, resulting devoid of any significant activity at the other histamine receptors and endowed with weak anti-cholinesterase activity (pIC_{50} = 5.23). Moreover, in ex-vivo binding experiments it displayed a prompt and long-lasting presence in rat CNS, and in the passive avoidance test it resulted as effective as the reference anti-Alzheimer drug donepezil in attenuating the scopolamine-induced amnesia in rats.[31]

With the aim to extend the structure–activity relationships (SAR) exploration for this class of biphenyl H_3-antagonists, we synthesized a larger series of molecules, testing their ability to selectively block histamine H_3 receptors and to inhibit cholinesterase activity in rat brain tissues. Accordingly, while conserving the 4,4′-bis-methyl-biphenyl core scaffold, we prepared both symmetrical compounds (**3b** and **3c)** and asymmetrical ones, carrying on one side a piperidine ring and on the other side a second group chosen to modulate its size, shape and basicity (**5a–5i**). The length of the linker connecting the biphenyl scaffold to the amine fragments was varied by introducing an ethylene chain, instead of the methylene spacers, in a symmetrical or asymmetrical way (**7b, 7c, 12, and 16**). Finally, we evaluated the effect produced by the presence of only one basic fragment in compounds with increased spacer length (**14**, **15**). The compounds were characterized for their binding affinity at human H_3 and H_4 receptors and at rat H_3 receptors; their H_3-antagonist behavior was assessed by functional tests performed on human and guinea-pig receptors, while H_1 and H_2 antagonism was measured on guinea-pig preparations. Inhibition of cholinesterase activity was evaluated on rat brain preparations and the effect of the compounds on cell viability was also assessed. Moreover, the synthesis, the characterization of H_1 and H_2 antagonist behavior, the binding affinity at H_4 receptor and the cholinesterase inhibitory activity of compounds **3a, 3d–3k, 5j–5l**, and **7a** are also reported for the first time. The results obtained for the newly synthesized compounds, merged with the data previously obtained for symmetric compounds, allow a better characterization of the structural features required to combine potent H_3 antagonism with moderate anti-cholinesterase activity for this class of biphenyl derivatives.

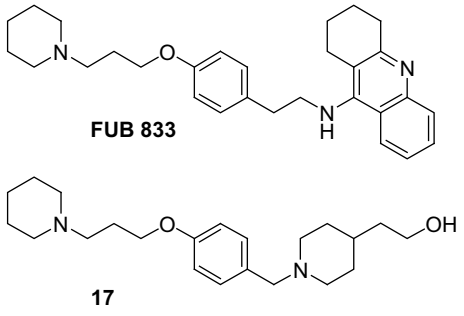


Figure 1. Histamine H_3 receptors antagonists-acetylcholinesterase inhibitors.

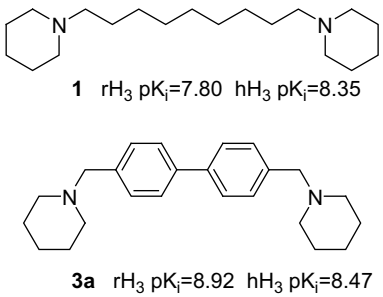
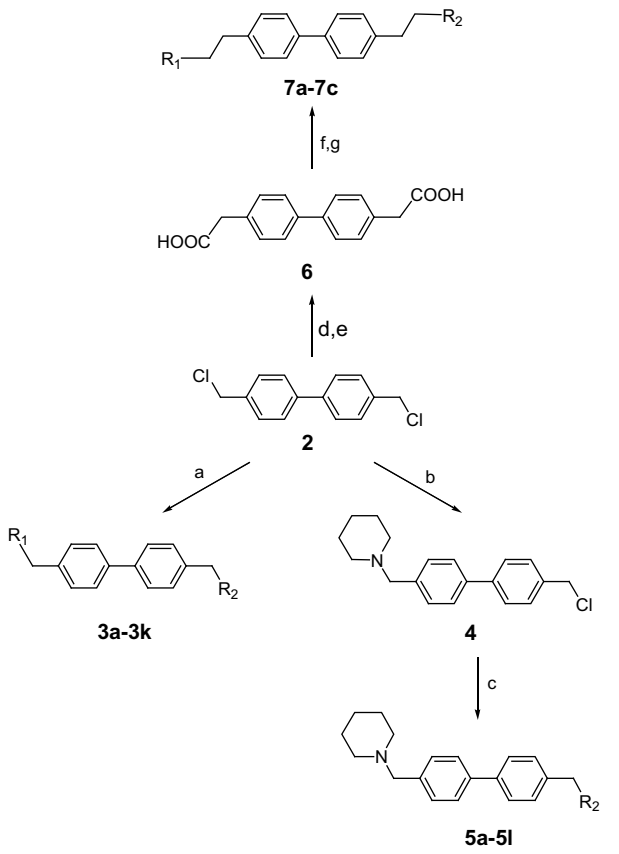


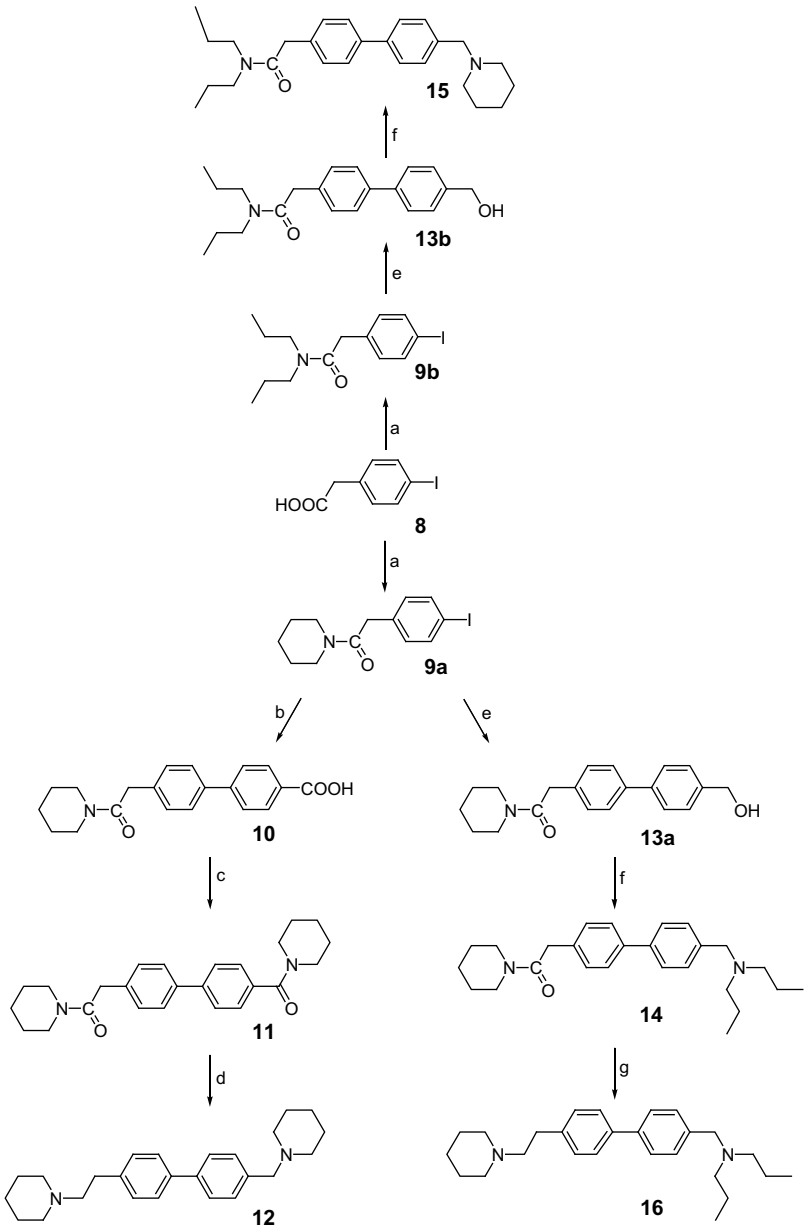
Chart 1.

2. Chemistry

The biphenyl derivatives were prepared following the synthetic routes outlined in Schemes 1 and 2.



Scheme 1. Reagents and conditions: (a) primary or secondary amine, THF, MW; (b) piperidine, Et_3N, THF, MW; (c) amine (THF, MW), or sodium salt of 2-piperidone or 4-hydroxybenzonitrile (anhydrous toluene, reflux); (d) NaCN , H_2O/CH_3CN, MW; (e) i—KOH , EtOH, MW; ii—HCl 2N; (f) i—CDI, THF, MW; ii—amine, THF, MW; (g) RedAl®, anhydrous toluene.



Scheme 2. Reagents and conditions: (a) i—CDI, CH_3CN, MW; ii—amine, CH_3CN, MW; (b) i—4-carboxyphenylboronic acid, $Pd(OAc)_2$, K_2CO_3, acetone, H_2O, 65 °C; ii—HCl 1N; (c) i—CDI, CH_3CN, MW; ii—piperidine, MW; (d) RedAl®, anhydrous toluene; (e) 4-hydroxymethylphenylboronic acid, $Pd(OAc)_2$, K_2CO_3, acetone, H_2O, 65 °C; (f) i—MsCl, Et_3N, CH_2Cl_2; ii—amine, CH_3CN; (g) RedAl®, anhydrous toluene.

As depicted in Scheme 1, the symmetrical compounds **3a–3k** were obtained by reacting the commercially available 4,4′-bis(chloromethyl)-biphenyl **2** with the appropriate amine.[32] The asymmetrical compounds **5a–5l** were prepared reacting **2** with piperidine to obtain the monosubstituted intermediate **4**. After isolation and purification, **4** was reacted with the suitable amine or the sodium salt of 2-piperidone or of 4-hydroxybenzonitrile to obtain the desired products. The synthesis of compounds **3b–3d** and **3f**, **3i**, and **3j** is described in Ref. 32.

Compounds **7a–7c** were synthesized reacting 4,4′-bis(chloromethyl)-biphenyl **2** with NaCN to obtain the dicyano derivative, that was hydrolyzed in ethanolic KOH; acidification provided the symmetrical dicarboxylic acid **6**. Intermediate **6** was activated with 1,1′-carbonyl-diimidazole (CDI) and reacted with the appropriate amine to give the corresponding tertiary diamides that were finally reduced with RedAl®[33] to obtain the target diamino compounds **7a–7c**.

