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Synthesis and structure–activity relationship of Huprine derivatives as human acetylcholinesterase inhibitors

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ABSTRACT

New series of Huprine (12-amino-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinolines) derivatives have been synthesized and their inhibiting activities toward recombinant human acetylcholinesterase (rh-AChE) are reported. We have synthesized two series of Huprine analogues; in the first one, the benzene ring of the quinoline moiety has been replaced by different heterocycles or electron-withdrawing or electron-donating substituted phenyl group. The second one has been designed in order to evaluate the influence of modification at position 12 where different short linkers have been introduced on the Huprine X, Y skeletons. All these molecules have been prepared from ethyl- or methyl-bicyclo[3.3.1]non-6-en-3-one via Friedländer reaction involving selected *o*-aminocyano aromatic compounds. The synthesis of two heterodimers based on these Huprines has been also reported. Activities from moderate to same range than the most active Huprines X and Y taken as references have been obtained, the most potent analogue being about three times less active than parent Huprines X and Y. Topologic data have been inferred from molecular dockings and variations of activity between the different linkers suggest future structural modifications for activity improvement.

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1. Introduction

The ubiquitous synaptic enzyme acetylcholinesterase (AChE) terminates transmission at cholinergic synapses by rapidly hydrolyzing acetylcholine. As a consequence of its key physiological role, AChE is not only the target of a repertoire of natural toxins [e.g., the alkaloids Galanthamine and (−)-Huperzine A, or the three-fingered polypeptide toxin Fasciculin], or man made pest control agents and warfare agents, but also the target of drugs designed to combat neuromuscular disorders, such as myasthenia gravis and glaucoma,[1] and most recently to alleviate the cholinergic deficiency associated with Alzheimer's disease.[2] With this latter aim in mind, a possible symptomatic treatment for this disease is to remedy the abnormal low rate of neurotransmitter acetylcholine by controlling the enzymatic activity of acetylcholinesterase. Indeed, AChE can be reversibly inhibited,[3–5] resulting in an increased neurotransmission where the synaptic connections are still intact and thus reducing the secondary neurodegenerative effects of the pathology. Recent results show that a specific inhibition of AChE is a potential therapeutic target also for prevention of the first step of Alzheimer disease, the assembly of β-amyloid peptide (Aβ) into amyloid fibrils.[6–9]

Since the inhibition of AChE based on the 'cholinergic hypothesis' remains the most powerful therapeutic strategy in treating Alzheimer's disease, four different AChE inhibitors have already been approved by the health authorities and are commercially available, Tacrine (Cognex®), Donepezil (or E2020, Aricept®), Rivastigmine (Exlon®), and Galanthamine (Reminyl®), whose structures are displayed in Figure 1.

Within these compounds, Tacrine has been the most studied compound and the first marketed product despite severe side effects. Today, Tacrine remains a reference structure even if the adverse troubles induced, such as hepatotoxicity and gastrointestinal disorders, led to its withdrawal from the market. Treatment having to be taken life-long, with the enhanced liver toxicity of those drugs, it is of prior importance to develop new more efficient, selective, and safe AChE inhibitors.

Huprines, empirically designed Tacrine–Huperzine A hybrids have been described a few years ago as highly potent AChE

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inhibitors (Scheme 1).[10] Surprisingly, if many efforts have been undertaken to develop new Tacrine analogues, the modifications on the Huprines have been scarcely studied.

With the goal in mind to develop efficient heterodimeric acylation site—peripheral site hAChE inhibitors, in a first step, we have decided to evaluate the possibility of further improvement of Huprine's binding ability toward AChE acylation site. First, docking experiments led on human AChE, and crystallographic data on *Torpedo California* AChE complexed with Huprine[11] allowed us to identify on Huprine structure two preferred moieties (positions 1–4 of benzene ring B of quinoline moiety and position 9 on the cyclohexene part) which could be favorably modified in order to improve the binding of these inhibitors to the acylation site. Previous results have shown that the modifications on the positions 1–3 on the quinoline benzene have led to tight binding AChE inhibitors, the most potent being Huprines X and Y displaying a chlorine atom at position 3 (Scheme 2).[10,12,13]

Moreover, investigation of structural modifications on positions 9[10] and 13[14,15] on the bicyclo[3.3.1]nonane moiety led to disappointing results. Taking into account that the nature of the B aromatic moiety has not been modified on the previous studies, we decided to focus our synthetic efforts on the modification of this aromatic ring size and on its electronic density as displayed in Scheme 3.

In a second step, aware of the interest of a homo- or heterodimeric dual inhibitor binding both AChE acylation and peripheral binding sites,[16] and thus of the necessity to introduce a reactive linker on the Huprine scaffold, another goal for us was to explore Huprines binding ability when modified with such a linker,

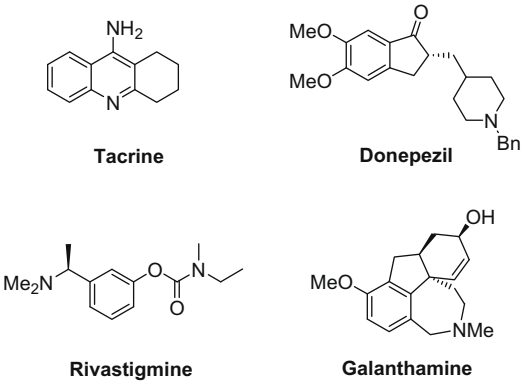
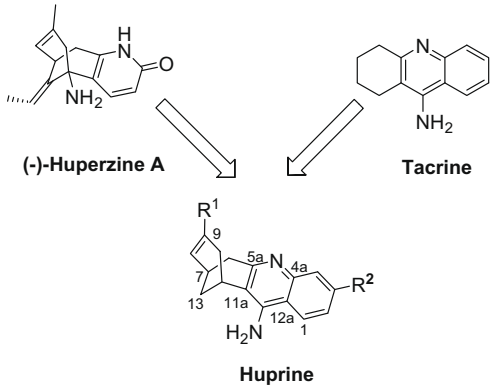
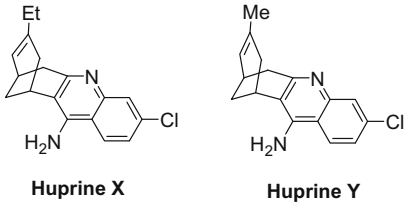


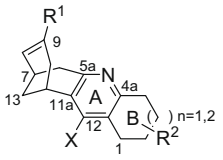
Figure 1. Commercially available AChE inhibitors.



Scheme 1. Structure of Tacrine–Huperzine A hybrids (Huprines) and their starting models. The atom numbering of the Huprine core has been addressed according to Camps et al.[10]



Scheme 2. Huprines X and Y.



Scheme 3. Sites of planned modifications on the Huprine scaffold. R^1 represents a methyl or an ethyl group. R^2 represents various π-donor or electron-donating groups. Aminopyridine cycle (X = NH_2) or N-substituted aminopyridine cycle (X = $(CH_2)_2$-Y, Y = OH, N_3, Cl, or α-chloroacetyl) or 4-substituted pyridine cycle (X = OH, Cl) is designed as cycle A and aromatic cycle fused to aminopyridine is designed as cycle B.

depending on the nature of the linker. We took as reference the previous results obtained on Huprine and Tacrine homodimers, and the docking experiments, both of which show that aromatic amino group on position 12 points toward the narrowest region of the gorge. We thus decided to modify this aniline on the best binding Huprine inhibitor obtained in the first step. These results, supported by both the inhibition activity measurements and by modelization data will enable us to determine the best structure for this part of the inhibitor following a progressive approach of the construction of the linker.

In this article, we want to disclose our efforts toward the synthesis and characterization of our first targets as well as the biological activity assays on the recombinant human acetylcholinesterase (IC_{50} measurements). A set of 24 new Huprine derivatives has been synthesized, which can be divided into two groups depending on the region modified: (1) the modulation of the aromatic part, (2) the interactions with the amino acid residues at the bottom of the gorge. Finally, these results have also been compared to those obtained from molecular modeling calculations on the same enzyme in order to rationalize the activities observed.

2. Results and discussion

2.1. Chemistry

2.1.1. Modulation of the aromatic part

As first targets, the following Huprines **3–11** (Fig. 2) were synthesized, with the goal in mind to screen a wide variety of chemical structures, and to get a better insight into their binding ability to AChE. In this way, taking into account the results reported by Camps et al. for the halogenated Huprines,[10,12] we have first introduced two electron-donor methoxy groups in order to increase the electron density of the aromatic ring (**3a,b**), then varied its distribution by introducing one or several heteroatoms within the aromatic ring. Thus, the quinoline moiety was replaced by 1,8 and 1,7 π-enriched naphthyridine moieties (**4a,b**, **5**), furo[2,3-*b*]pyridine (**6a,b**), oxazolo[5,4-*b*]pyridine (**7a,b**, **8**), thiazolo[4,5-*b*]pyridine **(9a,b),** and 2*H*-pyrazolo[3,4-*b*]pyridine (**10**, **11**).

These Huprine analogues have been readily synthesized in four steps from symmetrical bicyclic diketone **1** as shown in Scheme 4. Indeed, it is well-known from the literature that nucleophilic additions of Grignard or organolithium reagents on this type of bicyclic

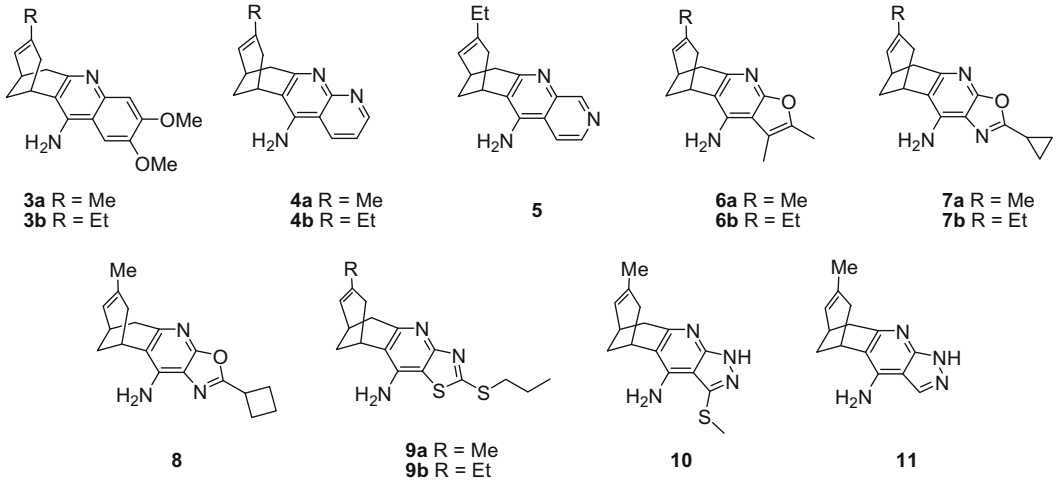
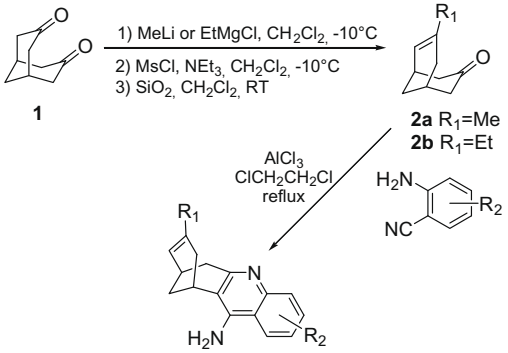


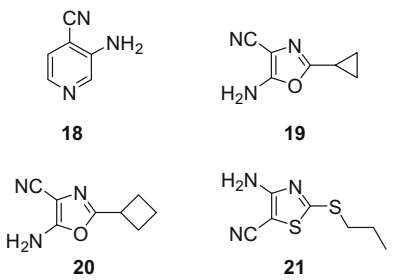
Figure 2. Huprine derivatives synthesized through the modulation of the aromatic moiety.



Scheme 4. Synthetic pathway used for the preparation of Huprines.

diketones afford polycyclic structures such as the oxaadamentanol intermediates required for the preparation of the key enones **2a,b**.[17] Mesylation of the oxaadamentanols followed by a Grob-like fragmentation[18] gives access to the enones **2a,b**. The key step has been then a remarkably regioselective Friedländer annulation of enones **2a,b** on the corresponding functionalized *o*-cyanoaniline derivatives.[19] Considering that the diketone **1** is commercially available but quite expensive, we have chosen to perform the synthesis of this intermediate in two steps from methyl acetonedicarboxylate and propanedial following the classical procedure described by Bertz et al.[20]

Concerning the accessibility of the second Friedländer reaction partner, some of them are commercially available (precursors of Huprines X, Y, **3–4**, **6a,b**, **10–11**) whereas *o*-aminocyanoaromatics **18–21** (Scheme 5) required for the preparation of Huprines **5** and



Scheme 5. *O*-aminocyanoaromatics **18–21** intermediates synthesized for the preparation of Huprines **5**, **7–9**.

Table 1
Regioselectivity observed for the synthesis of Huprines **6–8** and **10–11**

Compound	*anti*/*syn* ratio[a]
6a	87/13
6b	80/20
7a	93/7
7b	88/12
8a	100/0
8b	96/4
10	95/5
11	82/18

[a] Determined by ^{1}H NMR.

7–9, respectively, were synthesized following described procedures (see Supplementary data).

The Friedländer condensation was then performed under classical conditions, using anhydrous $AlCl_3$ in refluxing 1,2-dichloroethane (DCE) overnight and afforded the desired Huprine analogues in moderate to very good isolated yields (30–90%). Interestingly, using such equilibration conditions (overnight reflux), the regioselectivity of this reaction proved to be excellent in the case of six-membered rings, yielding exclusively the thermodynamically favored *anti* Huprines.[19] In an opposite manner, the minor regioisomer *syn* could be observed in some five-membered rings. The ratios *anti*/*syn* obtained for the Huprine derivatives including a five-membered ring are displayed in Table 1. Apparently, the *syn*/*anti* regioselectivity between Huprines depends on the nature of the second aromatic ring.

These compounds correspond to original modifications of the Huprine scaffold while only analogues bearing different substituents on a quinoline scaffold have been reported to date.[10,12] All the new Huprines were fully characterized through their spectroscopic (IR, MS, ^{1}H and ^{13}C NMR spectra) and analytic data (elemental analysis), and their purity was checked by RP-HPLC.

