Tacrine−Huperzine A Hybrids (Huprines): A New Class of Highly Potent and Selective Acetylcholinesterase Inhibitors of Interest for the Treatment of Alzheimer's Disease

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Abstract: Tacrine−huperzine A hybrids (huprines) are a new class of very potent and selective acetylcholinesterase (AChE) inhibitors. Huprines were designed from tacrine and (−)-huperzine A through a conjunctive approach. They combine the 4-aminoquinoline substructure of tacrine with the carbobicyclic substructure of (−)-huperzine A. Structural variations on several parts of a lead structure have allowed to complete a structure−activity relationship exploration of this new structural family and have led to several huprines more active than other known AChE inhibitors.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, responsible for over 50% of all cases of dementia, which affects up to 5% of those people over 65 years, its prevalence increasing to more than 20% of those over 80 years [1]. Three main stages can be clinically characterized in AD [2]. The first stage, the so-called amnesia stage, involves initial loss of short-term memory and lack of emotional spontaneity. In the second stage, the confusion stage, the patient exhibits time and space disorientation, severe mental confusion and personality changes. The last stage, the dementia stage, involves the total mental incapacity and full dependence of the patient. While the disease itself is not fatal, medical complications associated with AD, usually viral or bacterial infections, lead to the death of the patient [3]. Thus, AD is considered to be the third largest cause of death in the western world, after cardiovascular diseases and cancer. Taking into account the increase in life expectancy, the fact that the incidence of AD increases with advancing age, and the devastating effects of this illness, AD represents nowadays a major public health problem and will presumably be the most important pathology of the XXI century in developed countries. Important efforts have been made in the last two decades in order to determine the etiopathogenesis of AD, and to carry out its early diagnosis and therapeutic control.

Most relevant pathogenic events in AD can be classified into four main categories [4]: primary events (genetic alterations, neuronal apoptosis-like processes leading to premature neuronal death and brain dysfunction), secondary events (β-amyloid deposition in senile plaques and brain vessels, neurofibrillary tangles due to hyperphosphorilation of tau proteins, synaptic loss), tertiary events (neurotransmitter deficits, neurotrophic alterations, neuroinmune dysfunction, neuroinflammatory processes) and quaternary events (accelerated neuronal death due to excitotoxic reactions, alterations in calcium homeostasis, free radical formation, cerebrovascular dysfunction), constituting all of these pathogenic events potential targets for treatment of AD. In spite of the multifactorial nature of AD, most treatment strategies have been directed to two main targets: the β-amyloid peptide and the cholinergic neurotransmission. Therefore, there are two main approaches for the treatment of AD.

The first approach is to prevent the neurodegenerative changes that ultimately cause irreversible damage to the brain. The β-amyloid peptide (Aβ) is the main component of the senile plaques, one of the neurohistopathological signs of AD [5]. Aβ derives from the proteolytic processing of the β-amyloid precursor protein (AβPP), which has been proposed to serve as a multifunctional protein that mediates both cell adhesion and neurite outgrowth [6]. While trace amounts of Aβ have been detected as part of the normal cellular metabolism of AβPP [7,8], an increase in the production of the peptide and its subsequent deposition as insoluble amyloid plaques may represent the key pathological event that triggers the disease process [9,10]. Therefore, any manipulation that diminishes or prevents the generation or deposition of Aβ may be a potential therapeutic strategy [11], which could serve to slow down the rate of progression of the disease and prevent further neuronal cell losses. It has been shown that immunization with Aβ essentially prevents the development of Aβ-plaque formation in young transgenic animal models of AD, while treatment of older animals reduces the extent and progression of AD-like neuropathologies [11]. These results raise the possibility that immunization with Aβ may prove beneficial for both the treatment and the prevention of AD. However, there is no evidence that such an approach is effective in patients.