The synthesis of asymmetrical compounds **12, 14–16** is outlined in Scheme 2. The commercially available 2-(4-iodophenyl)acetic acid (**8**) was activated with CDI and reacted with piperidine or dipropylamine to give the intermediates **9a and b**. These intermediates were submitted to Suzuki coupling with commercially available 4-carboxyphenylboronic acid or with 4-hydroxymethylphenylboronic acid to give the carboxylic acid **10** and the hydroxymethylbiphenyl derivatives **13a and b**, respectively. Compound **10** was activated with CDI and reacted with piperidine to give the diamide **11** that was reduced with RedAl® to obtain the final compound **12**. Compounds **13a and b** were activated with mesyl chloride (MsCl) and reacted with the appropriate amine to obtain the final compounds **14** and **15**. Reduction of compound **14** with RedAl® gave derivative **16**.

3. Pharmacology

3.1. Materials and methods

Drugs used were purchased from Sigma (St. Louis, MA, USA). Cultured SK-N-MC cells stably expressing human histamine H_3 or H_4 receptors and the reporter gene β-galactosidase (Johnson & Johnson Pharmaceutical Research and Development, L.L.C.) were used for binding and functional studies. Functional experiments were also performed on isolated organs excised from guinea-pigs (250–350 g) whereas binding and colorimetric assays were carried out using brain preparations from male Wistar rats (150–200 g) (Charles River, Italy). Animals were housed, handled and cared for according to the European Community Council Directive 86 (609) EEC and the experimental protocols were carried out in compliance with Italian regulations (DL 116/92) and with the local Ethical Committee Guidelines for Animal Research.

3.2. Rat histamine H_3 receptor binding assay

Rat brain membranes, prepared according to Kilpatrick's method,[34] were incubated for 45 min with [^{3}H]-(*R*)-α-methylhistamine 0.5 nM ([^{3}H]RAMHA) and the compounds under study (0.03 nM to 10 μM), in Tris–HCl 50 mM, pH 7.4, NaCl 50 mM, EDTA 0.5 mM, then rapidly filtered (AAWP Millipore filters 0.8 μm) under vacuum and rinsed twice with the same ice-cold buffer. Nonspecific binding was defined by 10 μM thioperamide as competing ligand.

3.3. Human histamine H_3 and H_4 receptor binding assay

Homogenates of SK-N-MC cells, a human neuroblastoma cell line stably expressing the human histamine H_3 or H_4 receptors, were used in radioligand displacement studies according to literature methods.[35,36] Membranes were incubated for 60 min at room temperature with 0.5 nM [^{3}H]-(*R*)-α-methylhistamine (47.0 Ci/mmol, Amersham Bioscience) or with 10 nM [^{3}H]histamine (18.1 Ci/mmol, Perkin Elmer) in the absence or in the presence of competing ligands (0.01 nM to 10 μM). 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). Nonspecific binding was defined by 10 or 100 μM histamine as competing ligand for H_3 and H_4 receptors, respectively.

3.4. Human histamine H_3 receptor functional assay

Compounds were added directly to the media containing SK-N-MC cells expressing the human histamine H_3 receptor as well as the construct gene (β-galactosidase), followed, 5 min later, by addition of forskolin (5 μM). The compounds (1 nM to 10 μM) were added 10 min prior to (*R*)-α-methylhistamine (0.1–100 nM). After a 6 h incubation at 37 °C, the medium was aspirated and the cells were lysed with 25 μl of 0.1× assay buffer (mM composition: NaH_2PO_4 10, Na_2HPO_4 10, pH 8, $MgSO_4$ 0.2, $MnCl_2$ 0.01) and after 10 min with 100 μl of 1× assay buffer (NaH_2PO_4 100, Na_2HPO_4 100, pH 8, $MgSO_4$ 2, $MnCl_2$ 0.1) containing 0.5% Triton and 40 mM β-mercaptoethanol. Color was developed using 25 μl of 1 mg/ml substrate solution (chlorophenol red β-D-galactopyranoside; Roche Molecular Biochemicals, Indianapolis, IN, USA) and quantitated on a microplate reader by measuring the absorbance at 570 nm (Biorad microplate reader 550, Segrate, MI, Italy).[37]

3.5. Cell viability

Cell viability was determined through colorimetric quantification of formazan derived from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolic reduction.[38] SK–N–MC cells were incubated with the compounds under study (1–10 μM) or with the vehicle for 6 h. At the end of the period of incubation, 10 μl of 5 mg/ml MTT solution were added to each well. After 3 h the culture medium was removed, the cells washed with phosphate buffer solution (PBS) and 200 μl of formazan solubilization solution (0.1 N HCl in anhydrous isopropanol) were added. Culture medium absorbance was spectrophotometrically read at 570 nm (Biorad microplate reader 550, Segrate, MI, Italy). Cell viability was expressed as relative viability compared to control.

3.6. Functional studies on isolated tissues

3.6.1. Field stimulated guinea-pig ileum

Portions of guinea-pig ileum were longitudinally mounted (1 g load) in organ chambers, filled with Krebs–Henseleit solution (mM composition: NaCl 118.9, KCl 4.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4 \cdot 7H_2O$ 1.2, glucose 11.1) and gassed with 95% O_2–5% CO_2 at 37 °C. The tissues were electrically stimulated (0.1 Hz, 1 ms, submax voltage) (LACE, Ospedaletto PI, Italy). The H_3-antagonist activity of the tested compounds (1 nM to 30 μM), was functionally determined on twitch contractions inhibition induced by (*R*)-α-methylhistamine cumulatively administered (1 nM to 1 μM) in the presence of 1 μM mepyramine.

3.6.2. Guinea-pig isolated ileum and atria

Guinea-pig terminal ileum portions (H_1 receptors) and atria (H_2 receptors) were isometrically suspended in organ baths filled with Krebs–Henseleit (37 °C) and Ringer–Locke solution (31 °C) (mM composition: NaCl 154.0, KCl 5.6, $CaCl_2$ 1.08, $NaHCO_3$ 5.95, glucose 11.1), respectively, and aerated with 95% O_2–5% CO_2. Atrial responses were determined as changes in the rate of spontaneous beating by means of a connected cardiotachograph. Cumulative dose–response curves of histamine in ileum (1 nM to 1 μM) or of H_2-agonist dimaprit in atria (0.1–100 μM) were obtained in the absence and in the presence of the compounds tested up to 10 μM concentration.[10]

3.6.3. Rat brain cholinesterase inhibition

The inhibition of brain cholinesterase was determined spectrophotometrically using acetylthiocholine as substrate according to the method of Ellman et al.[39] Aliquots of rat brain homogenates were incubated in phosphate buffer 0.1 M (pH 8.0) with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (5 mM) and test compounds at appropriate concentrations (1–100 μM). The reaction was started at 37 °C by adding 20 μl of acetylthiocholine (75 mM). The reaction was stopped after 15 min by adding formalin (4%). The hydrolysis of acetylthiocholine catalyzed by the enzyme was determined by monitoring the formation of the yellow 5-thio-2-nitrobenzoate anion at a wavelength of 412 nm (Biorad microplate reader 550, Segrate, MI, Italy). The percent inhibition of cholinesterase was calculated as follows: $[(\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}})/\text{absorbance}_{\text{control}}] \times 100$. Results are provided as pIC_{50} (−Log concentration causing half-maximal inhibition of cholinesterase).

3.7. Data analysis

The data are presented as means ± SEM. In functional tests results are expressed as percentage of the maximum response induced by the agonist. The pEC_{50} values (−Log of the concentration giving half-maximal effect) and slopes of regression lines were calculated by analyzing agonist concentration–response curves by the least-squares method. The antagonistic affinities were estimated determining pK_B ('apparent pA_2') as described by Furchgott's equation.[40] When non-surmountable antagonism was detected, the antagonist potency of the drugs was expressed by pD_2' values determined according to Van Rossum's equation.[41]

In in vitro binding assays, pIC_{50} values were estimated from the displacement curves of the tested compounds versus [^{3}H]-(*R*)-α-methylhistamine or [^{3}H]histamine and converted to pK_i values according to Cheng and Prusoff's equation.[42]

4. Results and discussion

The binding and functional characterization of the newly synthesized biphenyl derivatives **3b**, **3c**, **5a–5i**, **7b**, **7c**, **12**, and **14–16** are reported in Table 1, together with the previously described data for compounds **3a, 3d–3k, 5j–5l**, and **7a**.[30,31] While all the previously reported biphenyl derivatives showed higher binding affinities for human H_3 receptors than for rat H_3 receptors, some of the newly synthesized compounds have similar affinity values (e.g. **3b and 5d**) or a slightly higher binding affinity for rat H_3 receptors (**3c, 5c, 5e, and 14**). The new compounds proved to be antagonists at human and guinea-pig H_3 receptors, with highly correlated pK_i and pK_B data at human H_3 receptors ($r = 0.83$). The symmetric compounds **3b** and **3c**, with diethylamine and dipropylamine substituents, respectively, confirmed the importance of tertiary amines for high H_3 binding affinity, since they roughly maintained the high potency of the dimethylamino derivative **3d**, compared to the bis-secondary amines **3e** and **3f**. However, the best arrangement for the lipophilic terminals of the basic groups remains that of the piperidine rings of compound **3a**, even if steric tolerance for the acyclic tertiary amine **3c** seems higher than for the more rigid substituted piperidine **3g**. As for the antagonist effect on human H_3 receptors, in a cAMP-responsive element (CRE) mediated β-galactosidase reporter gene assay and on guinea-pig native H_3 receptors, compound **3b** was the most potent among the symmetrical compounds ($pK_B = 8.96$ and 9.34, respectively).

The asymmetric derivatives **5a–5l**, maintaining a piperidine ring on the 4,4′-bis-methyl-biphenyl scaffold, generally exhibited higher H_3 affinity and antagonist potency than the corresponding symmetric compounds (**3c**, **3d**, and **3f–3k**). A relevant increase in binding affinity at both rat and human H_3 receptors and in the inhibitory potency at guinea-pig and human H_3 receptors is evident for compounds **5a, 5b, 5j**, and **5k**. Compound **5b**, in particular, was characterized by remarkable binding affinity, particularly at human H_3 receptors, showing that the binding sites for the two basic groups have different steric tolerance. On the other hand, inclusion of electron-withdrawing fragments in close proximity to one of the basic groups (**5c and 5j**) led to a significant decrease of receptor affinity. This trend is confirmed by the lower affinity observed for compounds **5d** and **5l**.