2.1.2. Derivatization with reactive linkers to probe key interactions with the amino acid residues in the AChE gorge

Taking into account that none of the newly described Huprines displayed better binding affinities than parent Huprines X and Y (vide infra) and with the goal in mind to evaluate the possibilities to further graft a linker on the Huprine scaffold aimed at preparing homo- or heterodimeric dual AChE binding sites inhibitors, the new Huprines **12–17** (Fig. 3), based on Huprine X and Y skeleton were synthesized. These molecules were chosen to achieve two main objectives: firstly, the synthesis of these molecules will allow

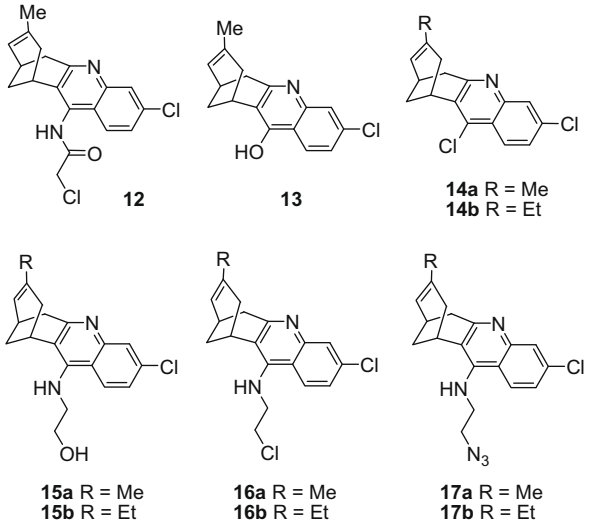


Figure 3. Huprine derivatives synthesized for the exploration of possible interactions with the amino acid residues of the enzyme gorge.

us to tackle the tricky problem of the alkylation of this type of fragile aminoquinoline derivatives.[21] Secondly, the biological evaluation of these derivatives will bring some interesting information on the molecular environment at this part of the gorge and the possibilities of further interactions with the amino acid residues present in the enzyme. We have chosen a two-carbon linker with a further functional group aiming at the covalent association with an AChE peripheral site binder.[22]

The prepared Huprine analogues have various heteroatoms (N from azido group, O, Cl) at the terminal position of either the grafted spacer unit or the amino group of the parent Huprine. As we have encountered serious difficulties to alkylate or acylate the amino group of Huprine (details in Supplementary data), we have chosen (except for acylated compound **12**) to introduce the linker by a S_NAr reaction on the 12-chloro-Huprine analogues **14a**,**b**. To the best of our knowledge, the preparation of these 12-chloro-derivatives has been only reported by Camps et al.,[21] but the conditions described by the authors showed important drawbacks such as the formation of significant amounts of regioisomers. To avoid this problem, we have decided to apply conditions used for the preparation of 9-chlorotacrine derivatives.[23,24] Reaction of enones **2a**,**b** with 2-amino-4-chlorobenzoic acid in refluxing $POCl_3$ afforded the pure desired 12-chloroquinoline derivatives **14a**,**b** in 27% and 46% yield, respectively.

Thereafter, S_NAr reactions have been undertaken. Treatment of **14a,b** with ethanolamine in refluxing *n*-pentanol gave mixture of compounds,[22] whereas the conditions described by Hu et al. (with catalytic amounts of NaI in phenol at 150 °C) allowed to furnish the desired products **15a,b** in 77% and 95% yield, respectively.[24] With **15a,b** in hand, introduction of different functional groups on the linker was envisaged. Upon treatment of alcohols **15a,b** with $SOCl_2$, the compounds **16a,b** were readily obtained in quantitative yields (97% and 99%, respectively). Finally, nucleophilic substitution of the chlorine atom of **16a** and **16b** by sodium azide gave products **17a** and **17b** in good yields (78% and 69%, respectively, over two steps from the alcohols **15a** and **15b**). In the other hand, we attempted selective reduction of carboxamide function of **12** with various hydride sources to access directly to **16a**, but all tested conditions failed.

2.1.3. Coupling of a peripheral site inhibitor to afford dual-site inhibition

As suggested above, the covalent association with an AChE peripheral site binder has finally been undertaken. Huprines **17a** and **17b** have respectively been coupled by a copper(I)-catalyzed Huisgen reaction with the tetrahydroquinoline derivatives **22** and **23** prepared following described procedures (see Supplementary data).[33] The *anti* regioisomers **24** and **25** were thus obtained exclusively (Fig. 4).

3. Structure–activity relationship

The AChE inhibitory efficiencies have been evaluated according to the standard Ellman methodology (see Section 5).[25] According to the targeted activity, we have chosen to perform our analyses with human AChE rather than with *Torpedo Californica* or *Electrophorus Electricus* AChE since significant differences in the structures and conformations of these enzymes exist. Most of the inhibitory activ-

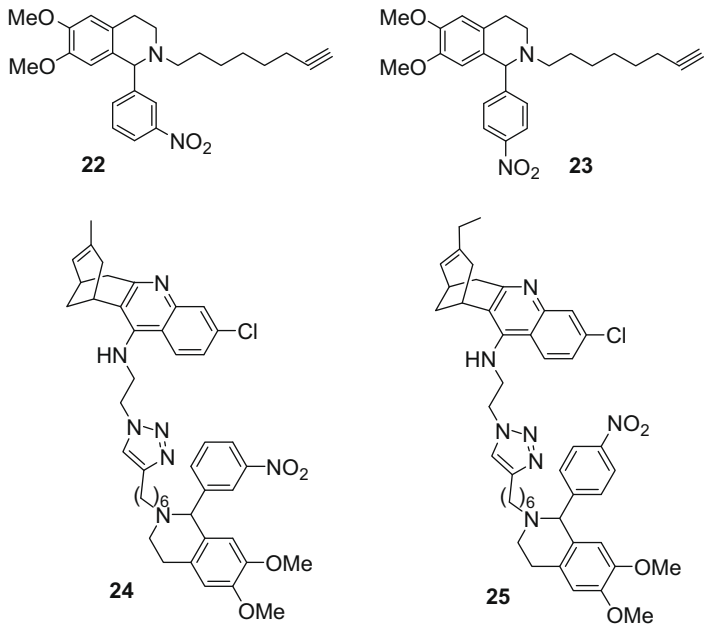


Figure 4. Dual-site inhibitors **24** and **25** and their respective tetrahydroquinoline derivative precursors **22** and **23**.

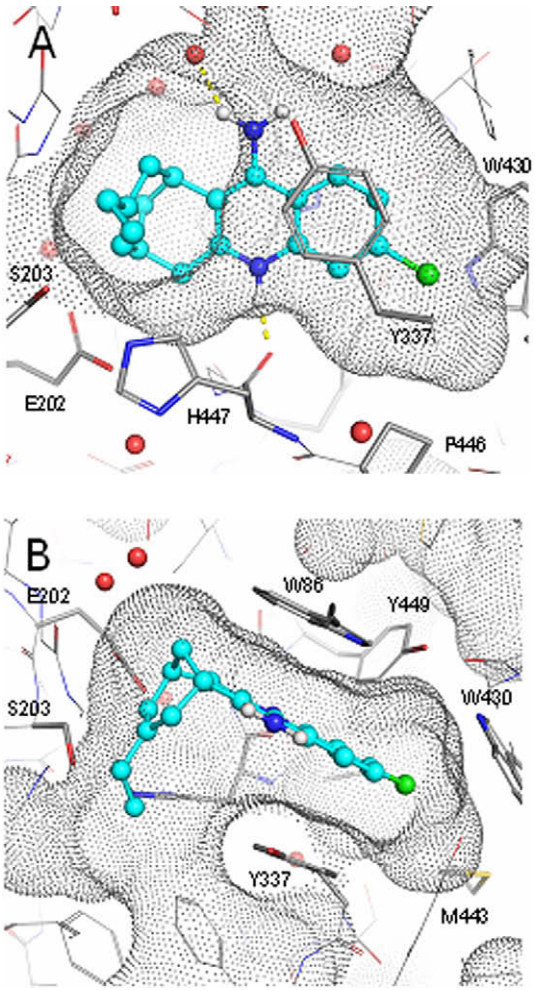


Figure 5. Side view (A) and top view (B) of Huprine X docked in human acetylcholinesterase. The solvent accessibility surface is represented as dots. Carbon atoms are represented in gray (*rh*-AChE) or cyan (Huprine X), oxygen in red, and nitrogen in blue. Atoms of Huprine X and water molecules are represented as spheres. Key hydrogen bonds are represented as yellow dashes. Key amino acid residues are labeled and represented in sticks.

ities toward human AChE, reported in the literature have been obtained from experiments performed on human erythrocytes. In order to get more reliable values, and since we have developed an efficient production protocol of conformationally active recombinant human AChE (*rh*-AChE), we have chosen to perform the inhibitory efficiency evaluation on this *rh*-AChE. Furthermore, only few reference values are available for this enzyme.

As this study is devoted to a first evaluation of the inhibitory activities of Huprine derivatives **3–17**, all the inhibitory potencies (IC_{50}) have been determined with the racemic mixtures, the (−) enantiomer of Huprines being reported to be about twice more active than the corresponding racemic compound. For comparative purposes, we also included the IC_{50} values of the most active known Huprines X and Y toward the same enzyme *rh*-AChE. The values obtained for these reference compounds were found to be in good agreement, but slightly weaker than those given in the literature[12] (1.10 nM vs 0.75 nM and 1.84 nM vs 0.78 nM for (± Huprines X and Y, respectively), probably due to different experimental conditions used for the assays. Especially, in vitro AChE inhibitory activities were measured at pH 7.4 whereas literature values are reported at pH 8.0.

In order to rationalize the interpretations of kinetic data, docking studies were performed on *rh*-AChE for each compound including the references Huprines X and Y. Docking experiments show

Table 2
Recombinant human AChE IC_{50} values for the inhibitory activity of Huprines **3–11**

Compound	IC_{50}[a] (M)	Calculated pK_a of protonated pyridine moiety[b]
Huprine X	$1.10 \pm 0.02 \times 10^{-9}$	7.95
Huprine Y	$1.84 \pm 0.03 \times 10^{-9}$	
3a	$1.17 \pm 0.01 \times 10^{-5}$	10.05
3b	$5.10 \pm 0.93 \times 10^{-6}$	
4a[c]	$8.10 \pm 0.24 \times 10^{-7}$	7.33
4b	$8.55 \pm 0.32 \times 10^{-7}$	
5	$6.64 \pm 0.78 \times 10^{-7}$	6.71
6a	$4.78 \pm 0.03 \times 10^{-6}$	6.90
6b	$6.45 \pm 0.28 \times 10^{-6}$	
7a	$>5.0 \times 10^{-5}$	5.70
7b	$>5.0 \times 10^{-5}$	
8	$2.71 \pm 0.33 \times 10^{-5}$	
9a	$7.69 \pm 0.09 \times 10^{-6}$	6.87
9b	$7.27 \pm 0.83 \times 10^{-6}$	
10	$>5.0 \times 10^{-5}$	10.02
11	$>5.0 \times 10^{-5}$	

[a] The conditions used for the tests were as follows: enzyme concentration of 125 pM in 0.1 M phosphate buffer containing 15 mM NaCl, 150 μM NaN_3, and 15 μM Bovine Serum Albumin (BSA) at pH 7.4, 25 °C (see Section 5).
[b] Calculated using the SPARC online calculator (http://ibmlc2.chem.uga.edu/sparc/).
[c] Inhibition obtained on recombinant human BuChE: $3.42 \pm 0.12 \times 10^{-7}$ M; Selectivity BuChE/AChE: 0.4.

that Huprines X and Y fit in the active site gorge of *rh*-AChE (Fig. 5), in a similar manner than observed in the X-ray structure of the complex Huprine X and *Torpedo californica* AChE.[11] The central 4-aminopyridine nucleus (cycle A) is stacked against Trp86 with the amino group H-bonded to a water molecule close to Ser125. The amino group is also at 3.7 Å of Tyr337 hydroxyl, a distance too long for strong H-bonding. Protonation of 4-aminopyridine is an important factor as the pyridinium proton is H-bonded to the main chain carbonyl of His447, and the delocalized positive charge leads to cation–π interactions with Trp86. The heterocyclic fused-ring onto the central 4-aminopyridine nucleus of Huprine (cycle B) fits in an aromatic/hydrophobic pocket formed by resi-

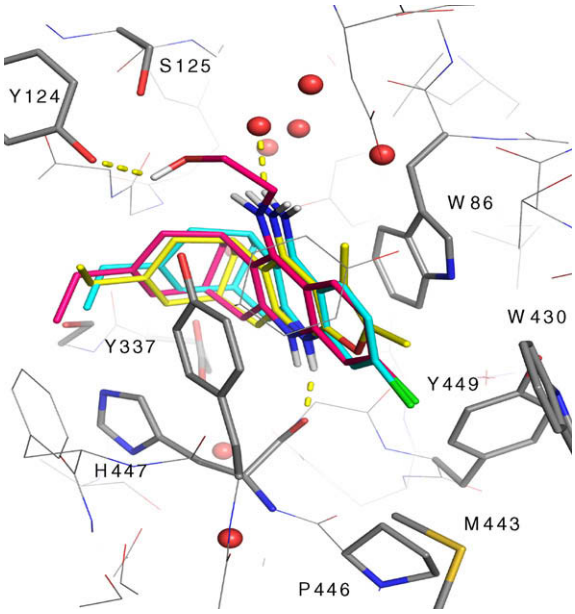


Figure 6. Huprine X, **6b** and **15b** docked in human acetylcholinesterase. Carbon atoms are represented in gray (*rh*-AChE), cyan (Huprine X), yellow (**6b**) or magenta (**15b**), oxygen in red, and nitrogen in blue. Atoms of Huprine X and water molecules are represented as spheres. Key hydrogen bonds are represented as yellow dashes. Key amino acid residues are labeled and represented in sticks.

dues Tyr337, Try341, Met443, Trp439, and Pro446. In particular, cycle B is stacked against Tyr337 and chlorine interacts with Trp439. The aliphatic bicycle fits in an elbow of the active site gorge with the ethyl/methyl at position 9 of Huprines X/Y close to the catalytic serine. Overall, dockings of Huprines X/Y allow evaluating the impact of the modifications made.

3.1. Modulation of the aromatic part

Compounds **3–11** were tested for their AChE inhibitory activity and the results are summarized in Table 2. Regarding modifications of the aromatic part, the best inhibitions were obtained for 4-amino-1,7-naphthyridine **4** and 4-amino-1,8-naphthyridine **5** derivatives which are however less active (two orders of magnitude) than the reference compounds.

Structure–activity relationship analysis regarding the heterocyclic fused-ring cycle B, led to the following conclusions: introduction of a cyclopropyl- and a cyclobutyl-1,3-oxazole moieties (compounds **7–8**) was found to decrease strongly the inhibition activity. It is important to note that during the course of our work, Marco et al. have reported the synthesis of substituted 1,3-oxazole Tacrine derivatives and reported similar negative results on the inhibition of AChE.[26] Docking results show that there is enough room in the pocket to fit the cyclopropyl and cyclobutyl groups. So the decrease in activity seems to be related to other factors than steric hindrance. There is a significant decrease of the calculated pK_a which suggests that **7–8** pyridiniums are deprotonated at pH 7.4, thus possibly leading to less favorable interactions with Trp86 and His447. Although the general orientations of the 6-atom ring and 5-atom ring analogues are virtually identical, 5-atom ring cycles have less surface contact with Tyr337, thus decrease aromatic–aromatic stacking interactions. Besides, despite being hydrophobic, the small aliphatic cycles might not optimally interact with the aromatic residues of the pocket.