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The second approach is to slow the decline of neuronal degeneration and to treat the symptoms of the disease by repletion of several deficient neurotransmitters. While, monoaminergic and neuropeptidergic enhancers have shown very limited use in AD [4], and aminoacidergic regulators (GABAergic regulators) have shown some interest as neuroprotective agents [4], until now, most therapeutic strategies have been based on the so-called cholinergic hypothesis of cognitive dysfunction [12]. This hypothesis postulates that at least some of the cognitive decline experienced by patients of AD results from a deficiency in neurotransmitter acetylcholine (ACh), and thus in cholinergic neurotransmission, which seems to play a fundamental role in memory. On the one hand, cholinergic neurons are markedly damaged in AD patient brains, together with a defect in choline acetyltransferase and acetylcholinesterase (AChE) [13,14]. On the other hand, the muscarinic antagonist scopolamine induces a marked deterioration in short-term memory, similar to that observed in the first stage of AD, which can be reversed by administration of the centrally active AChE inhibitor physostigmine [3]. Although, cholinergic neurotransmission can be enhanced by drugs acting at the pre-synaptic level (choline precursors, ACh releasers, presynaptic muscarinic autoreceptor antagonists) [15] and at the post-synaptic level (muscarinic and nicotinic agonists) [15], the only drugs currently approved for the treatment of the cognitive deficit in AD act at the synaptic level by inhibiting AChE [15,16]. The enzyme AChE plays a key role in the hydrolysis of the neurotransmitter ACh. Therefore, its inhibition leads to an increase in the bioavailability of ACh at the synaptic cleft, thus improving cholinergic neurotransmission. On the other hand, it has been recently shown that AChE tends to be deposited within amyloid plaques associated with Aβ, resulting in the formation of stable complexes that cause an increase in the neurotoxicity of Aβ [17], suggesting that AChE could play a pathogenic role in AD, thus increasing the interest for the development of potent AChE inhibitors for the treatment of AD.

The prototype of centrally active AChE inhibitor was tacrine (Cognex®, **1**) [12,18], which was the first drug approved by the FDA for the treatment of AD in 1993 [Fig. (**1**)]. However, severe side effects, such as hepatotoxicity and gastrointestinal upset, represent an important drawback [19]. The positive effects observed in tacrine spurred the development of other drugs with the pharmacological profile of AChE inhibitors, such as the recently marketed donepezil (Aricept®, **3**) [15,20], galanthamine (Remenyl®, **4**) [21], rivastigmine (Exelon®, **5**) [22], and metrifonate (Nivalin®, **6**) [23,24] [Fig. (**1**)]. (−)-Huperzine A (**2**), an alkaloid isolated from the Chinese medicinal herb *Huperzia serrata*,