To further explore the SAR of the biphenyl series, we synthesised a few symmetrical compounds with a longer spacer between the central core and the basic fragments (compounds **7a–7c**). As previously observed for **7a**, this modification is tolerated for receptor binding, only producing a slight reduction of affinity and antagonist potency compared to those of the corresponding shorter compounds, while maintaining the same rank order for the substituent effect (see compounds **3a, 3d**, and **3h**, respectively). The introduction of one monomethylene and one two-carbon linker between the biphenyl scaffold and the nitrogen atoms led to asymmetric compounds **12** and **14–16**. Compound **12**, having two piperidine rings, showed a limited decrease in receptor affinity, compared to its shorter and longer analogs, but it showed the highest potency on human H_3 receptors in the CRE-mediated β-galactosidase reporter gene assay. One of its piperidine rings can be replaced by a dipropylamino group (**16**) with negligible reduction of receptor affinity, while transformation of one of the two basic amines into an amide fragment led to higher decreases of activity, even if the *N*,*N*-dipropylamino-carbonyl group of **15** was better tolerated than other neutral groups on the piperidino-methyl-biphenyl scaffold (see **5d** and **5l**).

Generally, the compounds exhibited a good selectivity profile, since some of them interacted with H_1, H_2, and H_4 receptors only at concentrations about 1000 or 10,000 times higher than those required to bind H_3 receptors. Only compounds **5b** and **7a** interacted at micromolar concentrations with H_1 and H_2 receptors, respectively. Moreover, none of the compounds affected SK-N-MC cell viability, since no alterations were observed in the MTT test at concentrations between 1 and 10 μM (data not shown).

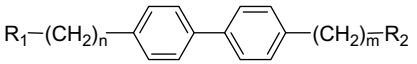
Different series of compounds carrying two basic centers have been described as cholinesterase inhibitors: they comprise polyamine derivatives,[43–45] meptazinol derivatives,[46] as well as H_3-antagonists.[24] The interesting anti-cholinesterase activity already described for symmetrical compounds **3a**, **3f**, **3h**, **3k**, and asymmetrical compounds **5j and 5k**[31] was maintained by asymmetrical compounds **5a, 5b, 5g, 5h, and 5i** and by compounds **12** and **14–16**. Comparing the inactivity of the symmetrical, longer compounds **7a** and **7c** with the anti-cholinesterase property of their shorter analogs **3a** and **3h** it can be speculated that the length of the spacer connecting the basic substituents to the biphenyl scaffold represents a critical feature for cholinesterase inhibition. Indeed, compound **12**, having an intermediate length, showed the highest cholinesterase inhibitory potency coupled to good human H_3 binding affinity and antagonist potency. Even if its pIC_{50} value on rat brain cholinesterase activity (5.96) is considerably lower than that of donepezil in the same experimental conditions ($pIC_{50} = 7.45$),[31] this compound may represent a starting point to develop new H_3-antagonists also endowed with anti-cholinesterase activity. In fact, its antagonist potency on cells expressing human H_3 receptors, its cholinesterase inhibitory potency and its selectivity profile on other histamine receptors are comparable, if not better, than those observed for compound **3a**, that showed promising efficacy in attenuating scopolamine-induced amnesia in rats.[31]

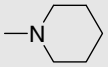
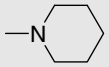
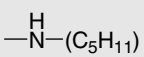
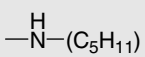
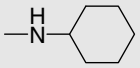
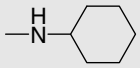
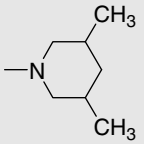
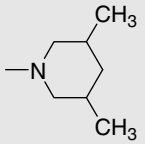
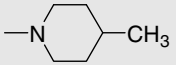
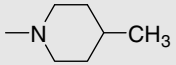
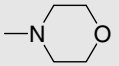
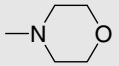
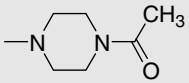
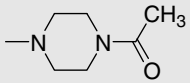
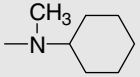
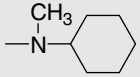
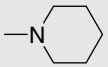
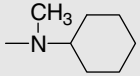
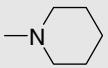
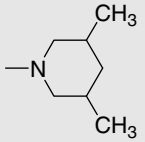
Two different docking solutions for the most potent compound **3a**, into a homology model of rat H_3 receptor, have been previously described by us.[30] In the best scoring pose, compound **3a** is oriented 'horizontally,' in a direction perpendicular to the axes of the transmembrane (TM) helices, and it forms two salt bridges between its protonated piperidines and the carboxylate groups of Asp114 in TM3 and Glu206 in TM5. Glu206 is important for the binding of the natural ligand histamine and is supposed to interact with the imidazole ring,[47] while Asp114, the conserved acidic residue present in many class A G protein-coupled receptors, is thought to bind the side chain amine of histamine. In the crystal structure of the β_2 adrenergic receptor Asp113, corresponding to the H_3 Asp114, binds the secondary amine group of the co-crystallized inverse agonist carazolol.[48] The docking protocol also provided an alternative, 'vertical' pose for **3a**, roughly parallel to the axes of the TM helices. In this pose, a piperidine nitrogen interacts with Asp114 and the central scaffold occupies a region delimited by TM3, 6, and 7, leading the second piperidine to form a hydrogen bond with the side chain of Asn404 in TM7. Both orientations have been proposed for other classes of non-imidazole H_3 receptor antagonists.[49–51]

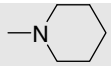
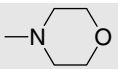
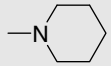
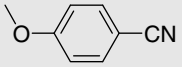
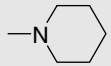
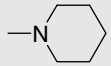
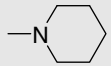
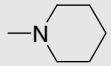
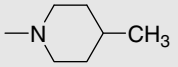
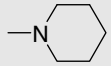
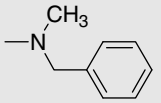
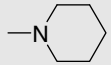
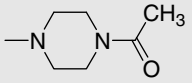
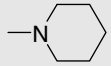
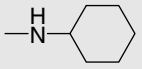
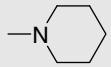
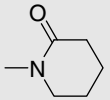
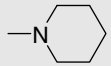
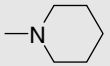
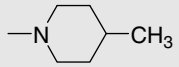
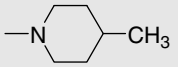
Other representatives of this series of compounds (**3g, 5b, 5d**, and **7a**), docked into the rat H_3 receptor model, gave alternative solutions corresponding to horizontal and vertical poses. As the distance between Glu206 and Asp114 is optimal for a double acid–base interaction with the two piperidine rings of compound **3a**, it was expected that the longer derivative **7a** would hardly assume the horizontal pose. Actually, it gave both vertical and horizontal docking solutions (Fig. 2), with the first ones preferred, according to Glide scoring function (see Section 6). On the other hand, compound **3g**, having two 3,5-dimethyl-piperidino-methyl groups and significantly lower binding affinity, showed no docking solution with horizontal poses and simultaneous interactions at

Table 1

[a]Affinity (pK_i) and antagonist potency (pK_B) of the compounds under study at human (hH_3), rat (rH_3), guinea-pig (gpH_3) histamine H_3 receptors, guinea-pig histamine H_1 (gpH_1) or H_2 (gpH_2) receptors, human histamine H_4 receptors (hH_4).

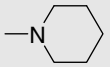
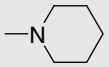
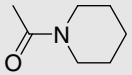
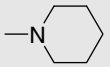
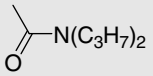
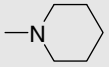


	n	*m*	R_1	R_2	rH_3 pK_i[b]	hH_3 pK_i[c]	hH_3 pK_B[d]	gpH_3 pK_B[e]	gpH_1 pK_B[f]	gpH_2 pK_B[g]	hH_4 pK_i[h]	Cholinesterase	
												pIC_{50}[i]	Max Eff. (%)[j]
3a[k]	1	1			8.92	9.47	8.77	8.58	$pD_2' = 4.84$	#	4.60	5.23	82
3b	1	1	$-N(C_2H_5)_2$	$-N(C_2H_5)_2$	8.62 ± 0.10	8.55 ± 0.14	8.96 ± 0.19	9.34 ± 0.27	#	#	#	#	
3c	1	1	$-N(C_3H_7)_2$	$-N(C_3H_7)_2$	8.50 ± 0.14	8.03 ± 0.00	7.80 ± 0.09	8.78 ± 0.18	5.54 ± 0.12	$pEC_{50} = 5.47 \pm 0.14$			
3d[k]	1	1	$-N(CH_3)_2$	$-N(CH_3)_2$	8.18	8.73	8.17	8.46	#	#	#	#	
3e[k]	1	1			5.48	6.12	6.51	§	§	§	5.19	#	
3f[k]	1	1			5.68	6.25	6.54	5.82	§	§	#	5.29	70
3g[k]	1	1			6.17	6.40	6.19	#	$pD_2' = 5.08$	#	4.86	#	
3h[k]	1	1			7.82	8.21	7.68	7.30	5.89	#	#	5.45	83
3i[k]	1	1			7.09	7.91	7.67	ND	#	#	#	#	
3j[k]	1	1			5.41	5.85	6.70	§	$pD_2' = 4.81$	#	#	#	
3k[k]	1	1			6.67	7.43	7.27	7.45	$pD_2' = 5.24$	§	5.19	5.78	57
5a	1	1			8.13 ± 0.13	8.56 ± 0.01	9.19 ± 0.15	8.46 ± 0.18	5.56 ± 0.02	#	#	5.86 ± 0.11	93
5b	1	1			8.62 ± 0.16	9.06 ± 0.06	8.48 ± 0.12	9.02 ± 0.17	6.15 ± 0.09	&	#	5.62 ± 0.09	91

5c	1	1			7.81 ± 0.08	7.71 ± 0.10	7.77 ± 0.08	9.07 ± 0.13	#	#	#	#	
5d	1	1			7.15 ± 0.23	7.12 ± 0.06	7.27 ± 0.09	7.27 ± 0.03	#	§	#	#	
5e	1	1		$-N(CH_3)_2$	8.88 ± 0.11	8.66 ± 0.05	8.92 ± 0.09	9.05 ± 0.21	#	&	#	#	
5f	1	1		$-N(C_2H_5)_2$	8.59 ± 0.08	8.71 ± 0.12	8.90 ± 0.16	9.41 ± 0.07	#	#	#	#	
5g	1	1		$-N(C_3H_7)_2$	8.50 ± 0.15	8.76 ± 0.05	8.18 ± 0.21	9.12 ± 0.20	5.48 ± 0.10	#	#	5.83 ± 0.08	93
5h	1	1			8.24 ± 0.10	8.62 ± 0.17	8.64 ± 0.09	8.75 ± 0.26	#	#	#	5.85 ± 0.08	93
5i	1	1			7.85 ± 0.03	8.17 ± 0.03	7.98 ± 0.13	7.34 ± 0.14	$pD_2' = 4.75 \pm 0.11$	£	#	5.50 ± 0.11	79
5j[k]	1	1			7.33	8.20	7.86	7.79	$pD_2' = 4.46$	#	#	4.94	79
5k[k]	1	1			8.04	8.77	8.03	7.59	#	§	#	5.24	73
5l[k]	1	1			6.94	7.33	7.46	7.57	#	#	#	#	
7a[k]	2	2			8.40	8.93	8.50	8.43	5.32	6.38	#	#	
7b	2	2	$-N(CH_3)_2$	$-N(CH_3)_2$	7.71 ± 0.11	8.29 ± 0.05	8.06 ± 0.13	8.04 ± 0.17	#	#	#	#	
7c	2	2			7.26 ± 0.09	7.78 ± 0.14	8.17 ± 0.23	7.43 ± 0.16	5.85 ± 0.01	£	#	#	