The placement of a pyrazole heterocyclic ring (compounds **10–11**) decreased significantly the inhibitory activity and led to some very disappointing results. In addition to less favorable stacking of the 5-atom ring with Tyr337, one hypothesis is that both nitrogens might be too much hydrophilic for the hydrophobic pocket.

Noteworthing, the thiazole derivatives **9a** and **9b** proved to be more active by at least one order of magnitude than the oxazole or pyrazole analogues, despite the propyl chain being too long for the pocket. This might be explained by favorable π–sulfur interactions between the thiazole sulfur and Tyr337 on one side and the thiopropyl sulfur and Trp439 on the other side.

Table 3
Recombinant human AChE IC_{50} values for the inhibitory activity of Huprines **12–17**

Compound	IC_{50}[a] (M)	Calculated pK_a of protonated pyridine moiety[b]
Huprine X	$1.10 \pm 0.00002 \times 10^{-9}$	7.95
Huprine Y	$1.84 \pm 0.03 \times 10^{-9}$	
12	$2.51 \pm 0.97 \times 10^{-6}$	4.63
13	$1.47 \pm 0.03 \times 10^{-5}$	11.86
14a	$2.24 \pm 0.39 \times 10^{-5}$	4.08
14b	$1.92 \pm 0.16 \times 10^{-5}$	
15a	$9.66 \pm 0.35 \times 10^{-9}$	8.58
15b[c]	$6.75 \pm 1.23 \times 10^{-9}$	
16a	$4.33 \pm 0.19 \times 10^{-8}$	8.50
17a	$8.69 \pm 0.03 \times 10^{-9}$	8.64
17b	$1.37 \pm 0.07 \times 10^{-8}$	

[a] The conditions used for the tests were as follows: enzyme concentration of 125 pM in 0.1 M phosphate buffer containing 15 mM NaCl, 150 μM NaN_3, and 15 μM Bovine Serum Albumin (BSA) at pH 7.4, 25 °C (see Section 5).

[b] Calculated using the SPARC online calculator (http://ibmlc2.chem.uga.edu/sparc/).

[c] Inhibition obtained on recombinant human BuChE: $3.64 \pm 0.03 \times 10^{-6}$ M; Selectivity BuChE/AChE: 540.

Finally, Huprines **6a** and **6b** showed the best result considering a 5-membered ring size possibly due to better hydrophobic interactions with residues in the pocket (Fig. 6).

Furthermore, the variation from methyl to ethyl group at position 9 always led to the same range of activity without significant differences, even if ethyl derivatives proved often slightly more active.

The inhibition of the most potent Huprine in this series on recombinant human butylcholinesterase (rh-BuChE) has been reported. Interestingly, the IC_{50} value proved better on this enzyme.

Despite lower activities than expected, these first analogues are very interesting as the large variety of synthesized structures allowed us to define which types of interactions were unfavorable and will guide us in view of further structural modifications of Huprine scaffold. As a general trend, the only significant data we could draw from these results is the influence of stacking interactions between cycle B and Tyr337, larger rings being better, and pK_a of 4-aminopyridine dependent on the nature of cycle B.

3.2. Derivatization with reactive linkers to probe key interactions with the amino acid residues in the gorge

Compounds **12–17** were submitted to the same in vitro assay to determine their potency to inhibit *rh*-AChE. The results are summarized in Table 3.

As previously mentioned, these structures have been designed: (1) to confirm former molecular modelization analyses on *Torpedo californica* AChE showing interactions between the amino group of the Huprine with a water molecule close to Ser125[10] and especially to determine the direct impact of the lack of this group for human AChE inhibition activity, (2) to study the influence of the attachment of a linker at this position and to probe the potential interactions of this added chemical moiety with some enzyme amino acid residues, in order to choose the less detrimental linker with the goal in mind to further design highly potent dual binding inhibitors.

As mentioned for the underivatized Huprines, changing a methyl group by an ethyl group at position 9 did not lead to significant differences in the inhibition activity.

As expected, compounds **14a** and **14b** bind the enzyme very weakly, the decrease in inhibitory potency being four orders of magnitude lower than that of Huprines X and Y. These results confirm the strong favorable effect induced by the amino group of central pyridine nucleus which is thus of prior importance for H-bonding, and protonation of the pyridine moiety.

In addition to this first result, we also studied the replacement of this amino group by another H-bond donor (i.e., hydroxyl group for compound **13**). However the hydroxyl is deprotonated and the pyridine is protonated at pH 7.4 resulting in a drastic modification of electrons distribution of the aromatic cycle. Thus there is both a loss of an H-bond donor at position 12 and a loss of cation–π interaction with Trp86. This likely explains that **13** is 8000-fold less active than Huprine X. This convinces us that the central 4-aminopyridine moiety of Huprine derivatives is essential for inhibition activity and cannot be replaced by an isosteric group.

Interestingly, modifying this key interaction through monoalkylation of amino group (suppressing possibility of double H-bonding) resulted in very active compounds, slightly less than parent Huprines X and Y, but inferior to 14 nM for all of them. In this latter series, the only exception was compound **12** which exhibits a micromolar inhibition activity (60-fold less potent than its reduced analogue **16**). This fact can be easily explained by the change of pK_a and to a lesser degree by the presence of the carbonyl group which is bulky enough to prevent the ability of inhibitor molecule **12** to fit within the narrowest region of the gorge. This valuable information should be taken into consideration when choosing the syn-

thetic methodology for the linker introduction. Thus, alkylation reactions of the 4-aminopyridine moiety with small hydrocarbyl chains will be preferred.

Chemical variations at the terminal position of the linker induced little changes in the inhibition activity (compounds **15–17**). For this sub-class of N-derivatized Huprines, the hydroxyl group containing inhibitor **15b** was found to be the most active, (only sixfold less active than Huprine X and 3.6-fold less active than Huprine Y). Moreover, the low inhibition value reported on rh-BuChE for this compound showed its good selectivity for rh-AchE (Table 3). According to docking experiments, the hydroxyl of **15b** is at H-bond distance to Tyr124, Ser125, and a conserved water molecule nearby (Fig. 6).

These findings could be of interest since the retained strategy to inhibit AChE involves dual-site binding, which requires the introduction of a linker attached on the amino group of the Huprines. Furthermore, these latter results clearly show that the linker must be very slim (i.e., linear alkyl chain) at the bottom of the gorge, but the nature of its terminal reactive group does not seem to be a crucial factor. Thus, a wide range of heterobifunctional cross-linkers bearing a linear carbon chain could be used in the context of homo- or heterodimeric dual inhibitors. Finally, further exploration of these interactions must be carried out in order to increase the number of contact points between the inhibitor molecule and the closest amino acid residues and so further improve its inhibitory efficiency.

3.3. Coupling of a peripheral site inhibitor to afford dual-site inhibition

Heterodimers **24** and **25** as well as their monomer precursors were also tested for inhibition potency on recombinant human AChE and BuChE. The results obtained are resumed in Table 4.

Taking these results into consideration, the following conclusions could be drawn. Despite no strong benefical synergic effect has been observed, these first heterodimers **24** and **25** proved a little (about two times) more efficient than the Huprines precursors. Several explanations are possible: the modest binding affinity of the tetrahydroisoquinoline derivatives perhaps limits the binding ability, but an inadequate length of the tether or a bad position of triazole inducing detrimental interactions with residues in AChE gorge can be also responsible of this relative activity for the heterodimers. However, similar results have been reported by Camps et al.[21a] in their exploration of Tacrine–Huprine heterodimers, the gain of introducing a second inhibitor being limited compared to the results of Sharpless et al. with Tacrine-based heterodimers on other acetylcholinesterases.[33]

Anyhow, more analyses (docking calculations) must be carried out in the future to have a better overview of the interactions and more heterodimeric inhibitors must be synthesized by rational approach.

Finally, the most interesting feature of these heterodimers proved to be their high selectivity toward AChE, since they are about 10,000-fold more potent on AChE than on BuChE. In comparison, the more selective Tacrine–Huprine heterodimer was 100-fold more potent on AChE.[21a]

Table 4
Recombinant human AChE and BuChE IC_{50} values for the inhibitory activity of heterodimers **24** and **25** and their precursors **17a**, **22**, **17b,** and **23**

Compound	IC_{50} (M)[a] human AChE	IC_{50} (M)[a] human BuChE	BuChE/AChE
17a	$8.69 \pm 0.03 \times 10^{-9}$	$2.35 \pm 0.06 \times 10^{-6}$	270
22	$8.08 \pm 0.47 \times 10^{-6}$	[b]	
24	$3.95 \pm 0.33 \times 10^{-9}$	[c]	$>10^4$
17b	$1.37 \pm 0.07 \times 10^{-8}$	$8.24 \pm 0.24 \times 10^{-7}$	60
23	$2.92 \pm 0.03 \times 10^{-6}$	[b]	
25	$5.96 \pm 0.36 \times 10^{-9}$	[d]	$>10^4$

[a] The conditions used for the tests were as follows: enzyme concentration of 125 pM in 0.1 M phosphate buffer containing 15 mM NaCl, 150 μM NaN_3, and 15 μM Bovine Serum Albumin (BSA) at pH 7.4, 25 °C (see Section 5).
[b] Less than 1% of reversion was obtained at 100 μM.
[c] Only 6% of reversion was obtained at 10 μM.
[d] Only 7% of reversion was obtained at 10 μM.

4. Conclusions

In this paper, we have reported the synthesis and structure–activity relationship, based on recombinant human AChE inhibitory activity and docking calculations, of 24 new Huprine derivatives and 2 Huprine-based heterodimers. The selectivity toward human BuChE has been evaluated for the most potent compounds. All these analogues have been prepared from various *o*-cyanoaromatic compounds (synthesized or commercially available) via Friedländer condensation with enones **2a,b**. Concerning the series where the aromatic part has been modified, the most active analogues were the naphthyridine analogues **4a,b,** and **5**. The replacement of the six-membered ring by a five-membered ring proved generally detrimental on the inhibitory activity. Structure–activity relationships have suggested that the influence of the pyridine pK_a is of prior importance on the binding efficiency. The study on the introduction of a short linker on the amino group of Huprines X and Y showed that the nature of the linker at this position is important, and that only compounds bearing a slim alkyl group on the nitrogen atom have shown a weak loss of activity (inferior to one order of magnitude). This finding is of particular relevance for further dual-binding inhibitors design. The heterodimers displayed better activities than the parent Huprines but without the strong synergic effect expected. However their high selectivity for AChE should be taken into consideration.

5. Experimental

5.1. Enzyme kinetic studies

AChE inhibitory activity was evaluated spectrophotometrically using a UV–vis Varian Cary 50 scan spectrophotometer equipped with a microplate reader at 25 °C by the method of Ellman,[25] using recombinant human AChE (*rh*-AChE) or recombinant human BuChE (*rh*-BuChE) and acetylthiocholine iodide or butyrylthiocholine (0.53 mM) as substrate. The reaction took place in the presence of 125 pM of *rh*-ChE in a final volume of 200 μL of 0.1 M phosphate-buffered solution (pH 7.4), containing 15 mM NaCl, 150 μM NaN_3, 15 μM Bovine Serum Albumin (BSA), and 333 μM 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB) solution used to produce the yellow anion of 5-thio-2-nitrobenzoic acid. The different inhibitory derivatives were pre-incubated with the enzyme at 25 °C for at least 30 min before measurement. One sample without inhibitor was always present to yield the 100% of ChE activity. The rate of change of absorbance (ΔA/min), reflecting the rate of hydrolysis of acetylthiocholine was recorded at $\lambda = 414$ nm for 20 min (kinetic mode). These experiments were generally done at least in triplicate and the values averaged. Data from concentration–inhibition experiments of the inhibitors were calculated by nonlinear regression analysis, which gave estimates of the IC_{50} (concentration of drug producing 50% of enzyme activity inhibition). Recombinant human acetylthiocholinesterase was purified as previously described.[27] DTNB and acetylthiocholine were purchased from Sigma.

5.2. Molecular docking

Docking calculations were carried out using AUTODOCK, version 4.0.1, with the Lamarckian genetic algorithm (LGA[28]). In order to

determine the protonation state of each derivative at pH 7.4, pK_a was calculated using the SPARC online calculator (http://ibmlc2.chem.uga.edu/sparc/). The molecular models of each derivative were built and minimized with the MM2 force field of Chem3D (CambridgeSoft.). Gasteiger total charges were calculated using the PETRA online (http://www2.chemie.uni-erlangen.de/software/petra/intro.phtml).[29] The structure of human AChE was prepared using MODELLER and CNS from the crystal structure of its complex with fasciculin (pdb code 1b41). In order to take into account the flexibility of the enzyme upon Huprines binding, the structure of *h*AChE was compared to the complex of Huprine X and *Torpedo californica* AChE (pdb code 1e66) and moving loops were rebuilt and minimized using MODELLER.[30] Structural water molecules were conserved in the model in order to improve docking accuracy. Huprines and *h*AChE were further prepared using AUTODOCK Tools 1.5.2.[31] The 3D affinity grid box was designed to include the full active site gorge of human AChE. The number of grid points in the *x*-, *y*-, and *z*-axes was 96, 115, and 115 with grid points separated by 0.2 Å. Docking calculations were set to 100 runs. At the end of the calculation, AUTODOCK performed cluster analysis. Docking solutions with ligand all-atom root-mean-square deviation (rmsd) within 1.0 Å of each other were clustered together and ranked by the lowest energy representative. Usually more than 85% of the runs lead to solutions belonging to the same cluster. The lowest-energy solution was accepted as the one most representative of the Huprine–AChE complex.

5.3. Synthesis

In general, column chromatography purifications were performed on silica gel (40–63 μm) from SdS. Thin-layer chromatography (TLC) was carried out on Merck DC Kieselgel 60 F-254 aluminum sheets. Compounds were visualized by one of the two following methods: (1) illumination with a short wavelength UV lamp (λ = 254 nm) or (2) staining with a 3.5% (w/v) phosphomolybdic acid solution in absolute ethanol. All solvents were dried following standard procedures (CH_2Cl_2, 1,2-dichloroethane, and CH_3CN: distillation over P_2O_5, DMF, and DMSO: distillation over BaO under reduced pressure, THF, toluene, and Et_2O: distillation over Na/benzophenone). Triethylamine (TEA) and pyridine were distilled from CaH_2 and stored over BaO or KOH.

Melting points were recorded on a LEICA VMHB Kofler system at atmospheric pressure and were uncorrected. Microanalyses were carried out on Carlo-Erba 1106. Infrared spectra were recorded as KBr pellets using a Perkin Elmer FT-IR Paragon 500 spectrometer with frequencies given in reciprocal centimeters (cm^{-1}). ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX 300 spectrometer (Bruker, Wissembourg, France). Chemical shifts are expressed in parts per million (ppm) from $CDCl_3$ (δ_H = 7.26, δ_C = 77.16), DMSO-d_6 (δ_H = 2.50, δ_C = 39.52) or CD_3OD (δ_H = 3.31, δ_C = 49.00).[32] ^1J values are expressed in hertz. Mass spectra were obtained with a Finnigan LCQ Advantage MAX (ion trap) apparatus equipped with an electrospray source. All analyses were performed in the positive mode. Analytical HPLC was performed on a Thermo Electron Surveyor instrument equipped with a PDA detector under the following conditions: Thermo Hypersil GOLD C18 column (5 μ, 4.6 × 150 mm) with CH_3CN and 0.1% aq TFA as eluents [90% TFA (5 min), linear gradient from 0% to 100% of CH_3CN (40 min)] at a flow rate of 1.0 mL min^{-1} dual UV detection at 254 and 270 nm.