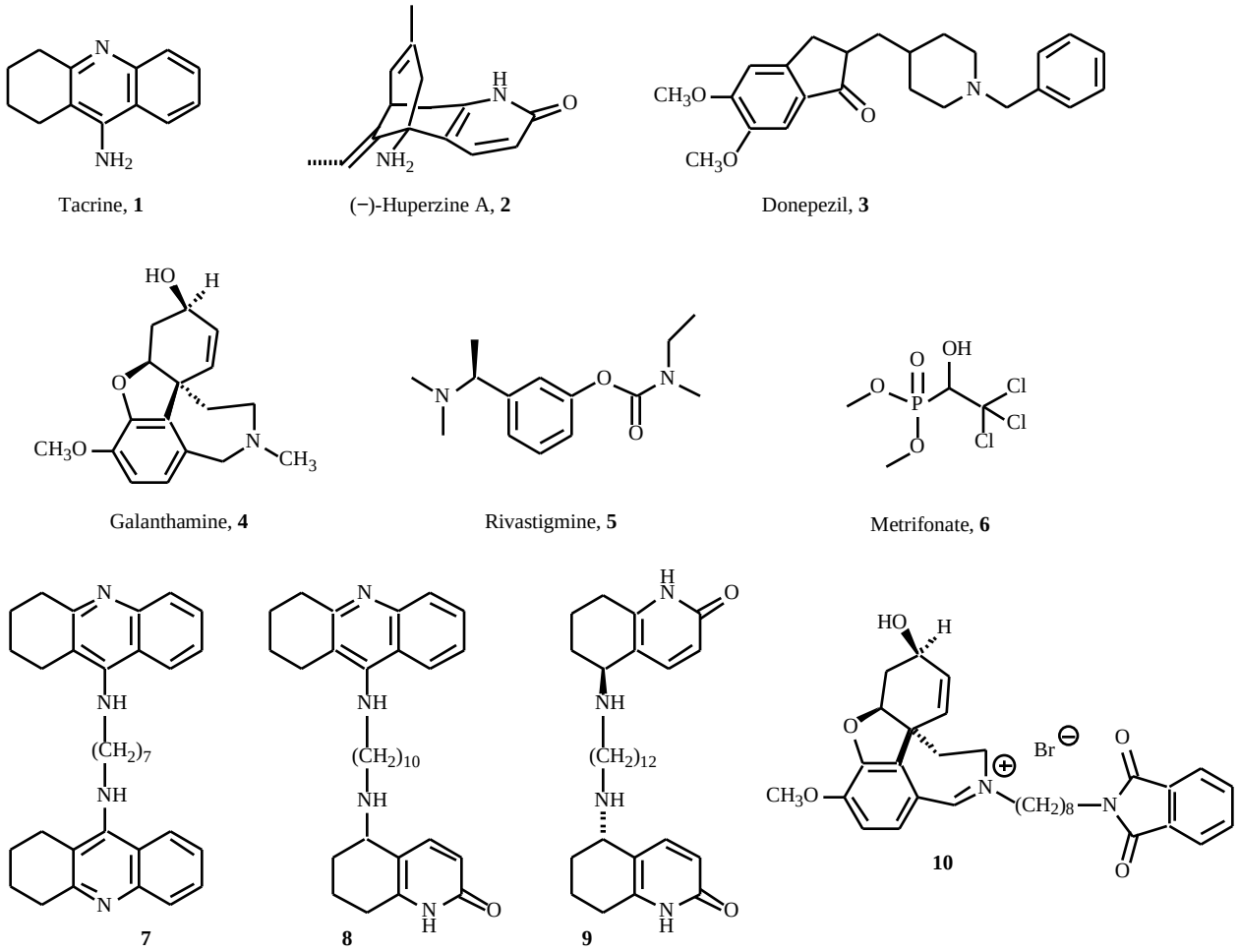
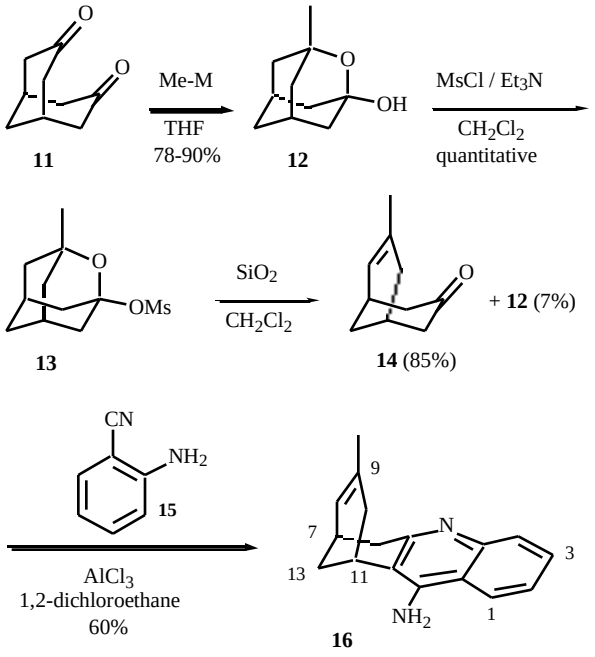


Fig. (1). Structures of commercial (**1**−**6**) and other very potent (**7**−**10**) AChE inhibitors.

is a very potent, selective and long-acting AChE inhibitor, which enhances cognitive function in animals and humans and exhibits a neuroprotective action on cortical neurons, and seems to be superior to other AChE inhibitors taking into account these combined actions [25-27] [Fig. (**1**)]. (−)-Huperzine A has been recently marketed in USA as a dietary supplement.

One of the strategies used in medicinal chemistry for the discovery of new lead compounds is based on the modification and improvement of known active compounds. In this sense, important efforts have been made to develop analogues of the above centrally acting AChE inhibitors with increased potency, selectivity and safety, by applying all the different possibilities for molecular variation of the lead structures. Several analogues designed by simplification of the lead structures (disjunctive approach) have been prepared [28,29] but the most active analogues have been obtained by keeping the same level of complexity (analogical approach) or by enlargement through additional structural elements (conjunctive approach). The analogical approach has led to the development of some analogues which are currently in phase III clinical trials in Japan, such as the tacrine-derivative amiridine (NIK-247) [30,31], the physostigmine-derivative eptastigmine (heptylphysostigmine, MF-201) [32] and the donepezil-derivative TAK-147 [5,33]. More recently, much effort has been invested in the development of new AChE inhibitors designed through a conjunctive approach, either by duplication of a parent drug (molecular duplication) or by association in the same molecule of structural fragments of different lead compounds (associative synthesis).