(*continued on next page*)

Table 1 (*continued*)

	n	*m*	R_1	R_2	rH_3 pK_i[b]	hH_3 pK_i[c]	hH_3 pK_B[d]	gpH_3 pK_B[e]	gpH_1 pK_B[f]	gpH_2 pK_B[g]	hH_4 pK_i[h]	Cholinesterase	
												pIC_{50}[i]	Max Eff. (%)[j]
12	1	2			8.08 ± 0.12	8.70 ± 0.14	9.28 ± 0.07	8.73 ± 0.14	#	§	#	5.96 ± 0.06	91
14	1	1	$-N(C_3H_7)_2$		6.75 ± 0.16	6.60 ± 0.04	6.96 ± 0.08	7.24 ± 0.05	5.44 ± 0.09	§	#	4.98 ± 0.08	65
15	1	1			7.27 ± 0.04	8.07 ± 0.15	8.16 ± 0.15	8.00 ± 0.19	5.44 ± 0.13	§	#	4.95 ± 0.02	80
16	1	2	$-N(C_3H_7)_2$		7.97 ± 0.07	8.41 ± 0.09	8.82 ± 0.21	7.98 ± 0.16	#	§	#	5.17 ± 0.01	80

(pIC_{50}) of the compounds on rat brain cholinesterase.
pD_2': −log reducing to 50% the maximal agonist effect.
ND, not determined.

[a] Values are means ± SEM of 4–6 independent experiments.
[b] Inhibition of [^{3}H]RAMHA binding to rat brain membranes.
[c] Inhibition of [^{3}H]RAMHA binding to SK-N-MC cells stably expressing the human histamine H_3-receptor.
[d] Antagonist potency at human histamine H_3-receptors expressed in SK-N-MC cells.
[e] Antagonist potency at H_3 receptors expressed in guinea-pig ileum.
[f] Antagonist potency at H_1 receptors expressed in guinea-pig ileum.
[g] Antagonist potency at H_2 receptors expressed in guinea-pig atrium.
[h] Inhibition of [^{3}H]RAMHA binding to SK-N-MC cells stably expressing the human histamine H_4-receptor.
[i] Rat brain cholinesterase inhibition.
[j] Maximum percent inhibition of rat brain cholinesterase.
[k] Ref. 30 and Ref. 31.
[#] Inactive up to 10 μM.
[§] Inactive up to 1 μM.
[&] Inactive up to 3 μM.
[£] Inactive up to 0.1 μM.

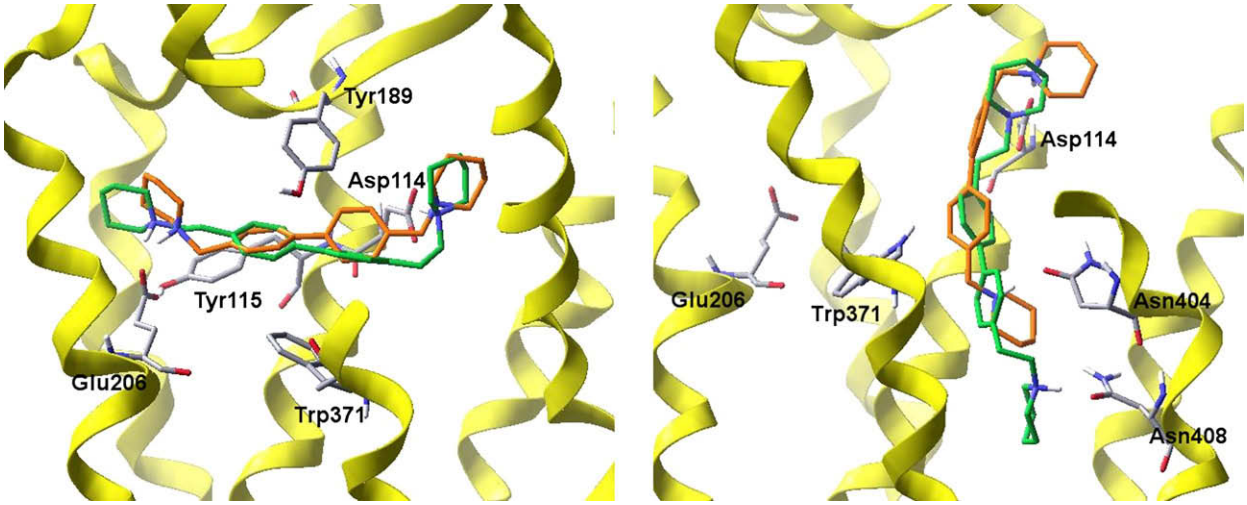


Figure 2. Representation of compound **3a** (orange carbons) and **7a** (green carbons) docked into the rat H_3 receptor model. Left: horizontal pose; right: vertical pose. Only polar hydrogens are shown. The protein backbone is represented by a ribbon; for clarity, a portion of TM6 and TM7 are not shown.

both Glu206 and Asp114, while its asymmetric analog **5b**, having remarkably higher affinity, could accommodate the unsubstituted piperidine ring close to Glu206 and take a second salt bridge between its 3,5-dimethyl-piperidine and Asp114. Moreover, compound **5d**, having only one basic group, can assume both horizontal and vertical poses. While the relationship between the limited decrease of binding affinity for the longer derivative **7a** and its docking preference is unclear, other solutions seem to support the horizontal pose as more consistent with affinity data. In fact, if di-basic compounds must bind to their binding site at H_3 receptors in a horizontal arrangement, the affinity loss for compound **3g**, compared to **5b**, may be related to the lack of high-scored horizontal poses, and that observed for the mono-basic derivatives (e.g. **5d**) can be explained by the lack of one salt bridge with Glu206 or Asp114.

Compound **12** was docked into the catalytic cavity of mouse acetylcholinesterase, whose molecular model was built refining a crystal structure available at the PDB site.[52] It gave a best scoring solution with the piperidino-ethyl group deeply inserted into the active site gorge, similarly to the benzyl group in the docking solution obtained for donepezil, which was in turn similar to its accommodation into *Torpedo californica* acetylcholinesterase active site, as observed for a crystal structure.[53] As represented in Figure 3, compound **12** occupies the acetylcholinesterase active site spanning from Trp86, near the catalytic triad, to Trp286 belonging to the periferic anionic site, with the two protonated nitrogen atoms pointing towards the tryptophan indole rings. This binding mode is similar to the one reported for the dual-acting flexible H_3-antagonist-acetylcholinesterase inhibitor **17** (Fig. 1),[24] having comparable inhibitory potency, and suggests that the more rigid and bulky biphenyl scaffold is tolerated by the acetylcholinesterase active site. The docking model represented in Figure 3, together with homology models of the H_3 receptor, can assist the design of new H_3-antagonists with anti-acetylcholinesterase activity.

5. Conclusion

In summary, a series of rigid H_3-antagonists characterized by good receptor affinity and selectivity profile for human and rodent histamine H_3 receptors has been described. Analyzing the biological data we can assert that, in di-basic compounds, the presence of one piperidine connected to the central biphenyl scaffold provides derivatives endowed with high H_3 binding affinity and antagonist potency. Introduction of non-basic moieties generally leads to a decrease of potency. Some H_3-antagonists showed an interesting, although mild, anti-cholinesterase activity, providing the basis for the development of dual-acting compounds. Moreover, the versatility of the synthetic routes employed allowed to prepare compounds with different combinations of substituents and of linkers bound to the central biphenyl core with good yields.

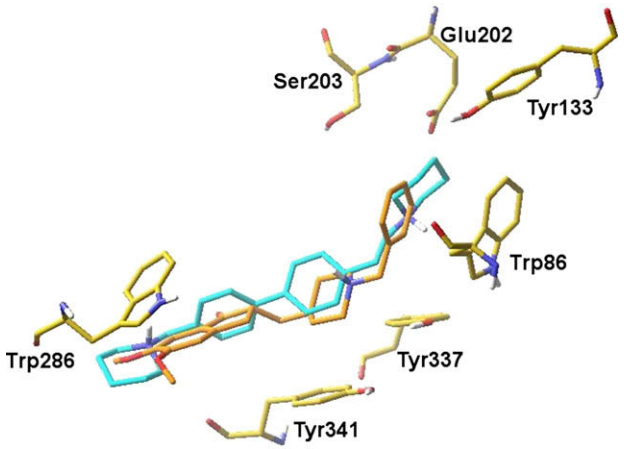


Figure 3. Compound **12** (cyan carbons) and donepezil (orange carbons) docked into the mouse acetylcholinesterase active site.

6. Experimental

6.1. Chemistry

6.1.1. General methods

Melting points were not corrected and were determined with a Gallenkamp melting point apparatus. The final compounds were analyzed on a ThermoQuest (Italia) FlashEA 1112 Elemental Analyzer, for C, H, and N. The percentages we found were within ±0.4% of the theoretical values. The ^{1}H NMR spectra were recorded on a Bruker 300 spectrometer (300 MHz); chemical shifts (δ scale) are reported in parts per million (ppm) relative to tetramethylsilane as internal standard. ^{1}H NMR spectra are reported in the following order: multiplicity, approximate coupling constants (J value) in Hertz (Hz) and number of protons; signals were characterized as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet) br s (broad signal). Mass spectra

were recorded using a API-150 EX with APCI interface (Applied Biosystem, Mds Sciex, Toronto, Canada). Reactions were monitored by TLC, on Kieselgel 60 F 254 (DC-Alufolien, Merck). Final compounds and intermediates were purified by preparative flash chromatography on BÜCHI Sepacore®, using SiO_2 column (Prepacked Cartridges, SiO_2 60, 40–63 μm, BÜCHI); the eluents were mixtures of CH_2Cl_2/CH_3OH at various volume ratios. When indicated, gaseous NH_3 was added to the methanolic phase to obtain a 5% w/w solution. Microwave reactions (MW) were conducted using a CEM Discover Synthesis Unit. Abbreviations are the following: EtOAc, ethyl acetate; THF, tetrahydrofuran; DMSO, dimethylsulfoxide; CDI, 1,1′-carbonyl-diimidazole; MsCl, mesyl chloride.