5.3.1. General procedure for the preparation of racemic Huprines

To a suspension of anhydrous $AlCl_3$ (1.5 equiv) and *o*-aminocyano aromatic heterocycle (1.53 equiv) in dry 1,2-dichloroethane (c = 0.5 M) was added dropwise a solution of (±)-7-alkylbicyclo[3.3.1]non-6-en-3-one (1 equiv) in dry 1,2-dichloroethane (c = 0.3 M) and the resulting reaction mixture was refluxed overnight. Thereafter, the mixture was cooled to room temperature and diluted by sequential addition of deionized water, THF, aqueous 5 N NaOH and then stirred at room temperature for 30 min. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The resultant crude product was purified by flash chromatography on a silica gel column to give the desired Huprine.

5.3.1.1. (±)-12-Amino-2,3-dimethoxy-9-methyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[b]quinoline (3a). This compound was prepared according to the general procedure described above using 2-amino-4,5-dimethoxybenzonitrile (1.81 g, 10.2 mmol), **2a** (1 g, 6.65 mmol). Purification by flash chromatography on a silica gel column (AcOEt to CH_2Cl_2/MeOH, 92:8) afforded the desired Huprine **3a** as a off-white solid (600 mg, 30%); mp: 247 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 3H, H_{14}), 1.94–2.04 (m, 3H, H_{10}, H_{13}), 2.42–2.52 (m, 1H, H_{13}), 2.68–2.78 (m, 1H, H_7), 2.93 (br d, J = 17.1 Hz, 1H, $H_{6'}$), 3.12 (dd, J = 17.1 Hz, J = 5.4 Hz, 1H, H_6), 3.16–3.26 (m, 1H, H_{11}), 3.96 (s, 3H, OMe), 3.98 (s, 3H, OMe), 4.57 (br s, 2H, NH_2), 5.49–5.59 (m, 1H, H_8), 6.89 (s, 1H, H_1), 7.26 (s, 1H, H_4); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.6 (C_{14}), 27.7 (C_{11}), 28.6 (C_7), 29.4 (C_{10}), 35.9 (C_{13}), 39.6 (C_6), 56.1 (OMe), 98.7 (C_1), 108.0 (C_4), 111.8 (C_{12a}), 114.9 (C_{11a}), 125.5 (C_8), 132.2 (C_9), 143.7 (C_{12} or C_{4a}), 144.7 (C_{12} or C_{4a}), 148.1 (C_2 or C_3), 151.7 (C_2 or C_3), 155.3 (C_{5a}); IR (KBr): ν = 3470, 2985, 1651, 1438, 1254, 1162, 1004 cm^{-1}; MS (ESI+): *m/z* (%): 311 (100) $[M+H]^+$; HPLC: t_R = 21.4 min (purity >95%).

5.3.1.2. (±)-12-Amino-2,3-dimethoxy-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[b]quinoline (3b). This compound was prepared according to the general procedure described above using 2-amino-3-cyanopyridine (454 mg, 2.54 mmol), **2b** (250 mg, 1.66 mmol). Purification by flash chromatography on a silica gel column (CH_2Cl_2/MeOH, 98:2) afforded the desired Huprine **3b** as a white-off solid (350 mg, 68%); mp: 149 °C (decomposition); ^1H NMR (300 MHz, $CDCl_3$): δ = 0.83 (t, 3H, H_{15}, J = 7.7 Hz), 1.76 (q, 2H, H_{14}, J = 7.7 Hz), 1.88 (m, 1H, H_{10}), 1.96 (d, J = 17.7 Hz, 1H, H_{13}), 1.98–2.08 (m, 1H, H_{10}), 2.40–2.50 (m, 1H, H_{13}), 2.67–277 (m, 1H, H_7), 2.90 (d, J = 17.4 Hz, 1H, $H_{6'}$), 3.10 (dd, J = 17.4 Hz, J = 5.4 Hz, 1H, H_6), 3.07–3.17 (m, 1H, H_{11}), 3.95 (s, 3H, OMe), 3.97 (s, 3H, OMe), 5.29 (br s, 2H, NH_2), 5.41–5.51 (m, 1H, H_8), 7.04 (s, 1H, H_1), 7.20 (s, 1H, H_4); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.0 (C_{15}), 27.4 (C_{11}), 28.0 (C_7), 29.4 (C_{10}), 30.0 (C_{14}), 34.2 (C_{13}), 38.2 (C_6), 56.2 (OMe), 99.7 (C_1), 105.7 (C_4), 111.4 (C_{12a}), 114.0 (C_{11a}), 123.1 (C_8), 137.8 (C_9), 146.7 (C_{12} or C_{4a}), 148.1 (C_2 or C_3, C_{12} or C_{4a}), 151.7 (C_2 or C_3), 155.3 (C_{5a}); IR (KBr): ν = 3364, 2963, 1645, 1514, 1412, 1284, 1122 cm^{-1}; MS (ESI+): *m/z* (%): 325 (100) $[M+H]^+$; HPLC: t_R = 19.4 min (purity 90%).

5.3.1.3. (±)-11-Amino-8-methyl-5,6,9,10-tetrahydro-6,10-methanocyclooctabenzo[b][1,8]naphthyridine (4a). This compound was prepared according to the general procedure described above using 2-amino-3-cyanopyridine (454 mg, 2.54 mmol), **2a** (250 mg, 1.66 mmol). Purification by flash chromatography on a silica gel column (CH_2Cl_2/MeOH, 98:2) afforded the desired Huprine **4a** as a white-off solid (350 mg, 68%); mp: 251 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 3H, H_{13}), 1.95–2.05 (m, 3H, H_9, H_{15}), 2.44–2.54 (m, 1H, H_{15}), 2.68–2.78 (m, 1H, H_6), 3.09 (br d, J = 17.9 Hz, 1H, H_4), 3.18 (dd, J = 17.9 Hz, J = 5.2 Hz, 1H, H_4), 3.15–3.25 (m, 1H, H_{10}), 4.98 (br s, 2H, NH_2), 5.49–5.59 (m, 1H, H_7), 7.28 (dd, J = 8.3 Hz, J = 4.2 Hz, 1H, H_2), 8.14 (br d, J = 8.3 Hz, 1H, H_1), 8.93 (dd, J = 4.2 Hz, J = 1.8 Hz, 1H, H_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.5 (C_{13}), 27.7 (C_{10}), 28.3 (C_6), 29.1 (C_9), 35.5 (C_{4a}), 39.9 (C_4), 111.9 (C_{11a}), 116.0 (C_{5a}), 119.2 (C_2), 125.5 (C_7), 130.3 (C_1), 132.1 (C_8), 147.2 (C_{11}), 152.5 (C_3), 154.7 (C_{12a}), 161.0 (C_{12}); IR (KBr):

ν = 3481, 2912, 1634, 1550, 1429, 1336 cm^{-1}; MS (ESI+): *m/z* (%): 252 (100) $[M+H]^+$; HPLC: t_R = 13.8 min (purity >95%).

5.3.1.4. (±)-11-Amino-8-ethyl-5,6,9,10-tetrahydro-6,10-methanocyclooctabenzo[*b*][1,8]naphthyridine (4b). This compound was prepared according to the general procedure described above using 2-amino-3-cyanopyridine (333 mg, 2.79 mmol), **2b** (300 mg, 1.82 mmol). Precipitation in acetone gave the desired Huprine **4b** as a white-off solid (270 mg, 48%); mp: 161–162 °C; ^{1}H NMR (300 MHz, DMSO-d_6):δ = 0.81 (t, J = 7.7 Hz, 3H, H_{14}), 1.75 (q, J = 7.7 Hz, 2H, H_{13}), 1.83–1.93 (m, 3H, H_9, H_{15}), 2.35–2.45 (m, 1H, H_{15}), 2.61–2.71 (m, 1H, H_6), 2.79 (d, J = 17.4 Hz, 1H, H_4), 3.02 (dd, J = 17.4 Hz, J = 5.4 Hz, 1H, H_4), 3.32–3.42 (m, 1H, H_{10}), 5.42–5.52 (m, 1H, H_7), 6.71 (br s, 2H, NH_2), 8.04 (d, J = 5.7 Hz, 1H, H_2), 8.29 (d, J = 5.7 Hz, 1H, H_1), 8.94 (s, 1H, H_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 11.9 (C_{14}), 26.5 (C_{10}), 27.8 (C_6), 28.9 (C_9), 29.4 (C_{13}), 33.9 (C_{4a}), 39.5 (C_4), 115.0 (C_2), 116.5 (C_{11a}), 120.6 (C_{5a}), 122.9 (C_7), 137.7 (C_8), 139.9 (C_1), 141.8 (C_{12a}), 146.4 (C_{11}), 152.1 (C_3), 158.4 (C_{12}); IR (KBr): ν = 3339, 2897, 1662, 1574, 1415, 1358 cm^{-1}; MS (ESI+): *m/z* (%): 266 (100) $[M+H]^+$; HPLC: t_R = 15.9 min (purity 93%).

5.3.1.5. (±)-11-Amino-8-ethyl-5,6,9,10-tetrahydro-6,10-methanocyclooctabenzo[*b*][1,7]naphthyridine (5). This compound was prepared according to the general procedure described above using 3-aminoisonicotinonitrile **19** (150 mg, 1.25 mmol), **2b** (135 mg, 0.82 mmol). Purification by flash chromatography on a silica gel column (CH_2Cl_2/MeOH, 98:2) afforded the desired Huprine **5** as a white-off solid (234 mg, 70%); mp: 192 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.84 (t, J = 7.4 Hz, 3H, H_{14}), 1.78 (q, J = 7.4 Hz, 2H, H_{13}), 1.87–1.97 (m, 1H, H_9), 1.97 (br d, J = 16.8 Hz, 1H, H_{15}), 1.99–2.09 (m, 1H, H_9), 2.43–2.53 (m, 1H, H_{15}), 2.69–2.79 (m, 1H, H_6), 3.07 (dt, J = 17.9 Hz, J = 1.8 Hz, 1H, H_4), 3.17 (dd, J = 17.9 Hz, J = 5.2 Hz, 1H, H_4), 3.16–3.26 (m, 1H, H_{10}), 5.00 (br s, 2H, NH_2), 5.47–5.57 (m, 1H, H_7), 7.25 (dd, J = 8.3 Hz, J = 4.2 Hz, 1H, H_2), 8.16 (dd, J = 8.3 Hz, J = 1.8 Hz, 1H, H_1), 8.92 (dd, J = 4.2 Hz, J = 1.8 Hz, 1H, H_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.1 (C_{14}), 27.8 (C_{10}), 28.3 (C_6), 29.4 (C_9), 30.0 (C_{13}), 34.1 (C_{4a}), 40.4 (C_4), 111.9 (C_{11a}), 116.2 (C_{5a}), 119.1 (C_2), 123.6 (C_7), 129.9 (C_1), 137.4 (C_8), 146.6 (C_{11}), 152.4 (C_3), 155.1 (C_{12a}), 161.5 (C_{12}); IR (KBr): ν = 3330, 2892, 1623, 1547, 1490, 1332 cm^{-1}; MS (ESI+): *m/z* (%): 266 (100) $[M+H]^+$; HPLC: t_R = 15.1 min (purity >95%).

5.3.1.6. (±)-9-Amino-7-methyl-2,3-dimethylfuro[2,3-b]-4,5,8,9-tetrahydro-5,9-methanocyclo octan[e]pyridine (6a). This compound was prepared according to the general procedure described above using 2-amino-4,5-dimethylfuran-3-carbonitrile (678 mg, 5.09 mmol), **2a** (500 mg, 3.33 mmol). Purification by flash chromatography on a silica gel column (cyclohexane/AcOEt, 3:2) afforded the desired Huprine **15a** as a white solid (510 mg, 57%, along with 13% of inseparable isomer); mp: 251 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.53 (s, 3H, H_{14}), 1.79–1.89 (m, 1H, H_{10}), 1.88 (br d, J = 17.1 Hz, 1H, H_{13}), 1.93–2.03 (m, 1H, H_{10}), 2.28 (s, 3H, 2-Me or 3-Me), 2.30 (s, 3H, 2-Me or 3-Me), 2.33–2.43 (m, 1H, H_{13}), 2.60–2.70 (m, 1H, H_7), 2.78 (br d, J = 17.9 Hz, 1H, H_6), 3.02 (dd, J = 17.9 Hz, J = 5.6 Hz, 1H, H_6), 3.03–3.09 (m, 1H, H_{11}), 5.46–5.56 (m, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 9.3 (2-Me or 3-Me), 10.3 (2-Me or 3-Me), 22.4 (C_{14}), 25.8 (C_{11}), 27.4 (C_7), 28.2 (C_{10}), 34.7 (C_{13}), 37.8 (C_6), 104.6 (C_2), 106.4 (C_1), 113.9 (C_{11a}), 124.5 (C_8), 130.8 (C_9), 143.9 (C_3), 145.1 (C_{4a}), 149.3 (C_{12}), 159.6 (C_{5a}); IR (KBr): ν = 3489, 2919, 1632, 1590, 1434, 1360, 1297, 1115 cm^{-1}; MS (ESI+): *m/z* (%): 269 (100) $[M+H]^+$; HPLC: t_R = 17.3 min (purity >95%).

5.3.1.7. (±)-9-Amino-7-methyl-2,3-dimethylfuro[2,3-b]-4,5,8,9-tetrahydro-5,9-methanocyclo octan[e]pyridine (6b). This compound was prepared according to the general procedure described above using 2-amino-4,5-dimethylfuran-3-carbonitrile (678 mg, 5.09 mmol), **2b** (250 mg, 1.52 mmol). Purification by flash chromatography on a silica gel column (cyclohexane/AcOEt, 3:2) afforded the desired Huprine **6b** as a white solid (223 mg, 52%, along with 20% of unseparable undesired isomer); mp: 218 °C (decomposition); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, J = 7.4 Hz, 3H, H_{15}), 1.82–1.92 (m, 4H, H_{10}, H_{14}, H_{13}), 2.28 (s, 3H, 2-Me or 3-Me), 2.30 (s, 3H, 2-Me or 3-Me), 2.40–2.50 (m, 1H, H_{13}), 2.63–2.73 (m, 1H, H_7), 2.78 (br d, J = 17.9 Hz, 1H, H_6), 3.02 (dd, J = 17.9 Hz, J = 5.6 Hz, 1H, H_6), 3.01–3.11 (m, 1H, H_{11}), 4.39 (br s, 2H, NH_2), 5.46–5.56 (m, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 10.5 (2-Me or 3-Me), 11.4 (2-Me or 3-Me), 12.2 (C_{15}), 25.8 (C_{11}), 27.9 (C_7), 29.6 (C_{10}), 30.0 (C_{14}), 34.4 (C_{13}), 39.1 (C_6), 105.7 (C_2), 107.6 (C_1), 115.1 (C_{11a}), 123.6 (C_8), 137.3 (C_9), 145.1 (C_3), 146.2 (C_{4a}), 150.5 (C_{12}), 160.7 (C_{5a}); IR (KBr): ν = 3496, 2923, 1631, 1591, 1433, 1296, 1115 cm^{-1}; MS (ESI+): *m/z* (%): 283 (100) $[M+H]^+$; HPLC: t_R = 19.0 min (purity 93%).