Ten years ago, we became interested in the synthesis of new AChE inhibitors. At the beginning of this work, the X-ray crystal structures of the complexes of tacrine (**1**) and (−)-huperzine A (**2**) with AChE were not yet known, and consequently the exact mode of interaction of both inhibitors



Scheme 1. Synthetic procedure for the first tacrine-huperzine A hybrid (huprine), **16**.

with the active site of the enzyme was also unknown. Taking into account the structures of both lead compounds, we initially assumed that their heterocyclic rings could be placed in a similar way in the active site of AChE, as well as the partially saturated ring of tacrine and the carbobicyclic subunit of (−)-huperzine A. Thus, we planned the synthesis of new compounds, such as **16** (Scheme 1), designed through a conjunctive approach by using these two important lead compounds (associative synthesis). These tacrine−huperzine A hybrids (huprines) combined the 4-aminoquinoline substructure of tacrine with the carbobicyclic substructure of (−)-huperzine A, with the idea of increasing their binding to the active site of AChE. The synthesis and the structure−activity relationship exploration of this new structural family are herein described.

While this work was being carried out, other AChE inhibitors were developed also through conjunctive approaches, but involving a different design. Bivalency is an effective strategy for improving drug potency and selectivity, when multiple recognition sites for the same substrate exist. In this sense, important efforts have been made to develop new AChE inhibitors of increased affinity, potency and selectivity, able to bind simultaneously to the catalytic and peripheral sites of AChE. The general structure of these bis-interacting ligands normally includes two components from the same lead compound (molecular duplication) or one component from a lead compound and another one from a second lead compound (associative synthesis), being both components linked by a spacer group (normally an oligomethylene chain) with a suitable length to locate both substructures at the most appropriate distance for interaction with both binding sites. Thus, a homodimer containing two substructures of tacrine connected by an heptamethylene chain, **7**, turned out to be 149-fold more potent as AChE inhibitor than tacrine hydrochloride [Fig. (**1**)] [34-36]. Also, some hybrid compounds such as **8**, composed of a substructure of tacrine and a key fragment of huperzine A, connected by an oligomethylene chain (optimum decamethylene), were up to 13-fold more potent than (−)-huperzine A, and 25-fold more potent than tacrine salt [Fig. (**1**)] [37]. Also, several compounds such as **9**, designed by dimerization of the same fragment of huperzine A, proved to be more than 2-fold more potent than (−)-huperzine A, and 4-fold more potent than tacrine salt [Fig. (**1**)] [38]. Some galanthamine-based heterodimers, such as **10**, were also up to 5-fold more potent than tacrine salt and up to 36-fold more potent than galanthamine [Fig. (**1**)] [39].