6.1.1.1. General method of preparation of [biphenyl-4,4′diyl-bis(methylene)]bis-amine derivatives (3a–3k). A solution of 4,4′-bis(chloromethyl)-biphenyl **2** (1 mmol) was reacted with the appropriate amine (4 mmol) in THF (2 mL) (MW, 100 °C, 150 W, 100 psi, 5 min). The solvent was removed in vacuo to give the crude products, which were purified by flash chromatography [SiO_2, CH_2Cl_2/CH_3OH (NH_3) 97:3].

*1,1′-[Biphenyl-4,4′-diylbis(methylene)]bis-piperidine (**3a**).* Crystallized from EtOH. Yield: 64%; mp: 128–130 °C. ^{1}H NMR ($CDCl_3$) δ 1.41–1.51 ppm (m, 4H), 1.55–1.66 ppm (m, 8H), 2.37–2.48 ppm (m, 8H), 3.50 ppm (s, 4H), 7.37 ppm (d, J = 8.1 Hz, 4H), 7.53 ppm (d, J = 8.1 Hz, 4H). MS (EI) 349 $[M+1]^+$. Anal. calcd for $C_{24}H_{32}N_2$: C, 82.71; H, 9.25; N, 8.04. Found: C, 82.51; H, 9.17; N, 7.84.

*N,N′-[biphenyl-4,4′diylbis(methylene)]bis-diethylamine (**3b·2HCl·H$_2$O**).* Crystallized from abs EtOH/Et_2O. Yield: 71%; mp: 262–264 °C. ^{1}H NMR (D_2O) δ 1.38 ppm (t, J = 14.6 Hz, 12H), 3.21–3.34 ppm (m, 8H), 4.41 ppm (s, 4H), 7.64 ppm (d, J = 8.2 Hz, 4H), 7.84 ppm (d, J = 8.3 Hz, 4H). MS (EI) 325 $[M+1]^+$. Anal. calcd for $C_{22}H_{32}N_2{\cdot}2HCl{\cdot}1H_2O$: C, 63.60; H, 8.73; N, 6.74. Found: C, 63.27; H, 8.46; N, 6.43.

*N,N′-[biphenyl-4,4′diylbis(methylene)]bis-dipropylamine (**3c·2HCl·0.5H$_2$O**).* Crystallized from abs EtOH/Et_2O. Yield: 68%; mp: 274–276 °C. ^{1}H NMR (D_2O) δ 0.95 ppm (t, J = 14.5 Hz, 12H), 1.78–1.80 ppm (m, 12H), 3.10–3.15 ppm (m, 8H), 4.41 ppm (s, 4H), 7.62 ppm (d, J = 7.8 Hz, 4H), 7.83 ppm (d, J = 7.7 Hz, 4H). MS (EI) 381 $[M+1]^+$. Anal. calcd for $C_{26}H_{40}N_2{\cdot}2HCl\ {\cdot}0.5H_2O$: C, 67.51; H, 9.37; N, 6.05. Found: C, 67.52; H, 9.00; N, 6.39.

*N,N′-[biphenyl-4,4′diylbis(methylene)]bis-dimethylamine (**3d·2HCl·0.25H$_2$O**).* Crystallized from abs EtOH/Et_2O. Yield: 78%; mp > 300 °C. ^{1}H NMR (D_2O) δ 2.89 ppm (s, 12H), 4.38 ppm (s, 4H), 7.61 ppm (d, J = 8.3 Hz, 4H), 7.83 ppm (d, J = 8.4 Hz, 4H). MS (EI) 269 $[M+1]^+$. Anal. calcd for $C_{18}H_{24}N_2{\cdot}2HCl{\cdot}0.25H_2O$: C, 62.51; H, 7.57; N, 8.10. Found: C, 62.36; H, 7.45; N, 7.60.

*N,N′-[biphenyl-4,4′diylbis(methylene)]bis-pentylamine (**3e·2HCl**).* Crystallized from abs EtOH. Yield: 55%; mp > 300 °C. Free base NMR: ^{1}H NMR (DMSO) δ 0.88 ppm (t, J = 13.15 Hz, 6H), 1.24–1.32 ppm (m, 8H), 1.60–1.70 ppm (m, 4H), 2.90 ppm (t, J = 14,12 Hz, 4H), 4.18 ppm (s, 4H), 7.63 ppm (d, J = 8.06 Hz, 4H), 7.79 ppm (d, J = 8.18 Hz, 4H). MS (EI) 353 $[M+1]^+$. Anal. calcd for $C_{24}H_{36}N_2{\cdot}2HCl$: C, 67.74; H, 9.00; N, 6.58. Found: C, 67.56; H, 8.90; N, 6.20.

*N,N′-[biphenyl-4,4′diylbis(methylene)]bis-cyclohexylamine (**3f**).* Crystallized from EtOH/H_2O. Yield: 66%; mp: 98–100 °C. ^{1}H NMR ($CDCl_3$) δ 1.10–1.34 ppm (m, 10H), 1.58–1.67 ppm (m, 2H), 1.69–1.82 ppm (m, 4H), 1.88–2.05 ppm (m, 4H), 2.45–2.63 ppm (m, 2H), 3.84 ppm (s, 4H), 7.38 ppm (d, J = 8.1 Hz, 4H), 7.54 ppm (d, J = 8.1 Hz, 4H). MS (EI) 377 $[M+1]^+$. Anal. calcd for $C_{26}H_{36}N_2$: C, 82.92; H, 9.64; N, 7.44. Found: C, 82.84; H, 9.39; N, 7.21.

*1,1′-[Biphenyl-4,4′-diylbis(methylene)]bis(3,5-dimethylpiperidine) (**3g**).* Crystallized from EtOH. Yield: 73%; mp: 129–131 °C. ^{1}H NMR ($CDCl_3$) δ 0.45–0.60 (m, 2H), 0.83 (d, J = 6.3 Hz, 12H), 1.47 (t, J = 11.1 Hz, 4H), 1.63–1.81 (m, 6H), 2.80–2.89 (m, 4H), 3.52 (s, 4H), 7.37 (d, J = 8.1 Hz, 4H), 7.56 (d, J = 8.1 Hz, 4H). MS (EI) 405 $[M+1]^+$. Anal. calcd for $C_{28}H_{40}N_2$: C, 83.10; H, 9.96; N, 6.92. Found: C, 82.86; H, 9.87; N, 6.86.

*1,1′-[Biphenyl-4,4′-diylbis(methylene)]bis-4-methylpiperidine (**3h·0.25H$_2$O**).* Crystallized from EtOH. Yield: 84%; mp: 98–100 °C. ^{1}H NMR ($CDCl_3$) δ 0.92 (d, J = 5.7 Hz, 6H), 1.19–1.40 (m, 6H), 1.60 (d, J = 12.0 Hz, 4H), 1.96 (t, J = 11.7 Hz, 4H), 2.88 (d, J = 11.7 Hz, 4H), 3.51 (s, 4H), 7.38 (d, J = 8.1 Hz, 4H), 7.53 (d, J = 8.1 Hz, 4H). MS (EI) 377 $[M+1]^+$. Anal. calcd for $C_{25e35}N_2{\cdot}0.25H_2O$: C, 81.58; H, 9.58; N, 7.61. Found: C, 81.55; H, 9.52; N, 7.60.

*4,4′-[Biphenyl-4,4′-diylbis(methylene)]bis-morpholine (**3i**).* Crystallized from EtOH. Yield: 60%; mp: 123–125 °C. ^{1}H NMR ($CDCl_3$) δ 2.47 (t, J = 9.1 Hz, 8H), 3.53 (s, 4H), 3.73 (t, J = 9.2 Hz, 8H), 7.38 (d, J = 8.1 Hz, 4H), 7.54 (d, J = 8.1 Hz, 4H). MS (EI) 353 $[M+1]^+$. Anal. calcd for $C_{22}H_{28}N_2O_2$: C, 74.97; H, 8.01; N, 7.95. Found: C, 74.91; H, 8.06; N, 7.76.

*4,4′-[Biphenyl-4,4′-diylbis(methylene)]bis-(piperazin-1yl-ethan-2-one) (**3j·H$_2$O**).* Crystallized from EtOH. Yield: 85%; mp: 188–190 °C. ^{1}H NMR ($CDCl_3$) δ 2.07 ppm (s, 6H), 2.59 ppm (m, 8H), 2.37–2.59 ppm (m, 8H), 3.46 ppm (t, 4H), 3.55 ppm (s, 4H), 3.63 ppm (t, 4H), 7.37 ppm (d, J = 7.8 Hz, 4H), 7.54 ppm (d, J = 7.8 Hz, 4H). MS (EI) 435 $[M+1]^+$. Anal. calcd for $C_{26}H_{34}N_4O_2{\cdot}H_2O$: C, 68.99; H, 7.57; N, 12.38. Found: C, 68.79; H, 8.00; N, 12.06.

*N,N′-[biphenyl-4,4′diylbis(methylene)]bis-(N-methyl-N-cyclohexylamine) (**3k**).* Crystallized from EtOH. Yield: 69%; mp: 113–115 °C. ^{1}H NMR ($CDCl_3$) δ 1.05–1.38 (m, 10H), 1.58–1.86 (m, 2H), 1.76–1.94 (m, 8H), 2.22 (s, 6H), 2.40–2.51 (m, 2H), 3.59 (s, 4H), 7.37 (d, J = 8.1 Hz, 4H), 7.53 (d, J = 8.1 Hz, 4H). MS (EI) 405 $[M+1]^+$. Anal. calcd for $C_{28}H_{40}N_2$: C, 83.11; H, 9.96; N, 6.92. Found: C, 83.24; H, 9.98; N, 6.83.