5.3.1.8. (±)-9-Amino-1-cyclopropyl-oxazolo[4,5-*b*]-6-methyl-3,4,7,8-tetrahydro-6,10-methano cycloocta[e]pyridine (7a). This compound was prepared according to the general procedure described above using 5-amino-2-cyclopropyloxazole-4-carbonitrile **20** (456 mg, 3.05 mmol), **2a** (300 mg, 2.00 mmol). Purification by flash chromatography on a silica gel column (cyclohexane/AcOEt, 1:1) afforded a yellow solid (270 mg, 48%, along with 7% of unseparable isomer). Recrystallization from Et_2O gave the desired Huprine **7a** as a white solid; mp: 166 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.06–1.16 (m, 2H, CH_2 cyclopropyl), 1.17–1.27 (m, 2H, CH_2 cyclopropyl), 1.53 (s, 3H, H_{14}), 1.82–1.92 (m, 2H, H_{10}, H_{13}), 1.92–2.02 (m, 1H, H_{10}), 2.06–2.16 (m, 1H, CH cyclopropyl), 2.37–2.47 (m, 1H, H_{13}), 2.61–2.71 (m, 1H, H_7), 2.77 (br d, 1H, H_6, J = 17.4 Hz), 3.02 (dd, J = 17.4 Hz, J = 5.5 Hz, 1H, H_6), 3.04–3.14 (br s, 1H, H_{11}), 4.66 (br s, 2H, NH_2), 5.46–5.56 (m, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 8.8 (CH_2 cyclopropyl), 9.77 (CH_2 cyclopropyl), 22.7 (C_{14}), 27.4 (C_{11}), 28.5 (C_7), 29.0 (C_{10}), 35.8 (C_{13}), 39.1 (C_6), 116.6 (C_{11a}), 118.3 (C_1), 125.5 (C_8), 132.0 (C_9), 142.1 (C_{4a}), 151.1 (C_{12}), 158.4 (C_{5a}), 164.5 (C_3); IR (KBr): ν = 3339, 2906, 1742, 1651, 1471, 1334, 1291, 1107 cm^{-1}; MS (ESI+): *m/z* (%): 282 (100) $[M+H]^+$; HPLC: t_R = 17.9 min (purity >95%).

5.3.1.9. (±)-9-Amino-1-cyclopropyl-oxazolo[4,5-*b*]-6-ethyl-3,4,7,8-tetrahydro-6,10-methano cycloocta[e]pyridine (7b). This compound was prepared according to the general procedure described above using 5-amino-2-cyclopropyloxazole-4-carbonitrile **20** (300 mg, 2.01 mmol), **2b** (216 mg, 1.31 mmol). Purification by flash chromatography on a silica gel column (cyclohexane/AcOEt, 1:1) afforded a yellow solid. Recrystallization from Et_2O furnished the desired Huprine **7b** as a pale orange solid (390 mg, 70%, along with 12% of unseparable undesired isomer); mp: 134 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.80 (t, J = 7.3 Hz, 3H, H_{15}), 1.01–1.11 (m, 2H, CH_2 cyclopropyl), 1.11–1.21 (m, 2H, CH_2 cyclopropyl), 1.80–1.90 (m, 4H, H_{10}, H_{13}, H_{14}), 1.92–2.02 (m, 2H, H_{10}, H_{13}), 2.06–2.16 (m, 1H, CH cyclopropyl), 2.37–2.47 (m, 1H, H_{16}), 2.61–2.71 (m, 1H, H_7), 2.73 (br d, J = 17.4 Hz, 1H, H_6), 2.97 (dd, J = 17.4 Hz, J = 5.5 Hz, 1H, H_6,), 3.00–3.10 (br s, 1H, H_{11}), 4.62 (br s, 2H, NH_2), 5.46–5.56 (m, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 8.8 (CH_2 cyclopropyl), 9.8 (CH_2 cyclopropyl), 12.2 (C_{15}), 27.4 (C_{11}), 28.4 (C_7), 29.3 (C_{10}), 30.0 (C_{14}), 34.2 (C_{13}), 39.2 (C_6), 116.6 (C_{11a}), 118.3 (C_1), 123.5 (C_8), 137.4 (C_9), 142.1 (C_{4a}), 151.2 (C_{12}), 158.4 (C_{5a}), 164.5 (C_3); IR (KBr): ν = 3339, 2906, 1742, 1651, 1471, 1334, 1291, 1107 cm^{-1}; HPLC: t_R = 17.8 min (purity >95%).

5.3.1.10. (±)-9-Amino-1-cyclobutyl-oxazolo[4,5-*b*]-6-methyl-3,4,7,8-tetrahydro-6,10-methano cycloocta[e]pyridine (8). This compound was prepared according to the general procedure de-

scribed above using **21** (415 mg, 2.54 mmol), **2a** (250 mg, 1.66 mmol). Flash chromatography on a silica gel column (cyclohexane/AcOEt, 7:3) gave the desired Huprine **8**, which was recrystallized from Et_2O to afford as a pale orange solid (210 mg, 45%); mp: 200 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 3H, H_{14}), 1.77–1.87 (m, 1H, H_{10}), 1.90 (br d, J = 17.5 Hz, 1H, H_{13}), 2.02–2.12 (m, 3H, H_{10}, $CH_2\beta$cyclobutyl), 2.39–2.49 (m, 5H, H_{13}, $CH_2\beta$cyclobutyl, $CH_2\gamma$cyclobutyl), 2.61–2.71 (m, 1H, H_7), 2.78 (br d, J = 17.5 Hz, 1H, H_6), 3.04 (dd, J = 17.5 Hz, J = 5.6 Hz, 1H, H_6), 3.04–3.14 (m, 1H, H_{11}), 3.69 (quintd, J = 8.3 Hz, J = 0.9 Hz, 1H, CHcyclobutyl), 4.73, (br s, 2H, NH_2), 5.45–5.55 (m, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.8 ($CH_{2\gamma}$cyclobutyl), 23.5 (C_{14}), 27.0 ($CH_2\beta$cyclobutyl), 27.1 ($CH_2\beta$cyclobutyl), 27.4 (C_{11}), 28.5 (C_7), 29.0 (C_{10}), 33.88 (CHcyclobutyl), 35.8 (C_{13}), 39.2 (C_6), 116.5 (C_{11a}), 118.1 (C_1), 125.4 (C_8), 132.0 (C_9), 142.7 (C_{4a}), 151.6 (C_{5a}), 158.9 (C_{12}), 165.4 (C_3); IR (KBr): ν = 3332, 2918, 1652, 1472, 1329, 1110 cm^{-1}; MS (ESI+): m/z (%): 296 (100) $[M+H]^+$; Anal. Calcd for $C_{18}H_{21}N_3O$: C, 73.19; H, 7.17; N, 14.23. Found: C, 73.28; H, 7.28; N, 14.19; HPLC: t_R = 18.1 min (purity >95%).

5.3.1.11. (±)-11-Amino-2-thiopropyl-5,6,9,10-tetrahydro-thiazolo[4,5-*b*]-8-methyl-6,10-methanocycloocta[e]pyridine (9a). This compound was prepared according to the general procedure described above using **22** (507 mg, 2.54 mmol), **2a** (250 mg, 1.66 mmol). Flash chromatography on a silica gel column (CH_2Cl_2/MeOH, 98:2) gave the desired Huprine **9a** as a pale orange solid (390 mg, 70%); mp: 205 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.00 (t, J = 7.5 Hz, 3H, CH_3 thioalkyl chain,), 1.49 (s, 3H, H_{14}), 1.84 (quint, J = 7.5 Hz, 2H, $CH_2\beta$ thioalkyl chain,), 1.81–1.91 (m, 1H, H_{13}), 1.85–1.95 (m, 1H, H_{10}), 1.92–2.02 (m, 1H, H_{13}), 2.37–2.47 (m, 1H, H_{10}), 2.63–2.73 (m, 1H, H_8), 2.91 (br d, J = 17.5 Hz 1H, H_6), 3.08 (dd, J = 17.5 Hz, J = 5.5 Hz, 1H, H_6), 3.08–3.18 (m, 1H, H_{11}), 3.32–3.42 (m, 2H, $CH_2\alpha$ thioalkyl chain), 4.24 (br s, 2H, NH_2), 5.51 (br d, J = 4.5 Hz, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.4 (CH_3 thioalkyl chain), 22.8 ($CH_2\beta$ thioalkyl chain), 23.4 (C_{14}), 27.3 (C_{11}), 28.4 (C_7), 29 (C_{13}), 35.4 ($CH_2\alpha$ thioalkyl chain), 35.7 (C_{10}), 39.2 (C_6), 111.3 (C_1), 115.8 (C_{11a}), 125.0 (C_8), 131.8 (C_9), 144.7 (C_{4a}), 154.9 (C_{5a}), 163.1 (C_{12}), 168.3 (C_3); IR (KBr): ν = 3340, 2921, 1618, 1548, 1452, 1353, 1285 cm^{-1}; MS (ESI+): m/z (%): 332 (100) $[M+H]^+$; Anal. Calcd for $C_{17}H_{21}N_3S_2$: C, 61.59; H, 6.39; N, 12.68. Found: C, 61.36; H, 6.32; N, 12.68; HPLC: t_R = 20.4 min (purity >95%).

5.3.1.12. (±)-11-Amino-2-thiopropyl-5,6,9,10-tetrahydro-thiazolo[4,5-*b*]-8-ethyl-6,10-methano cycloocta[e]pyridine (9b). This compound was prepared according to the general procedure described above using **22** (507 mg, 2.54 mmol), **2b** (250 mg, 1.52 mmol). Flash chromatography on a silica gel column (CH_2Cl_2/AcOEt, 4:1) gave the desired Huprine **9b** as a white-off solid (290 mg, 56%); mp: 196 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.00 (t, J = 7.5 Hz, 3H, CH_3 thioalkyl chain), 1.49 (s, 3H, H_{14}), 1.84 (quint, J = 7.5 Hz, 2H, $CH_2\beta$ thioalkyl chain,), 1.81–1.91 (m, 1H, H_{14}), 1.85–1.95 (m, 1H, H_{10}), 1.92–2.02 (m, 1H, H_{13}), 2.37–2.47 (m, 1H, H_{10}), 2.63–2.73 (m, 1H, H_7), 2.91 (br d, J = 17.5 Hz, 1H, H_6), 3.08 (dd, J = 17.5 Hz, J = 5.5 Hz, 1H, H_6), 3.11–3.21 (m, 1H, H_{11}), 3.32–3.42 (m, 2H, $CH_2\alpha$thioalkyl chain), 4.24 (br s, 2H, NH_2), 5.51 (br d, J = 4.5 Hz, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.1 (CH_3 thioalkyl chain), 13.4 (C_{15}), 22.8 (CH_2 thioalkyl chain), 27.3 (C_{11}), 28.4 (C_7), 29.3 (C_5), 30.0 (C_{14}), 34.2 (C_{13}), 35.4 (CH_2 thioalkyl chain), 39.2 (C_6), 111.3 (C_1), 115.9 (C_{11a}), 123.5 (C_8), 137.2 (C_9), 144.6 (C_{13}), 155.0 (C_{5a}), 163.1 (C_{12}), 168.3 (C_3); IR (KBr): ν = 3346, 2893, 1619, 1549, 1452, 1353, 1287 cm^{-1}; MS (ESI+): m/z (%): 346 (100) $[M+H]^+$; Anal. Calcd for $C_{18}H_{23}N_3S_2$: C, 62.57; H, 6.71; N, 12.16; S, 18.56. Found: C, 62.73; H, 7.14; N, 11.91; S, 17.68; HPLC: t_R = 21.9 min (purity 81%).

5.3.1.13. (±)-9-Amino-1-(methylthio)-1H-pyrazolo[3,4-b]-3,4,7,8-tetrahydro-6-methyl-4,8-methanocycloocta[e]pyridine (10). This compound was prepared according to the general procedure described above using 3-amino-5-(methylthio)-1*H*-pyrazole-4-carbonitrile (785 mg, 5.09 mmol), **2a** (500 mg, 3.33 mmol). Purification by flash chromatography on a silica gel column (AcOEt) afforded the desired Huprine **10** as a yellow solid (320 mg, 33%, along with 5% of unseparable undesired isomer); mp: 256 °C (decomposition); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.55 (s, 3H, H_{14}), 1.83–1.93 (m, 1H, H_{10}), 1.89–1.99 (m, 1H, H_{13}), 1.95–2.05 (m, 1H, H_{10}), 2.40–2.50 (m, 1H, H_{13}), 2.57 (s, 3H, SMe), 2.65–2.75 (m, 1H, H_7), 3.01 (br d, J = 17.4 Hz, 1H, H_2), 3.07–3.17 (m, 1H, H_{11}), 3.19 (dd, J = 17.4 Hz, J = 5.5 Hz, 1H, H_6), 5.42 (br s, 2H, NH_2), 5.49–5.59 (m, 1H, H_8); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 19.3 (SMe), 23.6 (C_{14}), 26.9 (C_{11}), 28.3 (C_7), 29.4 (C_{10}), 35.9 (C_{13}), 39.5 (C_6), 104.1 (C_1), 111.6 (C_{11a}), 125.5 (C_8), 132.3 (C_9), 137.2 (C_2), 145.9 (C_{4a}), 153.2 (C_{12}), 157.5 (C_{5a}); IR (KBr): ν = 3364, 2923, 1611, 1582, 1425, 1373, 1278, 1168, 1031 cm^{-1}; MS (ESI+): m/z (%): 287 (100) $[M+H]^+$; Anal. Calcd for $C_{15}H_{18}N_4S$: C, 62.91; H, 6.33; N, 19.56; S, 11.20. Found: C, 62.89; H, 6.46; N, 19.63; S, 11.13; HPLC: t_R = 15.4 min (purity >95%).