SYNTHESIS OF TACRINE−HUPERZINE A HYBRIDS (HUPRINES) AND PRIMARY STRUCTURE−ACTIVITY RELATIONSHIP EXPLORATION

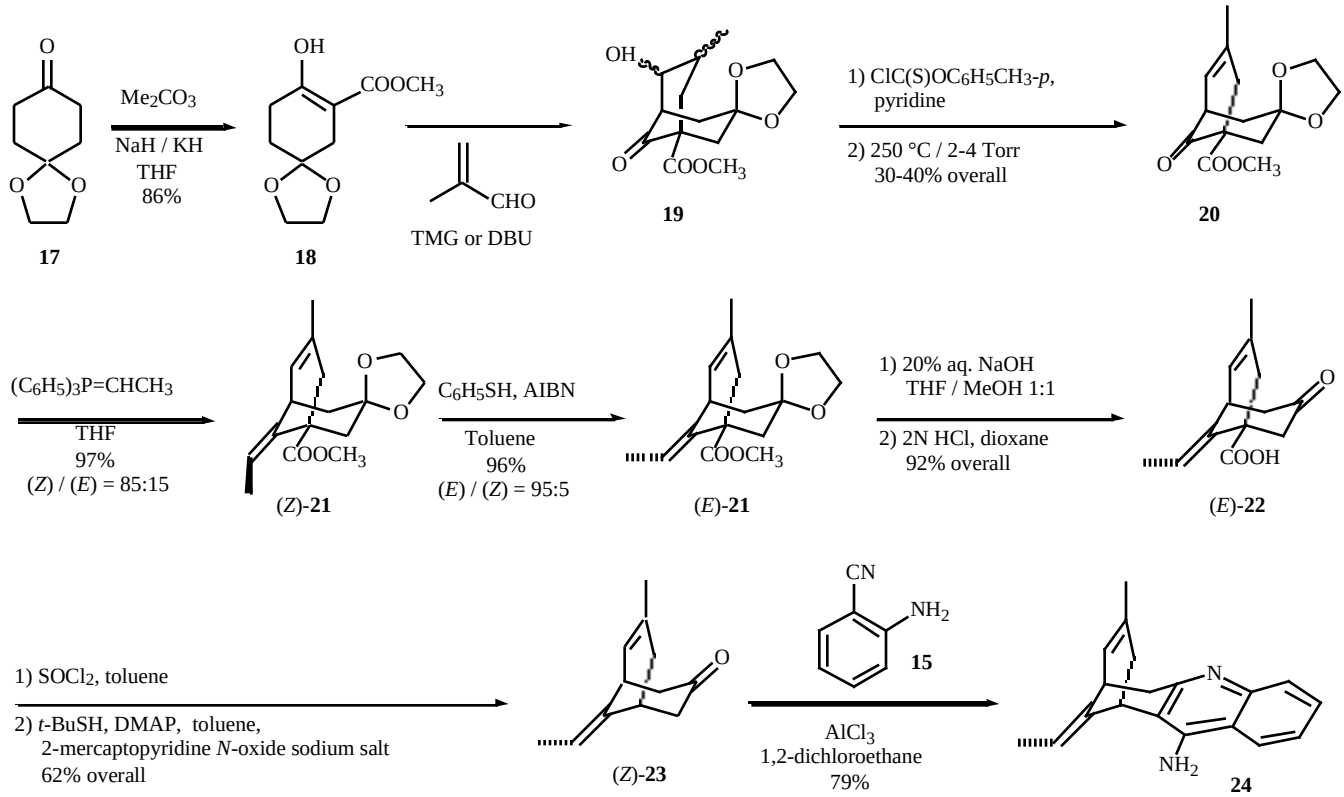
The synthesis of these compounds was envisaged by Friedländer condensation of a suitable polycyclic ketone with 2-aminobenzonitrile, **15**. The first huprine to be synthesized was compound **16**, lacking the ethylidene appendage characteristic of huperzine A (Scheme 1). Several three- or four-step syntheses of enone **14** were known [40,41], starting from adamantane, but the complexity of the involved methods prompted us to develop a simpler and more general

procedure to prepare 7-alkylbicyclo[3.3.1]non-6-en-3-ones, which is shown in Scheme 1 for the synthesis of enone **14**. Nucleophilic addition of a suitable organometallic reagent to the known bicyclo[3.3.1]nonane-3,7-dione, **11**, gave 3-methyl-2-oxa-1-adamantanol, whose corresponding mesylate was submitted to silica-gel promoted fragmentation to afford the desired enone **14** in 76% overall yield [42]. The highest yield of oxaadamantanol **12** was obtained when an organocerium reagent was used [43]. Friedländer condensation of **14** with 2-aminobenzonitrile, **15**, in the presence of $AlCl_3$ in refluxing 1,2-dichloroethane led efficiently to the expected huprine **16** [44], whose AChE inhibitory activity was assayed by the method of Ellman et al. [45] on AChE from bovine erithrocytes. This compound (IC_{50} of 65.0 nM) turned out to be 2-fold more potent than tacrine hydrochloride and slightly more potent than (−)-huperzine A as AChE inhibitor [44], constituting therefore a new AChE lead inhibitor, adequate for further molecular variations to carry out a primary structure-activity relationship exploration.

Thus, a first generation of huprines was designed by modification of three parts of the lead structure **16**: the methylene bridge between positions 7 and 11, the three-carbon unsaturated bridge between positions 7 and 11 and the benzene ring of the 4-aminoquinoline substructure.