6.1.1.2. Preparation of 4′-chloromethyl-biphenyl-4-ylmethylpiperidine (4). A solution of 4,4′-bis(chloromethyl)-biphenyl **2** (1 mmol) in THF (2 mL) was treated with piperidine (0.5 mmol) and triethylamine (0.5 mmol) (MW, 100 °C, 150 W, 100 psi, 5 min). The solvent was evaporated under reduced pressure; the residue was purified by flash chromatography [SiO_2, CH_2Cl_2:CH_3OH (NH_3) = 98:2]. Crystallized from EtOH/H_2O. Yield: 63%; mp: 174–176 °C. ^{1}H NMR ($CDCl_3$) δ 1.39–1.49 (m, 2H), 1.53–1.63 (m, 4H), 2.38–2.44 (m, 4H), 3.51 (s, 2H), 4.63 (s, 2H), 7.39 (d, J = 8.1 Hz, 2H), 7.45 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 8.1 Hz, 2H) 7.58 (d, J = 8.1 Hz, 2H). MS (EI) 300 $[M+1]^+$. Anal. calcd for $C_{19}H_{22}NCl$: C, 76.11; H, 7.39; N, 4.67. Found: C, 76.34; H, 7.67; N, 4.40.

6.1.1.3. General method of preparation of (4′-piperidin-1-ylmethyl-biphenyl-4-ylmethyl)amine derivatives (5a–5c and 5e–k). A solution of 4′-chloromethyl-biphenyl-4-ylmethylpiperidine **4** (1 mmol) in THF (2 mL) was treated with the appropriate amine (2 mmol) (MW, 100 °C, 150 W, 100 psi, 5 min). Removal of the solvent in vacuo gave the crude products, which were purified by flash chromatography [SiO_2, CH_2Cl_2/CH_3OH (NH_3) = 98:2].

*Cyclohexyl-(4′-piperidin-1-ylmethyl-biphenyl-4-ylmethyl)methylamine (**5a·2HCl·H$_2$O**).* Crystallized from abs EtOH/Et_2O. Yield: 65%; mp: 264–266 °C. ^{1}H NMR (D_2O) δ 1.15–2.07 (m, 16H), 2.75 (s, 3H), 2.91–3.07 (m, 2H), 3.25–3.35 (m, 1H), 3.44–3.54 (m, 2H), 4.21 (d, J = 1.3 Hz, 1H), 4.33 (s, 2H), 4.49 (d, J = 1.3 Hz, 1H), 7.61 (dd, J = 8.4 Hz, J = 1.6 Hz, 4H), 7.82 (dd, J = 8.4 Hz, 4H). MS (EI) 377 $[M+1]^+$. Anal. calcd for $C_{26}H_{36}N_2{\cdot}2HCl{\cdot}H_2O$: C, 66.79; H, 8.62; N, 5.99. Found: C, 66.83; H, 8.45; N, 5.90.

*4′-Piperidin-1-ylmethyl-biphenyl-4-ylmethyl-3,5-dimethylpiperidine (**5b·2HCl·0.25H$_2$O**).* Crystallized from abs EtOH/Et_2O. Yield: 82%; mp > 300 °C. ^{1}H NMR (D_2O) δ 0.96 (d, J = 6.6 Hz, 6H), 1.41–2.14 (m, 10H), 2.60 (t, J = 12 Hz, 2H), 2.93–3.15 (m, 2H), 3.35–3.62 (m, 4H), 4.36 (s, 2H), 4.38 (s, 2H), 7.62 (d, J = 8.1 Hz, 4H), 7.84 (d, J = 8.1 Hz, 4H). MS (EI) 377 $[M+1]^+$. Anal. calcd for $C_{26}H_{36}N_2{\cdot}2HCl{\cdot}0.25H_2O$: C, 68.78; H, 8.55; N, 6.17. Found: C, 68.86; H, 8.46; N, 6.41.

*4-(4′-Piperidin-1-ylmethyl-biphenyl-4-ylmethyl)morpholine (**5c·2HCl·0.5H$_2$O**).* Crystallized from abs EtOH/Et$_2$O. Yield: 76%; mp > 300 °C. ^{1}H NMR (D$_2$O) δ 1.39–2.08 (m, 6H), 2.88–3.10 (m, 2H), 3.16–3.60 (m, 6H), 3.66–4.21 (m, 4H), 4.33 (s, 2H), 4.43 (s, 2H), 7.61 (m, 4H), 7.82 (dd, *J* = 8.1 Hz, *J* = 3.3 Hz, 4H). MS (EI) 351 [M+1]$^+$. Anal. calcd for $C_{23}H_{30}N_2$·2HCl·0.5H$_2$O: C, 63.88; H, 7.46; N, 6.48. Found: C, 63.79; H, 7.40; N, 6.73.

*4′-Piperidin-1-ylmethyl-biphenyl-4-ylmethyldimethylamine (**5e·0.25H$_2$O**).* Crystallized from EtOH/H$_2$O. Yield: 67%; mp: 78–79 °C. ^{1}H NMR (CDCl$_3$) δ 1.39–1.49 (m, 2H), 1.55–1.66 (m, 4H), 2.27 (s, 6H), 2.36–2.49 (m, 4H), 3.46 (s, 2H), 3.52 (s, 2H), 7.33–7.42 (m, 4H), 7.50 (dd, *J* = 8.1 Hz, *J* = 2.1 Hz, 4H). MS (EI) 309 [M+1]$^+$. Anal. calcd for $C_{21}H_{28}N_2$·0.25H$_2$O: C, 80.59; H, 9.02; N, 8.95. Found: C, 80.74; H, 8.99; N, 8.79.

*4′-Piperidin-1-ylmethyl-biphenyl-4-ylmethyldiethylamine (**5f·2HCl·0.5H$_2$O**).* Crystallized from abs EtOH/Et$_2$O. Yield: 73%; mp > 300 °C. ^{1}H NMR (D$_2$O) δ 1.33 (t, *J* = 7.3 Hz, 6H), 1.42–2.01 (m, 6H), 2.88–3.06 (m, 2H), 3.24 (q, *J* = 7.2 Hz, 4H), 3.43–3.58 (m, 2H), 4.32 (s, 2H), 4.38 (s, 2H), 7.54–7.66 (m, 4H), 7.80 (dd, *J* = 8.4 Hz, *J* = 2.1 Hz, 4H). MS (EI) 337 [M+1]$^+$. Anal. calcd for $C_{23}H_{32}N_2$·2HCl·0.5H$_2$O: C, 66.01; H, 8.19; N, 6.69. Found: C, 66.28; H, 8.08; N, 6.45.

*4′-Piperidin-1-ylmethyl-biphenyl-4-ylmethyldipropylamine (**5g·2HCl·H$_2$O**).* Crystallized from abs EtOH/Et$_2$O. Yield: 68%; mp: 240–242 °C. ^{1}H NMR (D$_2$O) δ 0.95 (t, *J* = 7.2 Hz, 6H), 1.38–2.05 (m, 10H), 2.94–3.08 (m, 2H), 3.13 (t, *J* = 8.1 Hz, 4H), 3.43–3.63 (m, 2H), 4.34 (s, 2H), 4.42 (s, 2H), 7.61 (dd, *J* = 8.4 Hz, *J* = 2.7 Hz, 4H), 7.83 (dd, *J* = 8.1 Hz, *J* = 2.4 Hz, 4H). MS (EI) 365 [M+1]$^+$. Anal. calcd for $C_{25}H_{36}N_2$·2HCl·H$_2$O: C, 65.92; H, 8.85; N, 6.15. Found: C, 66.17; H, 8.55; N, 6.33.

*4′-Piperidin-1-ylmethyl-biphenyl-4-ylmethyl-4-methylpiperidine (**5h·2HCl·0.5H$_2$O**).* Crystallized from abs EtOH/Et$_2$O. Yield: 82%; mp > 300 °C. ^{1}H NMR (D$_2$O) δ 0.99 (d, *J* = 6 Hz, 3H) 1.33–1.60 (m, 3H), 1.60–2.05 (m, 8H), 2.89–3.13 (m, 4H), 3.38–3.62 (m, 4H), 4.35 (s, 4H), 7.62 (d, *J* = 8.1 Hz, 4H), 7.83 (d, *J* = 8.1 Hz, 4H). MS (EI) 363 [M+1]$^+$. Anal. calcd for $C_{25}H_{34}N_2$·2HCl·0.5H$_2$O: C, 67.55; H, 8.39; N, 6.30. Found: C, 67.39; H, 8.10; N, 6.39.

*Phenylmethyl-(4′-piperidin-1-ylmethyl-biphenyl-4-ylmethyl) methylamine (**5i·2HCl**).* Crystallized from abs EtOH. Yield: 64%; mp: 281–283 °C. ^{1}H NMR (D$_2$O) δ 1.37–1.56 (m, 1H), 1.56–1.88 (m, 3H), 1.88–2.08 (m, 2H), 2.79 (s, 3H), 2.92–3.12 (m, 2H), 3.41–3.62 (m, 2H), 4.34 (s, 2H), 4.39 (s, 2H), 4.42 (s, 2H), 7.46–7.65 (m, 9H), 7.82 (d, *J* = 8.1 Hz, 4H). MS (EI) 385 [M+1]$^+$. Anal. calcd for $C_{25}H_{36}N_2$·2HCl: C, 70.88; H, 7.49; N, 6.12. Found: C, 70.78; H, 7.43; N, 5.86.

*1-[4-(4′-Piperidin-1-ylmethyl-biphenyl-4-ylmethyl)-piperazin-1yl]ethanone (**5j·2HCl·0.5H$_2$O**).* Crystallized from abs EtOH/Et$_2$O. Yield: 65%; mp > 300 °C. ^{1}H NMR (D$_2$O) δ 1.40–1.55 (m, 2H), 1.58–1.99 (m, 6H), 2.15 (s, 3H), 2.91–3.55 (m, 8H), 4.32 (s, 2H), 4.43 (s, 2H), 7.55–7.65 (m, 4H), 7.76–7.86 (m, 4H). MS (EI) 392 [M+1]$^+$. Anal. calcd for $C_{25}H_{33}N_3O$·2HCl·0.5H$_2$O: C, 63.42; H, 7.45; N, 8.87. Found: C, 63.68; H, 7.56; N, 8.68.

*Cyclohexyl-(4′-piperidin-1-ylmethyl-biphenyl-4-ylmethyl)amine (**5k**).* Crystallized from EtOH/H$_2$O. Yield: 71%; mp: 76–78 °C. ^{1}H NMR (CDCl$_3$) δ 1.09–1.29 (m, 5H), 1.41–1.49 (m, 2H), 1.53–1.64 (m, 5H), 1.69–1.78 (m, 2H), 1.89–1.98 (m, 2H), 2.32–2.47 (m, 4H), 2.47–2.57 (m, 1H), 3.50 (s, 2H), 3.84 (s, 2H), 7.37 (d, *J* = 7.2 Hz, 4H), 7.49–7.58 (m, 4H). MS (EI) 363 [M+1]$^+$. Anal. calcd for $C_{25}H_{34}N_2$: C, 82.82; H, 9.45; N, 7.73. Found: C, 82.56; H, 9.48; N, 7.65.