5.3.1.14. (±)-9-Amino-1H-pyrazolo[3,4-*b*]-3,4,7,8-tetrahydro-6-methyl-4,8-methanocycloocta[e]pyridine (11). This compound was prepared according to the general procedure described above using 3-amino-4-pyrazolecarbonitrile (550 mg, 5.09 mmol), **2a** (500 mg, 3.33 mmol). Purification by flash chromatography on a silica gel column (AcOEt) afforded the desired Huprine **11** as a white solid (400 mg, 41% along with 9% of unseparable undesired isomer); mp: 224 °C (decomposition); ^{1}H NMR (300 MHz, CD_3OD): δ = 1.54 (s, 3H, H_{14}), 1.83–2.01 (m, 3H, H_{10}, H_{13}, H_{10}), 2.41 (dd, J = 17.4 Hz, J = 4.3 Hz, 1H, H_{13}), 2.58–2.68 (m, 1H, H_7), 2.74 (br d, J = 17.4 Hz, 1H, H_6), 3.00 (dd, J = 17.4 Hz, J = 5.5 Hz, 1H, H_6), 3.19–3.29 (m, 1H, H_{11}), 5.50 (d, J = 4.5 Hz, 1H, H_8), 8.11 (s, 1H, H_2); ^{13}C NMR (75 MHz, CD_3OD): δ = 23.7 (C_{14}), 27.8 (C_{11}), 29.7 (C_7), 30.3 (C_{10}), 37.0 (C_{13}), 39.7 (C_6), 105.3 (C_1), 111.9 (C_{11a}), 125.9 (C_8), 132.3 (C_9), 134.1 (C_2), 149.7 (C_{4a}), 151.8 (C_{12}), 156.9 (C_{5a}); IR (KBr): ν = 3342, 3216, 2918, 1654, 1589, 1495, 1435, 1398, 1372, 1333, 1274, 1154, 951, 920, 854, 831, 782 cm^{-1}; HPLC: t_R = 13.7 min (purity >95%).

5.3.1.15. (±)-2-Chloro-*N*-(3-chloro-6,7,10,11-tetrahydro-12-ylamido-9-methyl-7,11-methanocyclo octan[*b*]quinoline)acetamide (12). To a mixture of (±)-Huprine Y (1 g, 3.96 mmol), Et_3N (1.22 mL, 8.72 mmol) dissolved in THF (40 mL) was added slowly chloroacetylchloride (0.63 mL, 7.93 mmol). The reaction was stirred overnight. To the mixture was added water (50 mL), aqueous saturated solution of $NaHCO_3$ (50 mL), and AcOEt (100 mL). The aqueous layer was extracted once with AcOEt (100 mL), dried over $MgSO_4$, and evaporated under *vacuum* to furnish a brown residue. The product was purified by flash chromatography on a silica gel column (cyclohexane/AcOEt, 4:1–7:3) to afford **12** as a yellow solid (350 mg, 24%); mp: 230 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.51 (s, 3H, H_{14}), 1.76 (d, J = 17.2 Hz, 1H, H_{13}), 1.96–2.06 (m, 2H, H_{10}), 2.59 (br dd, J = 17.2 Hz, J = 4.6 Hz, 1H, H_{13}), 2.74–2.84 (m, 1H, H_7), 3.11 (br d, J = 17.7 Hz, 1H, H_6), 3.20 (dd, J = 17.7 Hz, J = 5.1 Hz, 1H, H_6), 3.41–3.51 (m, 1H, H_{11}), 4.38 (s, 2H, H_{19}), 5.51–5.61 (m, 1H, H_8), 7.40 (dd, J = 9.0 Hz, J = 2.0 Hz 1H, H_2), 7.63 (d, J = 9.0 Hz, 1H, H_1), 8.00 (d, J = 2.0 Hz, 1H, H_4), 8.30 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.4 (C_{14}), 28.2 (C_7), 28.4 (C_{10}), 29.0 (C_{11}), 38.1 (C_{13}), 40.1 (C_6), 43.0 (C_{19}), 122.5 (C_{12a}), 124.3 (C_1), 125.6 (C_8), 127.3 (C_2), 127.8 (C_4), 132.0 (C_3), 132.6 (C_9), 135.1 (C_{11a}), 137.3 (C_{4a}), 147.9 (C_{12}), 160.6 (C_{5a}), 165.3 (C_{15}); IR (KBr): ν = 3232, 2836, 1673, 1509, 1404, 1215 cm^{-1}; MS (ESI+): m/z (%): 361 (100) $[M+H]^+$; HPLC: t_R = 16.7 min (purity 94%).

5.3.2. General procedure for the preparation of racemic 12-chloro-Huprines

To a mixture of *(±)*-7-alkylbicyclo[3.3.1]non-6-en-3-one (1 equiv) and *o*-aminobenzoic acid derivative (1 equiv) was slowly added $POCl_3$ (10 equiv) at 0 °C. The resulting reaction mixture was heated under reflux for 3 h, then cooled to room temperature and poured carefully onto a mixture of ice and aqueous saturated solution of K_2CO_3. The aqueous layer was extracted twice with AcOEt, dried over $MgSO_4$, and concentrated under reduced pressure. Recrystallization or flash chromatography on a silica gel column afforded the desired 12-chloro-Huprine.

5.3.2.1. (±)-3-Chloro-12-hydroxy-6,7,10,11-tetrahydro-9-methyl-7,11-ethanocyclooctan[*b*]quinoline (13). This compound was prepared according to the general procedure described above using **2a** (1.4 g, 9.3 mmol) and 2-amino-4-chlorobenzoic acid (1.6 g, 9.3 mmol). Neutralization of the reaction was carried out in the presence of 5 M KOH solution (instead of saturated K_2CO_3 solution). Stirring at rt for 30 min completed the S_NAr substitution. Recrystallization from diethyl ether afforded **13** as a white solid (720 mg, 27%); mp > 280 °C; ^{1}H NMR (300 MHz, CD_3OD): δ = 1.55 (s, 3H, H_{14}), 1.76–1.80 (m, 1H, H_{10}), 1.91–2.03 (m, 2H, H_{10}, H_{13}), 2.39 (dd, J = 18.0 Hz, J = 4.5 Hz, 1H, H_{13}), 2.61 (d, J = 18.0 Hz 1H, H_6), 2.67 (br s, 1H, H_7), 3.03 (dd, J = 18.0 Hz, J = 5.5 Hz 1H, H_5), 3.46 (br s, 1H, H_{11}), 5.51 (d, J = 4.5 Hz, 1H, H_8), 7.29 (dd, J = 9.0 Hz, J = 1.9 Hz, 1H, H_2), 7.48 (d, J = 1.9 Hz, 1H, H_4), 8.19 (d, J = 9.0 Hz, 1H, H_1); ^{13}C NMR (75 MHz, CD_3OD): δ = 23.7 (C_{14}), 26.9 (C_7), 28.9 (C_{11}), 29.8 (C_{10}), 35.6 (C_{13}), 37.1 (C_6), 117.9 (C_4), 122.7 (C_{12a}), 124.8 (C_2), 125.0 (C_8), 128.4 (C_1), 135.4 (C_{11a}), 138.7 (C_3), 138.9 (C_9), 141.5 (C_{12}), 150.2 (C_{4a}), 159.4 (C_{5a}); IR (KBr): ν = 3244, 3096, 2986, 2925, 2820, 1634, 1604, 1555, 1504, 1464, 1410, 1359, 1249, 1172, 1079, 952, 838, 765 cm^{-1}; MS (ESI+): *m/z* (%): 286 (100) $[M+H]^+$, 288 (30); HPLC: t_R = 20.4 min (purity 91%).

5.3.2.2. (±)-3,12-Dichloro-6,7,10,11-tetrahydro-9-methyl-7,11-ethanocyclooctan[*b*]quinoline (14a). This compound was prepared according to the general procedure described above using **2a** (1.4 g, 9.3 mmol) and 2-amino-4-chlorobenzoic acid (1.6 g, 9.3 mmol). Flash chromatography on a silica gel column (CH_2Cl_2), followed by a recrystallization from acetone afforded **14a** as a white solid (730 mg, 27%); mp: 136 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 3H, H_{14}), 2.01–2.11 (m, 3H, H_{10}, H_{13}), 2.50–2.60 (m, 1H, H_{13}), 2.73–2.83 (m, 1H, H_7), 3.09 (d, J = 17.9 Hz, 1H, H_6), 3.19 (dd, J = 17.9 Hz, J = 5.3 Hz, 1H, H_5), 3.69–3.79 (m, 1H, H_{11}), 5.49–5.59 (m, 1H, H_8), 7.47 (dd, J = 8.9 Hz, J = 1.9 Hz, 1H, H_2), 7.97 (d, J = 1.9 Hz, H_4, 1H), 8.12 (d, J = 8.9 Hz, 1H, H_1); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.5 (C_{14}), 28.4 (C_7), 28.7 (C_{10}), 30.8 (C_{11}), 37.2 (C_{13}), 40.6 (C_6), 124.2 (C_{12a}), 125.1 (C_8), 125.6 (C_4), 127.4 (C_1 or C_2), 127.7 (C_1 or C_2), 132.9 (C_{11a}), 133.7 (C_3), 135.4 (C_9), 141.0 (C_{12}), 147.5 (C_{4a}), 160.4 (C_{5a}); IR (KBr): ν = 2927, 1609, 1544, 1476, 1301, 1195, 1072 cm^{-1}; MS (ESI+): *m/z* (%): 304 (100) $[M+H]^+$, 306 (25); HPLC: t_R = 24.6 min (purity >95%).

5.3.2.3. (±)-3,12-Dichloro-6,7,10,11-tetrahydro-9-ethyl-7,11-ethano-cyclooctan[*b*]quinoline (14b). This compound was prepared according to the general procedure described above using **2b** (800 mg, 4.9 mmol) and 2-amino-4-chlorobenzoic acid (836 mg, 9.3 mmol). Flash chromatography on a silica gel column (CH_2Cl_2), followed by recrystallization from acetone afforded **14b** as a white solid (720 mg, 46%); mp: 86 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 (t, J = 7.5 Hz, 3H, H_{15}), 1.73 (q, J = 7.5 Hz, 2H, H_{14}), 1.83–1.93 (m, 1H, H_{13}), 2.02 (br d, J = 17.2 Hz, 2H, H_{10}), 2.50 (br dd, J = 17.4 Hz, J = 4.7 Hz, 1H, H_{13}), 2.68–2.78 (m, J = 17.7 Hz, 1H, H_7), 3.01(br d, J = 17.7 Hz, 1H, H_6), 3.13 (dd, J = 17.7 Hz, J = 5.3 Hz 1H, H_6), 5.40–5.50 (m, 1H, H_8), 7.40 (dd, J = 9.0 Hz, J = 2.2 Hz, 1H, H_2), 7.89 (d, J = 2.2 Hz, 1H, H_4), 8.04 (d, J = 9.0 Hz, 1H, H_1); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.1 (C_{15}), 28.3 (C_7), 29.0 (C_{10}), 30.0 (C_{14}), 30.9 (C_{11}), 35.7 (C_{13}), 40.7 (C_6), 123.1 (C_8), 124.2 (C_{12a}), 125.6 (C_1), 127.5 (C_4), 127.7 (C_2), 133.7 (C_{11a}), 135.3 (C_3), 138.2 (C_9), 140.9 (C_{12}), 147.5 (C_{4a}), 160.5 (C_{5a}); IR (KBr): ν = 2908, 1769, 1508, 1452, 1210 cm^{-1}; MS (ESI+): *m/z* (%): 340 (100) $[M+Na]^+$, 342 (30); HPLC: t_R = 26.9 min (purity >95%).

5.3.3. Synthesis of (±)-*N*-(2-Hydroxyethyl)-3-chloro-6,7,10,11-tetrahydro-12-ylamino-9-methyl-7,11-methanocyclooctan[*b*]-quinoline (15a)

A mixture of **14a** (800 mg, 2.6 mmol), ethanolamine (160 μL, 2.6 mmol), phenol (1.14 g, 12 mmol), and NaI (55 mg, 370 μmol) was carefully heated at 150 °C under Ar for 2.5 h and then cooled to room temperature. The mixture was diluted with AcOEt (50 mL), H_2O (40 ml), and basified (pH 14) with NaOH (5 M) solution. The aqueous phase was extracted with AcOEt (2 × 50 mL) then the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give a pale brown solid. The product was purified by flash chromatography on a silica gel column (AcOEt) to afford **15a** as a white solid (670 mg, 77%); mp: 80 °C (decomposition); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.81 (t, J = 7.5 Hz, 3H, H_{15}), 1.75 (q, J = 7.5 Hz, 2H, H_{14}), 1.78–1.88 (m, 1H, H_{13}), 1.83–1.93 (m, 1H, H_{10}), 1.94–2.04 (m, 1H, H_{10}), 2.54 (br d, J = 17.4 Hz, 1H, H_{13}), 2.71 (br s, 1H, H_7), 2.97 (br d, J = 17.6 Hz, 1H, H_6), 3.12 (dd, J = 17.6 Hz, J = 5.5 Hz, 1H, H_6), 3.38–3.48 (m, 1H, H_{11}), 3.59–3.69 (m, 2H, H_{19}), 3.83–3.93 (m, 2H, H_{20}), 4.91 (br t, J = 5.3 Hz, 1H, NH or OH,), 5.47 (m, 1H, H_8), 7.25 (dd, J = 9.0 Hz, J = 2.2 Hz, 1H, H_2), 7.87 (d, J = 2.2 Hz, 1H, H_4), 7.95 (d, J = 9.0 Hz, 1H, H_1); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.0 (C_{15}), 27.4 (C_{11}), 28.2 (C_7), 29.4 (C_{10}), 30.0 (C_{14}),35.7 (C_{13}), 40.1 (C_6), 52.1 (C_{19}), 61.8 (C_{20}), 119.4 (C_{12a}), 122.4 (C_{11a}), 123.4 (C_8), 124.5 (C_2), 125.6 (C_1), 127.3 (C_4), 134.2 (C_3), 137.5 (C_9), 148.4 (C_{12} or C_{4a}), 150.6 (C_{12} or C_{4a}), 158.8 (C_{5a}); IR (KBr): ν = 3394, 3148, 2927, 1736, 1558, 1417, 1279, 1076 cm^{-1}; MS (ESI+): *m/z* (%): 329 (100) $[M+H]^+$, 331 (33); HPLC: t_R = 17.4 min (purity 93%).