6.1.1.4. Preparation of 4′-(4-cyanophenoxymethyl)-biphenyl-4-ylmethylpiperidine (5d·0.25H$_2$O). Starting from 4-hydroxybenzonitrile and the intermediate **4**, the desired compound **5d** was obtained according to the procedure described for the synthesis of **5l**, Crystallized from EtOH. Yield: 63%; mp: 157–159 °C. ^{1}H NMR (CDCl$_3$) δ 1.44–1.47 (m, 2H), 1.62–1.65 (m, 4H), 2.45–2.48 (m, 4H), 3.57 (s, 2H), 5.15 (s, 2H), 7.04 (d, *J* = 8.8 Hz, 2H), 7.40–7.64 (m, 10H). (EI) 385 [M+1]$^+$. Anal. calcd for $C_{26}H_{26}N_2O$·0.25H$_2$O: C, 80.69; H, 6.77; N, 7.23. Found: C, 80.89; H, 6.66; N, 7.18.

6.1.1.5. Preparation of 1-(4′-piperidin-1-ylmethyl-biphenyl-4-ylmethyl)piperidin-2-one (5l). A solution of 2-piperidone (3.32 mmol) in anhydrous toluene (2 mL, under an atmosphere of N$_2$) was treated with NaH (0.14 g, 60% dispersion in mineral oil); this mixture was added to a solution of **4** (1.67 mmol) in anhydrous toluene (3 mL). The reaction was stirred at 100 °C for 5 h and the solid residue was filtered off. Removal of the solvent in vacuo gave the crude products, which was purified by flash chromatography [SiO$_2$, CH$_2$Cl$_2$:CH$_3$OH (NH$_3$) = 99:1]. Crystallized from EtOH/H$_2$O. Yield: 62%; mp: 108–110 °C. ^{1}H NMR (CDCl$_3$) δ 1.39–1.49 (m, 2H), 1.53–1.64 (m, 4H), 1.74–1.85 (m, 4H), 2.36–2.44 (m, 4H), 2.48 (t, *J* = 5.7 Hz, 2H), 3.23 (t, *J* = 5.7 Hz, 2H), 3.50 (s, 2H), 4.63 (s, 2H), 7.23–7.41 (m, 4H), 7.48–7.57 (m, 4H). MS (EI) 363 [M+1]$^+$. Anal. calcd for $C_{24}H_{30}N_2O$: C, 79.52; H, 8.34; N, 7.73. Found: C, 79.31; H, 8.31; N, 7.53.

6.1.1.6. Preparation of 2,2′-biphenyl-4,4′-diylbis-acetic acid (6). A solution of 4,4′-bis(chloromethyl)-biphenyl **2** (1 mmol) in CH$_3$CN (2 mL) was reacted with NaCN (4 mmol) in H$_2$O (1 mL) to give [1,1′-biphenyl]-4,4′-diacetonitrile (MW, 100 °C, 150 W, 100 psi, 5 min). Yield: 94%. ^{1}H NMR (DMSO) δ 4.09 (s, 4H), 7.45 (d, *J* = 8.2 Hz, 4H), 7.72 (d, *J* = 8.2 Hz, 4H). MS (EI) 233 [M+1]$^+$.

A solution of [1,1′-biphenyl]-4,4′-diacetonitrile (1 mmol) in EtOH (2 mL) was reacted with KOH (4 mmol) in EtOH (2 mL) (MW, 150 °C, 200 W, 145 psi, 5 min). After cooling, the reaction mixture was acidified with HCl (1 M) and the desired product was obtained as precipitate. Yield: 91%. ^{1}H NMR (DMSO) δ 3.60 (s, 4H), 7.34 (d, *J* = 8.2 Hz, 4H), 7.59 (d, *J* = 8.2 Hz, 4H). MS (EI) 271 [M+1]$^+$.

6.1.1.7. General method of preparation of [biphenyl-4,4′diyl-bis(ethylene)]bis-amine derivatives (7a–7c). A solution of 2,2′-(1,1′-biphenyl)-4,4′-diylbis-acetic acid **6** (0.3 mmol) in THF (3 mL) were reacted with CDI (1.28 mmol) (MW, 150 °C, 200 W, 145 psi, 5 min). After this heating cycle, the suitable amine (1.2 mmol) was added to the reaction and the mixtures were submitted to a second heating cycle as described above. After cooling, THF was evaporated under reduced pressure; the residues were taken up in ethyl acetate, washed with HCl (1 N), NaOH (10% w/v), and dried over Na$_2$SO$_4$. The organic layers were concentrated to yield the desired amides which were characterized by ^{1}H NMR and mass spectra.

To the stirred solutions of the appropriate amide (1 mmol) in anhydrous toluene (15 mL, under an atmosphere of N$_2$), RedAl® (8.0 mmol, 70% solution in toluene, 2.3 mL) was carefully added. The resulting mixtures were stirred for 1 h at room temperature, then heated to reflux for 3 h.[33] After cooling, NaOH (10 mL, 3 N) was added dropwise and the organic phases were evaporated under reduced pressure to afford the crude products which were purified by flash chromatography [SiO$_2$, CH$_2$Cl$_2$/MeOH(NH$_3$) = 15:1].

*1,1′-[Biphenyl-4,4′-diylbis(ethylene)]bis-piperidine (**7a·2HCl·H$_2$O**).* Crystallized from abs EtOH/Et$_2$O. Yield: 70%; mp > 300 °C. ^{1}H NMR (CDCl$_3$) δ 1.46–1.50 (m, 4H), 1.60–1.69 (m, 8H), 2.54 (m, 8H), 2.56–2.62 (m, 4H), 2.82–2.88 (m, 4H), 7.26 (d, *J* = 8.1 Hz, 4H), 7.49 (d, *J* = 8.1 Hz, 4H). MS (EI) 377 [M+1]$^+$. Anal. calcd for $C_{26}H_{36}N_2$·2HCl·H$_2$O: C, 66.79; H, 8.62; N, 5.99. Found: C, 66.68; H, 8.07; N, 6.01.

*N,N′-[biphenyl-4,4′diylbis(ethylene)]bis-dimethylamine (**7b·2HCl·0.5H$_2$O**).* Crystallized from abs EtOH/Et$_2$O. Yield: 81%; mp > 300 °C. ^{1}H NMR (D$_2$O) δ 2.93 (s, 12H), 3.12 (t, *J* = 15.7 Hz, 4H), 3.46 (t, *J* = 15.7 Hz, 4H), 7.43 (d, *J* = 8.1 Hz, 4H), 7.69 (d,

J = 8.1 Hz, 4H). MS (EI) 297 $[M+1]^+$. Anal. calcd for $C_{20}H_{28}N_2 \cdot 2HCl \cdot 0.5H_2O$: C, 63.48; H, 8.26; N, 7.40. Found: C, 63.84; H, 7.80; N, 7.37.

1,1′-[Biphenyl-4,4′-diylbis(ethylene)]bis-4-methylpiperidine (***7c·2HCl·H₂O***). Crystallized from abs EtOH/Et_2O. Yield: 73%; mp > 300 °C. ^{1}H NMR (D_2O) δ 0.96 (d, J = 6.4 Hz, 6H), 1.42 (m, J = 13.4 Hz, 4H), 1.71 (m, 2H), 1.94–1.99 (m, J = 14.45 Hz, 4H), 2.97–3.05 (m, J = 12.68 Hz, 4H), 3.11–3.16 (m, 4H), 3.37–3.47 (m, 4H), 3.61–3.65 (m, J = 12.32 Hz, 4H), 7.44 (d, J = 8.2 Hz, 4H), 7.70 (d, J = 8.2 Hz, 4H). MS (EI) 405 $[M+1]^+$. Anal. calcd for $C_{28}H_{40}N_2 \cdot 2HCl \cdot H_2O$: C, 67.86; H, 8.94; N, 5.65. Found: C, 67.95; H, 8.34; N, 5.68.

6.1.1.8. General method of preparation of 4-iodophenyl-1-aminoethanone derivatives (9a and b). A solution of 2-(4-iodophenyl)acetic acid **8** (1 mmol) in CH_3CN (3 mL) was reacted with CDI (1.2 mmol) (MW, 130 °C, 150 W, 145 psi, 5 min). After this heating cycle, piperidine or dipropylamine (1.2 mmol) were added to the reaction, and the mixtures were submitted to a second heating cycle as described above. After cooling, H_2O was added and the crude products were obtained as precipitates.

4-Iodophenyl-1-piperidin-1-ylethanone (***9a***). Yield: 90%. ^{1}H NMR (DMSO) δ 1.31–1.42 (m, 4H), 1.50–1.57 (m, 2H), 3.38–3.42 (m, 4H), 3.65 (s, 2H), 7.03 (d, J = 8.2 Hz, 4H), 7.63 (d, J = 8.2 Hz, 4H). MS (EI) 319 $[M+1]^+$.

4-Iodophenyl-1-dipropylaminoethanone (***9b***). Yield: 85%. ^{1}H NMR ($CDCl_3$) δ 0.85–0.92 (m, 6H), 1.49–1.61 (m, 4H), 3.18 (t, 2H), 3.28 (t, 2H), 3.63 (s, 2H), 7.01 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H). MS (EI) 346 $[M+1]^+$.

6.1.1.9. Preparation of 4′-(2-oxo-2-piperidino-ethyl)biphenyl-4-carboxylic acid (10). A solution of **9a** (1mmol), 4-carboxyphenylboronic acid (1.1 mmol) and K_2CO_3 (3 mmol) in acetone (15 mL) and bidistilled H_2O (15 mL) was degassed bubbling nitrogen for 10 min. To this solution $Pd(OAc)_2$ (0.12 g) was added, and the reaction mixture was stirred at 65 °C for 1 h.[54] After filtration of the catalyst, HCl (5 mL 1 N) was added and the product was obtained as precipitate. Yield: 99%. ^{1}H NMR (DMSO) δ 1.36–1.41 (m, 4H), 1.52–1.54 (m, 2H), 3.41–3.45 (m, 4H), 3.74 (s, 2H), 7.33–8.07 (m, 8H), 12.99 (bs, 1H). MS (EI) 324 $[M+1]^+$.

6.1.1.10. Preparation of 2-(4′-piperidinocarbonyl-biphenyl-4-yl)-1-piperidinoethanone (11). A solution of **10** (1 mmol) in 3 mL of CH_3CN (3 mL) was reacted with CDI (1.2 mmol) (MW, 130 °C, 150 W, 145 psi, 5 min). After this heating cycle, piperidine (1.2 mmol) was added to the reaction, and the mixture was submitted to a second heating cycle as described above. After completion of reaction, H_2O was added and the solution was extracted with ethyl acetate; the organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was used in the successive reaction without further purification. Yield: 97%. ^{1}H NMR ($CDCl_3$) δ 1.40–1.66 (m, 12H), 3.40–3.69 (m, 8H), 3.74 (s, 2H), 7.29–7.62 (m, 8H). MS (EI) 391 $[M+1]^+$.

6.1.1.11. Preparation of 1-[2-(4′-piperidinomethyl-biphenyl-4-yl)ethyl]piperidine (12·2HCl·0.5H₂O). Reduction of amide **11** was performed as described in the preparation of compounds **7a–7c**. The crude product **12** was purified by flash chromatography [SiO_2, CH_2Cl_2/MeOH(NH_3) = 130:1]. Crystallized from abs EtOH/Et_2O. Yield: 90%; mp > 300 °C. ^{1}H NMR (D_2O) δ 1.36–1.39 (m, 2H), 1.58–1.67 (m, 6H), 1.79–1.81 (m, 4H), 2.90 (t, J = 10.8 Hz, 4H), 2.99–3.04 (m, 2H), 3.20–3.23 (m, 2H), 3.28 (d, J = 12.1 Hz, 2H), 3.48 (d, J = 11.8 Hz, 2H), 4.22 (s, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.56 (d, J = 8.2 Hz, 2H), 7.64 (d, J = 8.2 Hz, 2H), 7.71 (d, J = 8.2 Hz, 2H). MS (EI) 363 $[M+1]^+$. Anal. calcd for $C_{25}H_{34}N_2 \cdot 2HCl \cdot 0.5H_2O$: C, 67.55; H, 8.39; N, 6.30. Found: C, 67.38; H, 8.28; N, 6.26.

6.1.1.12. General method of preparation of (4′-hydroxymethyl-biphenyl-4-yl)-1-aminoethanone derivatives (13a and b). Using the same method described for the synthesis of (4′-carboxy-1,1′-biphenyl-4-yl)-1-piperidin-1-yl-ethanone 10, the title compounds were obtained starting from 4-iodophenyl-derivatives 9a,b and 4-hydroxymethylphenylboronic acid. After filtration over celite and evaporation of the solvent, the crude products were dissolved in H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the colorless solids.

(4′-Hydroxymethyl-biphenyl-4-yl)-1-piperidin-1-ylethanone (***13a***). Yield: 70%. ^{1}H NMR ($CDCl_3$) δ 1.37–1.44 (m, 2H), 1.54–1.60 (bs, 4H), 3.41 (t, 2H), 3.59 (t, 2H), 3.76 (s, 2H), 4.73 (s, 2H), 7.30 (d, J = 8.3 Hz, 2H), 7.42 (d, J = 8.3 Hz, 2H), 7.52–7.57 (m, 4H). MS (EI) 310 $[M+1]^+$.

(4′-Hydroxymethyl-biphenyl-4-yl)-1-dipropylaminoethanone (***13b***). Yield: 73%. ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H), 1.52–1.62 (m, 4H), 3.22 (t, 2H), 3.30 (t, 2H), 3.72 (s, 2H), 4.69 (s, 2H), 7.29 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 7.50–7.54 (m, 4H). MS (EI) 326 $[M+1]^+$.

6.1.1.13. General method of preparation of (4′-aminomethyl-biphenyl-4-yl)-1-aminoethanone derivatives (14 and 15). To a stirred solution of **13a** and **b** (1.54 mmol) in CH_2Cl_2 (33 mL), MsCl (3.08 mmol) was added; after cooling to 0 °C, Et_3N (3.84 mmol) was added and the resulting mixture was stirred at room temperature for 90 min. The solvent was evaporated to dryness and the crude product, dissolved in CH_3CN (2 mL), was reacted with the appropriate amine (1.7 mmol) (MW, 100 °C, 90 W, 100 psi, 5 min). After cooling, CH_3CN was evaporated under reduced pressure and the crude product was purified by chromatography [SiO_2, CH_2Cl_2:MeOH(NH_3) = 100:1].

2-(4′-Dipropylaminomethyl-biphenyl-4-yl)-1-piperidin-1-ylethanone (***14·HCl·0.5H₂O***). Crystallized from abs EtOH/Et_2O. Yield: 80%; mp: 174–176 °C. ^{1}H NMR (D_2O) δ 0.82 (t, 6H), 1.32–1.55 (m, 10H), 2.30–2.31 (m, 4H), 3.42–3.45 (m, 4H), 2.99–3.04 (m, 2H), 3.53 (s, 2H), 3.72 (s, 2H), 7.29 (d, J = 8.2 Hz, 2H), 7.36 (d, J = 8.2 Hz, 2H), 7.59 (d, J = 8.2 Hz, 2H). MS (EI) 393 $[M+1]^+$. Anal. calcd for $C_{26}H_{36}N_2O \cdot HCl \cdot 0.5H_2O$ C, 71.29; H, 8.74; N, 6.39. Found: C, 71.45; H, 8.71; N, 6.41.

2-(4′-Piperidin-1-ylmethyl-biphenyl-4-yl)-1-dipropylaminoethanone (***15·HCl***). Crystallized from abs EtOH/Et_2O. Yield: 85%; mp: 158–159 °C. ^{1}H NMR (DMSO) δ 0.76–0.88 (m, 6H), 1.30–1.78 (m, 10H), 2.83–2.87 (m, 2H), 3.17–3.30 (m, 6H), 4.27 (d, J = 4.9, 2H), 3.53 (s, 2H), 7.33 (d, J = 8.2 Hz, 2H), 7.62–7.68 (m, 4H), 7.75 (d, J = 8.2 Hz, 2H), 10.40 (s, 1H). MS (EI) 393 $[M+1]^+$. Anal. calcd for $C_{26}H_{36}N_2O \cdot HCl$ C, 72.78; H, 8.69; N, 6.53. Found: C, 73.03; H, 8.79; N, 6.54.

6.1.1.14. Preparation of [4′-(2-piperidin-1-yl-ethyl)-biphenyl-4-ylmethyl]dipropylamine (16·2HCl·0.5H₂O). Reduction of amide **14** was performed as described in the preparation of compounds **7a–7c**. The crude product **16** was purified by flash chromatography [SiO_2, CH_2Cl_2/MeOH(NH_3) = 130:1]. Crystallized from abs EtOH/Et_2O. Yield: 60%; mp: 260–262 °C. ^{1}H NMR (DMSO) δ 0.86 (t, 6H), 1.36–1.83 (m, 10H), 2.90–2.94 (m, 6H), 3.08–3.15 (m, 2H), 3.20–3.27 (m, 2H), 3.46–3.57 (m, 2H), 4.32 (d, J = 4.9, 2H), 7.37–7.40 (d, J = 8.2 Hz, 2H), 7.67–7.77 (m, 4H), 10.70 (s, 1H), 10.83 (s, 1H). MS (EI) 379 $[M+1]^+$. Anal. calcd for $C_{26}H_{38}N_2 \cdot 2HCl \cdot 0.5H_2O$ C, 67.81; H, 8.97; N, 6.08. Found: C, 67.71; H, 9.05; N, 6.04.

6.2. Molecular modeling

Molecular modeling studies were performed with Schröedinger software suite (Schröedinger L.L.C., NY). Ligand structures were optimized with Macromodel 8.6, applying MMFF94s force field[55] to an energy gradient of 0.05 kJ/mol Å.

6.2.1. Docking into the rat H_3 receptor model

Docking studies were performed with Glide 3.5,[56] employing a previously developed rat H_3 receptor model.[57] Protein structure was subjected to the Protein Preparation procedure implemented in Glide, applying the 'Preparation Only' procedure without neutralization of amino acid residues. Docking experiments were performed starting from minimum-energy conformations of the ligands placed in arbitrary positions, within a region centered on amino acid Asp114 in TM3, using enclosing and bounding boxes of 46 and 14 Å on each side, respectively. Compounds **3g**, **5b**, and **7a** were docked in their diprotonated state, compound **5d** in the protonated form. Van der Waals radii of the protein were not scaled, whereas van der Waals radii of the ligand atoms with partial atomic charge lower than |0.15| were scaled by 0.80. Standard precision mode was applied and poses with a Coulomb–van der Waals score greater than 0 kcal/mol were rejected. Docking solutions were ranked according to their Emodel value.

The horizontal and vertical poses of compound **7a** were merged into the H_3 receptor model. The complexes were energy-minimized with MMFF94s force field to a gradient of 0.05 kJ/mol Å with fixed protein backbone and they were submitted to molecular dynamics (MD) simulation to evaluate their stability. MD was performed with MMFF94 force field,[58] with a time step of 1 fs, a dielectric constant of 1 and a non-bonded cutoff of 8 Å, for 1 ns at 310 K after equilibration for 100 ps, with fixed position of the backbone atoms. The last conformation of each MD simulation was energy-minimized as already described, and the resulting structures are depicted in Figure 2.

6.2.2. Docking into the mouse acetylcholinesterase active site

Since the three-dimensional coordinates of rat acetylcholinesterase are not available, the structure of the mouse acetylcholinesterase was used for docking studies, given the high sequence similarity and the identical composition of amino acids facing the active site in the rat and mouse enzyme.[59] The crystallographic coordinates of acetylcholinesterase complex with succinylcholine (PDB ID: 2HA2)[52] were taken from the RCSB Protein Data Bank.[60] This crystal structure has already been used to perform docking studies for di-basic acetylcholinesterase inhibitors.[46] The acetylcholinesterase structure was prepared by deleting succinylcholine and water molecules and by adding hydrogen atoms. Protein structure was subjected to the Protein Preparation procedure implemented in Glide 3.5,[56] applying the standard 'Preparation and Refinement' protocol, without neutralization of amino acid residues. Docking experiments were performed starting from minimum-energy conformations of the ligands placed in arbitrary positions, within a region centered on the inhibitor succinylcholine, using enclosing and bounding boxes of 26 and 14 Å on each side, respectively. Compound **12** was docked in the diprotonated form and donepezil with protonated piperidine ring. Van der Waals radii of the protein were not scaled, whereas van der Waals radii of the ligand atoms with partial atomic charge lower than |0.15| were scaled by 0.80. Standard precision mode was applied and poses with a Coulomb–van der Waals score greater than 0 kcal/mol were rejected. Docking solutions were ranked according to their Emodel value. The best scoring solutions of compound **12** and donepezil were merged into acetylcholinesterase and the complexes are shown in Figure 3.

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