5.3.4. Synthesis of (±)-*N*-(2-Hydroxyethyl)-3-chloro-6,7,10,11-tetrahydro-12-ylamino-9-ethyl-7,11-methanocyclooctan[*b*]-quinoline (15b)

A mixture of **14b** (400 mg, 1.25 mmol), ethanolamine (76 μL, 1.25 mmol), phenol (544 mg, 5.78 mmol), and NaI (26 mg, 176 μmol) was carefully heated at 150 °C under Ar for 2.5 h and then cooled to room temperature. The mixture was diluted with AcOEt (40 mL), H_2O (30 ml), and basified (pH 14) with 5 M NaOH solution. The aqueous phase was extracted three times with AcOEt (40 mL) then the organic layer was washed with brine, dried over $MgSO_4$, and concentrated under *vacuum* to give a pale orange solid. The product was purified by flash chromatography on a silica gel column (AcOEt/cyclohexane, 1:1) to afford **15b** as a white solid (410 mg, 95%); mp: 90 °C (decomposition); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 3H, H_{14}), 1.77–1.87 (m, 1H, H_{10}), 1.81–1.91 (m, 1H, H_{13}), 1.89–1.99 (m, 1H, H_{10}), 2.45 (br d, J = 17.1 Hz 1H, H_{13}), 2.59–2.69 (m, 1H, H_7), 2.94 (br d, J = 17.5 Hz, 1H, H_6), 3.10 (dd, J = 17.5 Hz, J = 5.3 Hz 1H, H_6), 3.36–2.46 (m, 1H, H_{11}), 3.61–3.71 (m, 2H, H_{15}), 3.85–3.95 (m, 2H, H_{19}), 4.90 (br t, J = 5.3 Hz, 1H, OH or NH), 5.37–5.47 (m, 1H, H_8), 7.20 (dd, J = 9.0 Hz, J = 2.1 Hz 1H, H_2), 7.85 (d, J = 2.1 Hz, 1H, H_4), 7.92 (d, J = 9.0 Hz, 1H, H_1); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.4 (C_{14}), 27.2 (C_{11}), 28.1 (C_7), 29.0 (C_{10}), 36.8 (C_{13}), 39.6 (C6), 52.0 (C_{15}), 61.2 (C_{19}), 119.1 (C_{12a}), 121.7 (C_{11a}), 124.3 (C_2), 125.1 (C_8), 125.7 (C_1), 126.6 (C_4), 132.2 (C_9), 134.3 (C_3), 148.1 (C_{4a}), 150.1 (C_{12}), 158.3 (C_{5a}); IR (KBr): ν = 3204, 2928, 2108, 1608, 1558, 1418, 1076 cm^{-1}; MS (ESI+): *m/z* (%): 343 (100) $[M+H]^+$, 345 (30); HPLC: t_R = 18.9 min (purity 92%).

5.3.5. Synthesis of (±)-*N*-(2-chloroethyl)-3-chloro-6,7,10,11-tetrahydro-12-ylamino-9-methyl-7,11-methanocyclooctan[*b*]-quinoline (16a)

A mixture of **15a** (450 mg, 1.37 mmol) and $SOCl_2$ (2.1 mL, 29 mmol) was heated at reflux for 45 min. The mixture was concentrated under reduced pressure and the residue was diluted in AcOEt (40 mL), H_2O (30 mL), and basified (pH 12–14) with KOH (5 M) solution. The aqueous layer was extracted with AcOEt (2 × 50 mL), dried over $MgSO_4$, and concentrated under reduced pressure to afford **16a** as a pale brown solid (460 mg, quantitative). This product was pure enough to avoid purification; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.51 (s, 3H, H_{14}), 1.81 (d, J = 15.5 Hz, 1H, H_{13}), 1.88–1.98 (m, 1H, H_{10}), 2.01–2.11 (m, 1H, H_{10}), 2.58 (br dd, J = 15.5 Hz, J = 4.3 Hz, 1H, H_{13}), 2.70–2.80 (m, 1H, H_7), 3.02 (dt, J = 1.6 Hz, J = 17.7 Hz, 1H, H_6), 3.16 (dd, J = 17.7 Hz, J = 5.3 Hz 1H, H_6), 3.42–3.52 (m, 1H, H_{11}), 3.66–3.76 (m, 2H, H_{19}), 3.71–3.81 (m, 2H, H_{15}), 4.48 (br s, 1H, NH), 5.48–5.58 (m, 1H, H_8), 7.30 (dd, J = 9.0 Hz, J = 2.3 Hz, 1H, H_2), 7.87 (d, J = 9.0 Hz, 1H, H_1), 7.91 (d, J = 2.3 Hz, 1H, H_4); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.5 (C_{14}), 27.6 (C_{11}), 28.3 (C_7), 29.1 (C_{10}), 37.5 (C_{13}), 40.0 (C_6), 45.4 (C_{19}), 51.3 (C_{15}), 119.7 (C_{12a}), 123.6 (C_{11a}), 125.0 (C_1), 125.1 (C_2), 125.6 (C_8), 127.8 (C_4), 131.9 (C_9), 134.4 (C_3), 148.3 (C4a), 149.1 (C_{12}), 159.1 (C_{5a}); IR (KBr): ν = 2929, 2108, 1607, 1557, 1486, 1412, 1346 cm^{-1}; MS (ESI+): *m/z* (%): 347 (100) $[M+H]^+$, 349.

5.3.6. Synthesis of (±)-*N*-(2-chloroethyl)-3-chloro-6,7,10,11-tetrahydro-12-ylamino-9-ethyl-7,11-methanocyclooctan[*b*]quinoline (16b)

A mixture of **15b** (300 mg, 836 μmol) and $SOCl_2$ (1.3 mL, 17 mmol) was heated at reflux for 45 min. The mixture was concentrated under reduced pressure and the residue was diluted with AcOEt (30 mL), H_2O (20 mL), and basified (pH 9–10) with 5 M KOH solution. The aqueous layer was extracted with AcOEt (3 × 30 mL), dried over $MgSO_4$, and concentrated under *vacuum* to afford **16b** as a pale brown solid (320 mg, 98%). This product was pure enough to avoid purification; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.85 (t, J = 7.3 Hz, 3H, H_{15}), 1.79 (br q, J = 7.3 Hz, 2H, H_{14}), 1.81–1.91 (m, 1H, H_{13}), 1.90–2.00 (m, 1H, H_{10}), 2.02–2.12 (m, 1H, H_{10}), 2.62 (br dd, J = 17.4 Hz, J = 4.8 Hz, 1H, H_{13}), 2.73–2.83 (m, 1H, H_7), 3.03 (br d, J = 17.6 Hz, 1H, H_6), 3.18 (dd, J = 17.6 Hz, J = 5.3 Hz, 1H, H_6), 3.43–3.53 (m, 1H, H_{11}), 3.69–3.79 (m, 2H, H_{19}), 3.74–3.84 (m, 2H, H_{20}), 5.47–5.57 (m, 1H, H_8), 7.30 (dd, J = 9.0 Hz, J = 2.0 Hz, 1H, H_2), 7.88 (d, J = 9.0 Hz, 1H, H_1), 7.93 (d, J = 2.0 Hz, 1H, H_4); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.0 (C_{15}), 27.6 (C_{11}), 28.1 (C_7), 29.4 (C_{10}), 30.0 (C_{14}), 35.9 (C_{13}), 39.9 (C_6), 45.3 (C_{20}), 51.2 (C_{19}), 119.5 (C_{12a}), 123.4 (C_{11a}), 123.5 (C_8), 125.1 (C_1 or C_2), 125.2 (C_1 or C_2), 127.5 (C_4), 134.6 (C_3), 137.3 (C_9), 148.0 (C_{12} or C_{4a}), 149.4 (C_{12} or C_{4a}), 158.9 (C_{5a}); IR (KBr): ν = 2829, 1622, 1557, 1422, 1348 cm^{-1}.

5.3.7. Synthesis of (±)-*N*-(2-azidoethyl)-3-chloro-6,7,10,11-tetrahydro-12-ylamino-9-methyl-7,11-methanocyclooctan[*b*]-quinoline (17a)

A mixture of **16a** (350 mg, 1 mmol) and NaN_3 (262 mg, 4 mmol) in DMF (5 mL) was heated overnight at 80 °C. The mixture was concentrated under reduced pressure and purified by flash chromatography on a silica gel column (AcOEt/cyclohexane, 1:1) to afford **17a** as a pale brown solid (280 mg, 78%); mp: 74 °C (decomposition); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.48 (s, 3H, H_{14}), 1.77 (br d, J = 15.5 Hz, 1H, H_{13}), 1.85–1.95 (m, 1H, H_{10}), 1.99–2.09 (m, 1H, H_{10}), 2.56 (br dd, J = 15.5 Hz, J = 4.0 Hz, 1H, H_{13}), 2.67–2.77 (m, 1H, H_7), 2.99 (br d, J = 17.7 Hz, 1H, H_6), 3.13 (dd, J = 17.7 Hz, J = 5.5 Hz, 1H, H_6), 3.32–3.42 (m, 1H, H_{11}), 3.49–3.59 (m, 4H, H_{15}, H_{19}), 4.26 (br s, 1H, NH), 5.45–5.55 (m, 1H, H_8), 7.27 (dd, J = 8.9 Hz, J = 2.0 Hz, 1H, H_2), 7.85 (d, J = 8.9 Hz 1H, H_1), 7.88 (d, J = 2.0 Hz, 1H, H_4); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.4 (C_{14}), 27.5 (C_{11}), 28.2 (C_7), 29.0 (C_{10}), 37.4 (C_{13}), 39.9 (C_6), 48.9 (C_{19}), 52.1 (C_{15}), 124.9 (C_2), 125.0 (C_1), 125.5 (C_8), 127.7 (C_4), 131.8 (C_9), 134.2 (C_3), 148.3 (C_{4a}), 149.2 (C_{12}), 159.1 (C_{5a}); IR (KBr): ν = 3340, 2928, 2100, 1607, 1558, 1415, 1281 cm^{-1}; MS (ESI+): *m/z* (%): 354 (100) $[M+H]^+$, 356 (30).

5.3.8. Synthesis of (±)-*N*-(2-azidoethyl)-3-chloro-6,7,10,11-tetrahydro-12-ylamino-9-ethyl-7,11-methanocyclooctan[*b*]-quinoline (17b)

A mixture of **16b** (300 mg, 830 μmol,) and NaN_3 (216 mg, 3.3 mmol) in DMF (5 mL) was heated overnight at 80 °C. The mixture was concentrated under reduced pressure and the residue was diluted with AcOEt (20 mL) and water (20 mL). The aqueous layer was extracted twice with AcOEt (20 mL), dried over $MgSO_4$, and evaporated under *vacuum* to furnish a pale brown solid. The product was purified by flash chromatography on a silica gel column (AcOEt/cyclohexane, 7:3) to afford the desired product **17b** as a pale brown solid (210 mg, 69%); mp: 60 °C (decomposition); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.85 (t, 3H, H_{15}, J = 7.5 Hz), 1.73–1.83 (m, 2H, H_{14}), 1.77–1.87 (m, 1H, H_{13}), 1.90–2.00 (m, 1H, H_{10}), 2.04–2.14 (m, 1H, H_{10}), 2.62 (br dd, 1H, H_{13}, J = 17.7 Hz, J = 4.6 Hz), 2.73–2.83 (m, 1H, H7), 3.01 (br d, 1H, H_6, J = 17.7 Hz), 3.20 (dd, 1H, H_6, J = 17.7 Hz, J = 5.5 Hz), 3.37–3.47 (m, 1H, H_{11}), 3.54–3.64 (m, 4H, H_{19}, H_{20}), 5.47–5.57 (m, 1H, H_8), 7.31 (dd, 1H, H_2, J = 9.0 Hz, J = 2.0 Hz), 7.88 (d, 1H, H_1, J = 9.0 Hz), 7.90 (d, 1H, H_4, J = 2.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.1 (C_{15}), 27.7 (C_{11}), 28.2 (C_7), 29.5 (C_{10}), 30.0 (C_{14}), 36.1 (C_{13}), 40.3 (C_6), 49.1 (C_{20}), 52.2 (C_{19}), 119.7 (C_{11a}), 123.6 (C_8), 125.1 (C_1, C_2), 128.0 (C_4), 134.3 (C_{12a}, C_3), 137.2 (C_9), 148.6 (C_{12} or C_{4a}), 149.2 (C_{12} or C_{4a}), 159.4 (C_{5a}); IR (KBr): ν = 3369, 2929, 2100, 1607, 1557, 1415, 1280 cm^{-1}. MS (ESI+): *m/z* (%): 368 (100) $[M+H]^+$, 370 (33).

5.3.9. Analyses for 1-(3-nitrophenyl)-6,7-dimethoxy-2-(oct-7-ynyl)-1,2,3,4-tetrahydroisoquinoline (22)

^{1}H NMR ($CDCl_3$) δ = 1.27 (m, 4H, H_{18}, H_{19}), 1.46 (m, 4H, H_{17}, H_{20}), 1.88 (t, J = 2.7 Hz, 1H, H_{23}), 2.05 (td, J = 2.7 Hz, J = 7.0 Hz, 2H, H_{21}), 2.39 (m, 2H, H_{16}), 2.55 (m, 1H, H_8), 2.75 (dt, J = 4.1 Hz, J = 16.0 Hz, 1H, H_9), 2.96 (m, 1H, H_9), 3.12 (dt, J = 4.1 Hz, J = 16.0 Hz, 1H, H_8), 3.61 (s, 3H, OMe), 3.85 (s, 3H, OMe), 4.60 (s, 1H, H_1), 6.12 (s, 1H, H_{14}), 6.60 (s, 1H, H_{11}), 7.44 (t, J = 7.8 Hz, 1H, H_6), 7.60 (dt, J = 1.3 Hz, J = 7.8 Hz, 1H, H_7), 8.10 (ddd, J = 1.3 Hz, J = 2.3 Hz, J = 7.8 Hz, 1H,H_5), 8.12 (t_{app}, J = 1.3 Hz, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ = 18.4 (C_{21}), 26.7 (C_{18} or C_{19}), 26.8 (C_{18} or C_{19}), 28.0 (C_9), 28.4 (C_{17} or C_{20}), 28.6 (C_{17} or C_{20}), 46.5 (C_8), 54.1 (C_{16}), 55.9 (OMe), 56.0 (OMe), 67.3 (C_1), 68.2 (C_{23}), 84.7 (C_{22}), 111.2 (C_{11}), 111.4 (C_{14}), 122.3 (C_5), 124.3 (C_3), 127.4 (C_{10}), 128.4 (C_6), 129.1 (C_7), 135.6 (C_{15}), 147.3 (C_{12} or C_{13}), 147.4 (C_4), 147.8 (C_{12} or C_{13}), 148.2 (C_2);); IR (KBr): ν = 3275, 2932, 1609, 1537, 1450, 1353, 1254, 1135 cm^{-1} MS (ESI+): *m/z* (%): 423 (100) $[M+H]^+$.

5.3.10. Analyses for 1-(4-nitrophenyl)-6,7-dimethoxy-2-(oct-7-ynyl)-1,2,3,4-tetrahydroisoquinoline (23)

^{1}H NMR ($CDCl_3$) δ = 1.30 (m, 4H, H_{18}, H_{19}), 1.45 (m, 4H, H_{17}, H_{20}), 1.96 (t, 1H, J = 2.7 Hz, H_{23}), 2.12 (td, 2H, J = 2.7 Hz, J = 7.0 Hz, H_{21}), 2.39 (m, 2H, H_{16}), 2.58 (m, 1H, H_8), 2.77 (dt, J = 4.1 Hz, J = 15.9 Hz, 1H, H_9), 2.96 (m, 1H, H_9), 3.17 (dt, J = 4.1 Hz, J = 15.9 Hz, 1H, H_8), 3.61 (s, 3H, OMe), 3.85 (s, 3H, OMe), 4.58 (s, 1H, H_1), 6.10 (s, 1H, H_{14}), 6.62 (s, 1H, H_{11}), 7.45 (dt, J = 1.9 Hz, J = 8.8 Hz, 2H, H_3, H_7), 8.15 (dt, J = 1.9 Hz, J = 8.8 Hz, 2H, H_4, H_6). ^{13}C NMR ($CDCl_3$) δ = 18.5 (C_{21}), 26.8 (C_{18} or C_{19}), 26.9 (C_{18} or C_{19}), 28.2 (C_9), 28.5 (C_{17} or C_{20}), 28.7 (C_{17} or C_{20}), 46.8 (C_8), 54.4 (C_{16}), 55.9 (OMe), 55.9 (OMe), 67.5 (C_1), 68.3 (C_{23}), 84.7 (C_{22}), 111.2 (C_{11} or C_{14}), 111.3 (C_{11} or C_{14}), 123.5 (C_4, C_6), 127.3 (C_{10}), 128.5 (C_{15}), 130.3 (C_3, C_7), 147.2 (C_5), 147.3 (C_{12} or C_{13}), 147.8 (C_{12} or C_{13}), 152.8 (C_2); IR (KBr):

ν = 3307, 2927, 1609, 1518, 1450, 1348, 1221, 1137 cm^{-1} MS (ESI+): *m/z* (%): 421 (100) $[M+H]^+$.

5.3.11. Synthesis of (±)-3-chloro-6,7,10,11-tetrahydro-*N*-(2-(4-(6-(3,4-dihydro-6,7-dimethoxy-1-(3-nitrophenyl)isoquinolin-2(1*H*)-yl)hexyl)-1*H*-1,2,3-triazol-1-yl)-9-methyl)-12-amino-6,10-methanocycloocta[*b*]quinoline (24)

A mixture of **17a** (22.8 mg, 64 μmol), tetrahydroisoquinoline **22** (27.2 mg, 64 μmol), and CuI (0.85 mg, 4.5 μmol) in dry MeCN (1 mL) was stirred at room temperature for 15 h. THF (1 mL) was then added to the solution and the mixture was concentrated under *vacuum*. The product was purified by flash chromatography on a silica gel column (CH_2Cl_2/MeOH, 100:0–7:3) to afford the desired product **24** as a yellow solid (24 mg, 48%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.23 (m, 6H, H_{17}, H_{18}, H_{19}), 1.46 (s, 3H, H_{42}), 1.57 (m, 2H, H_{20}), 1.65 (d, *J* = 15.7 Hz, 1H, H_{41}), 1.83 (m, 1H, H_{38}), 1.97 (m, 1H, H_{38}), 2.40 (m, 2H, H_{16}), 2.52 (m, 2H, H_8, H_{41}), 2.65 (t, *J* = 7.5 Hz, 2H, H_{21}), 2.73 (m, 1H, H_9), 2.79 (m, 1H, H_{35}), 2.97 (m, 2H, H_9, H_{34}), 3.09 (m, 1H, H_{34}), 3.12 (m, 2H, H_{34}, H_{39}), 3.61 (s, 3H, OMe), 3.85 (s, 3H, OMe), 3.94 (m, 2H, H_{25}), 4.56 (m, 3H, H_1, H_{24}), 4.65 (br s, 1H, NH), 5.49 (m, 1H, H_{36}), 6.11 (s, 1H, H_{14}), 6.62 (s, 1H, H_{11}), 7.28 (m, 2H, H_{23}, H_{29}), 7.45 (t, *J* = 7.7 Hz, 1H, H_6), 7.58 (br d, *J* = 7.7 Hz, 1H, H_7), 7.76 (d, *J* = 8.7 Hz, 1H, H_{28}), 7.88 (d, *J* = 1.9 Hz, 1H, H_{31}), 8.09 (br d, *J* = 7.7 Hz, 1H, H_7), 8.15 (br s, 1H, H_3). ^{13}C NMR ($CDCl_3$) δ = 23.5 (C_{42}), 25.6 (C_{21}), 26.9 (C_{17}), 27.0 (C_{18}), 27.5 (C_{39}), 28.1 (C_{35}), 28.3 (C_9), 29.1 (C_{19}), 29.2 (C_{38}), 29.6 (C_{10}), 37.4 (C_{41}), 40.1 (C_{34}), 46.6 (C_8), 49.3 (C_{25}), 50.8 (C_{24}), 55.9 (OMe), 56.0 (OMe), 67.4 (C_1), 111.2 (C_{11}), 111.5 (C_{14}), 119.6 (C_{27}), 121.5 (C_{29}), 122.3 (C_5), 123.7 (C_{40}), 124.3 (C_3), 124.8 (C_{28}), 125.0 (C_{23}), 125.4 (C_{36}), 127.4 (C_{10}), 128.0 (C_3), 128.4 (C_{15}), 129.1 (C_6), 132.0 (C_{37}), 134.2 (C_{30}), 135.7 (C_7), 147.3 (C_{12} or C_{13}), 147.5 (C_4), 147.9 (C_{12} or C_{13}), 148.3 (C_{22}), 148.5 (C_2), 148.9 (C_{26} or C_{33}), 149.0 (C_{26} or C_{33}), 159.3 (C_{32}). MS (ESI+): *m/z* (%): 776 (100) $[M+H]^+$, 778 (35).

5.3.12. Synthesis of (±)-3-chloro-6,7,10,11-tetrahydro-*N*-(2-(4-(6-(3,4-dihydro-6,7-dimethoxy-1-(4-nitrophenyl)isoquinolin-2(1*H*)-yl)hexyl)-1*H*-1,2,3-triazol-1-yl)-9-ethyl)-12-amino-6,10-methanocycloocta[*b*]quinoline (25)

A mixture of **17b** (18.4 mg, 50 μmol), tetrahydroisoquinoline **23** (21.2 mg, 50 μmol), and CuI (1.1 mg, 5.5 μmol) in dry MeCN (1 mL) was stirred at room temperature for 15 h. THF (1 mL) was added to the solution and the mixture was concentrated under *vacuum*. The product was purified by flash chromatography on a silica gel column (CH_2Cl_2/MeOH, 100:0–7:3) to give the desired product **25** as yellow solid (39 mg, 98%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.83 (t, *J* = 7.4 Hz, 3H, H_{43}), 1.24 (m, 4H, H_{18}, H_{19}), 1.46 (m, 2H, H_{17}), 1.59 (m, 2H, H_{20}), 1.74 (m, 3H, H_{41}, H_{42}), 1.84 (m, 1H, H_{38}), 2.00 (m, 1H, H_{38}), 2.37 (m, 2H, H_{16}), 2.55 (m, 2H, H_{41}, H_8), 2.66 (t, *J* = 7.5 Hz, 2H, H_{21}), 2.75 (m, 2H, H_9, H_{35}), 2.94 (m, 1H, H_9), 3.02 (m, 1H, H_{34}), 3.13 (m, 3H, H_8, H_{34}, H_{39}), 3.60 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.95 (m, 2H, H_{25}), 4.57 (m, 3H, H_1, H_{24}), 4.74 (br s, 1H, NH), 5.47 (m, 1H, H_{36}), 6.08 (s, 1H, H_{14}), 6.61 (s, 1H, H_{11}), 7.26 (m, 2H, H_{23}, H_{29}), 7.44 (d, *J* = 8.7 Hz, 2H, H_3, H_7), 7.76 (d, *J* = 9.0 Hz, 1H, H_{28}), 7.93 (d, *J* = 1.9 Hz, 1H, H_{31}), 8.13 (d, *J* = 8.7 Hz, 2H, H_4, H_6). ^{13}C NMR ($CDCl_3$) δ = 12.0 (C_{43}), 25.7 (C_{21}), 26.9 (C_{17}), 30.0 (C_{18}), 27.4 (C_{39}), 28.1 (C_{35}), 28.2 (C_9), 29.1 (C_{19}), 29.3 (C_{38}), 29.6 (C_{20}), 29.9 (C_{42}), 35.8 (C_{41}), 40.2 (C_{34}), 46.8 (C_8), 49.3 (C_{25}), 50.7 (C_{24}), 54.3 (C_{16}), 55.9 (OMe), 60.0 (OMe), 67.6 (C_1), 111.2 (C_{11}), 111.3 (C_{14}), 119.4 (C_{27}), 121.5 (C_{29}), 123.4 (C_{36}), 123.5 (C_4, C_6), 123.6 (C_{40}), 124.9 (C_{28}), 125.0 (C_{23}), 127.3 (C_{10}), 127.6 (C_{31}), 128.4 (C_{15}), 130.3 (C_3, C_7), 134.3 (C_{30}), 137.4 (C_{37}), 147.1 (C_5), 147.3 (C_{12} or C_{13}), 147.8 (C_{12} or C_{13}), 148.2 (C_{22}), 148.9 (C_{26} or C_{33}), 149.3 (C_{26} or C_{33}), 152.9 (C_2), 159.2 (C_{32}). IR (KBr): ν = 3361, 2929, 1607, 1517, 1346, 1223, 1134 cm^{-1}. MS (ESI+): *m/z* (%): 790 (100) $[M+H]^+$, 792 (36).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.05.005.

References and notes

1. Taylor, P. Anticholinesterase Agents. In *The Pharmacological Basis of Therapeutics*; Hardman, J. G., Limbird, L. E., Molinoff, P. B., Ruddon, R. W., Gilman, A. G., Eds.; McGraw-Hill: New York, 1996; pp 161–176.
2. Giacobini, E. *Cholinesterases and Cholinesterase Inhibitors*; Martin Dunitz: London, 2000.
3. Casida, J. E.; Quistad, G. B. *Annu. Rev. Entomol.* **1998**, *43*, 1.
4. Du, D. M.; Carlier, P. R. *Curr. Pharm. Des.* **2004**, *10*, 3141.
5. Francotte, P.; Graindorge, E.; Boverie, S.; de Tullio, P.; Pirotte, B. *Curr. Med. Chem.* **2004**, *11*, 1757.
6. Munoz-Torrero, D.; Camps, P. *Curr. Pharm. Des.* **2006**, *12*, 4281.
7. Inestrosa, N. C.; Alvarez, A.; Perez, C. A.; Moreno, R. D.; Vicente, M.; Linker, C.; Casanueva, O. I.; Soto, C.; Garrido, C. *Neuron* **1996**, *16*, 881.
8. Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. *Biochem. Pharmacol.* **2003**, *65*, 407.
9. Thacker, P. D. *Drug Discovery Today* **2003**, *8*, 379.
10. Camps, P.; El Achab, R.; Görbig, D. M.; Morral, J.; Munõz-Torrero, D.; Badia, A.; Banõs, J. E.; Vivas, N. M.; Barril, X.; Orozco, M.; Luque, F. J. *J. Med. Chem.* **1999**, *42*, 3227.
11. Dvir, H.; Wong, D. M.; Harel, M.; Barril, X.; Orozco, M.; Luque, F. J.; Muñoz-Torrero, D.; Camps, P.; Rosenberry, T. L.; Silman, I.; Sussman, J. L. *Biochemistry* **2002**, *41*, 2970.
12. Camps, P.; El Achab, R.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J. E.; Vivas, N. M.; Barril, X.; Orzco, M.; Luque, F. J. *J. Med. Chem.* **2000**, *43*, 4657.
13. del Mar Alcala, M.; Vivas, N. M.; Hospital, S.; Camps, P.; Muñoz-Torrero, D.; Badia, A. *Neuropharmacology* **2003**, *44*, 749.
14. Camps, P.; Gómez, E.; Muñoz-Torrero, D.; Font-Bardia, M.; Solans, X. *Tetrahedron* **2003**, *59*, 4143.
15. Camps, P.; Gómez, E.; Muñoz-Torrero, D.; Badia, A.; Clos, M. V.; Curutchet, C.; Muñoz-Muriedas, J.; Luque, F. J. *J. Med. Chem.* **2006**, *49*, 6833.
16. (a) Sussman, J. L.; Harel, M.; Silman, I. *Chem. Biol. Interact.* **1993**, *87*, 187; (b) Rydberg, E. H.; Brumshtein, B.; Greenblatt, H. M.; Wong, D. M.; Shaya, D.; Williams, L. D.; Carlier, P. R.; Pang, Y. P.; Silman, I.; Sussman, J. L. *J. Med. Chem.* **2006**, *49*, 5491; (c) Guillou, C.; Mary, A.; Renko, D. Z.; Gras, E.; Thal, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 637.
17. (a) Gagneux, A. R.; Meier, R. *Tetrahedron Lett.* **1969**, *10*, 1365; (b) Stetter, H.; Tacke, P.; Gärtner, J. *Chem. Ber.* **1964**, *97*, 3480; (c) Stetter, H.; Tacke, P.; Gärtner, J. *Chem. Ber.* **1966**, *99*, 1435; (d) Meyer, W. P.; Martin, J. C. *J. Am. Chem. Soc.* **1976**, *98*, 1231.
18. Camps, P.; Achab, R. E.; Font-Bardia, M.; Görbig, D.; Morral, J.; Muñoz-Torrero, D.; Solans, X.; Simon, M. *Tetrahedron* **1996**, *52*, 5867.
19. Camps, P.; Gomez, E.; Muñoz-Torrero, D.; Arno, M. *Tetrahedron: Asymmetry* **2001**, *12*, 2909.
20. Bertz, S. H. *J. Org. Chem.* **1985**, *50*, 3585.
21. (a) Camps, P.; Formosa, X.; Muñoz-Torrero, D.; Petrignet, J.; Badia, A.; Clos, M. V. *J. Med. Chem.* **2005**, *48*, 1701; (b) Jung, M.; Tak, J.; Lee, Y.; Jung, Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1082.
22. Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radić, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 1053.
23. Hu, M.-K.; Wu, L.-J.; Hsiao, G.; Yen, M.-H. *J. Med. Chem.* **2002**, *45*, 2277.
24. Hu, M.-K.; Lu, C.-F. *Tetrahedron Lett.* **2000**, *41*, 1815.
25. Ellman, L. G.; Courtney, K. D.; Andres, V. J.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
26. Marco, J. L.; De los Rios, C.; Garcia, A. G.; Villarroya, M.; Carreiras, M. C.; Martins, C.; Eleutério, A.; Morreale, A.; Orozco, M.; Luque, F. J. *Bioorg. Med. Chem.* **2004**, *12*, 2199.
27. Carletti, E.; Li, H.; Li, B.; Ekström, F.; Nicolet, Y.; Loiodice, M.; Gillon, E.; Froment, M. T.; Lockridge, O.; Schopfer, L. M.; Masson, P.; Nachon, F. *J. Am. Chem. Soc.* **2008**, *130*, 16011.
28. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
29. Eramian, D.; Eswar, N.; Shen, M.-Y.; Sali, A. *Protein Sci.* **2008**, *17*, 1881.

30. Adams, P. D.; Grosse-Kunstleve, R. W.; Hung, L.-W.; Ioerger, T. R.; Mc Coy, A. J.; Moriarty, N. W.; Read, R. J.; Sacchettini, J. C.; Sauter, N. K.; Terwilliger, T. C. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2002**, *58*, 1948.
31. Sanner, M. F. *J. Mol. Graphics Modell.* **1999**, *17*, 57.
32. Gottelieb, H. E.; Kotlyar, V.; Nudelma, A. *J. Org. Chem.* **1997**, *62*, 7512.
33. Krasiński, A.; Radić, Z.; Manetsch, R.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. *J. Am. Chem. Soc.* **2005**, *127*, 6686.