STRUCTURAL VARIATIONS AT THE METHYLENE BRIDGE OF HUPRINES

Several types of huprines modified at the methylene bridge between positions 7 and 11, but keeping intact the three-carbon unsaturated bridge and the 4-aminoquinoline substructure, were designed and prepared [44]. The first one was the huprine bearing at position 13 the ethylidene appendage characteristic of huperzine A, **24**. For the synthesis of compound **24**, the intermediate keto ester **20** was required (Scheme 2). This compound was first described by Kozikowski et al. in connection with the synthesis of huperzine A analogues [46]. Later, we published an improved synthesis of this compound, differing from that of Kozikowski in the dehydration of the diastereomeric mixture of alcohols **19** to the olefin **20**, which was best performed by pyrolytic *syn*-elimination of the diastereomeric mixture of *O*-(*p*-tolyl)thiocarbonates derived from **19**, taking into account the *trans*-arrangement of the hydroxy and methyl substituents in the two main diastereomers of **19** (X-ray diffraction analysis) [47]. Compound **20** was then transformed into keto acid (*E*)-**22** following Kozikowski's methodology [46], that involves: i) Wittig reaction of **20** with triphenylethylidenephosphorane to give a mixture of alkenes containing mainly (*Z*)-**21**, ii) thiophenol-induced isomerization to (*E*)-**21**, iii) saponification of the ester group, and iv) hydrolysis of the acetal function. Barton's decarboxylation [48-52] of keto acid (*E*)-**22** afforded in good yield the enone (*Z*)-**23**, whose Friedländer condensation with 2-aminobenzonitrile, **15**, under standard conditions led to the expected huprine **24** [44]. Analogously, direct ester saponification and acetal hydrolysis of (*Z*)-**21**, followed by Barton's decarboxylation of the resulting keto acid, led to the (*Z*)-stereoisomer **25** [Fig. (**2**)] [44]. The configuration of the ethylidene group that was directed by the neighboring ester group was very important for the AChE inhibitory activity. Thus, the (*E*)-stereoisomer **24**, bearing the ethylidene group with the same configuration of huperzine A, was 3.6-fold



Scheme 2. Synthetic procedure for huprine **24**.

more potent (IC_{50} of 0.32 μM) than the (*Z*)-stereoisomer **25** (IC_{50} of 1.15 μM) [44]. However, compound **24** showed an AChE inhibitory activity almost 5-fold lower than that of the lead compound **16**. This was a surprising result because the ethylidene appendage had proved to be an essential structural feature for the AChE inhibitory activity of huperzine A analogues [25], although at that moment it had not been prepared any huperzine A analogue unsubstituted at this methylene bridge.

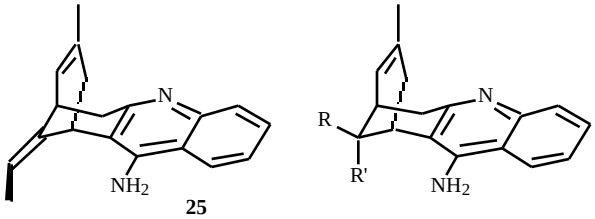
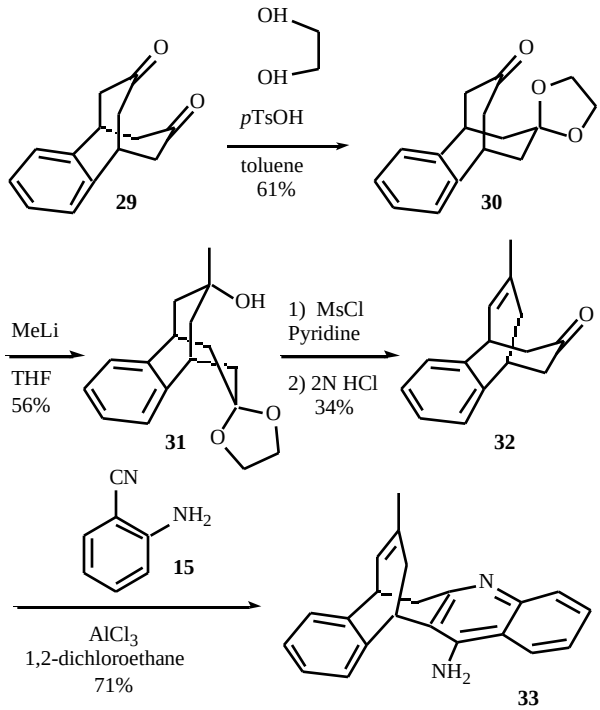


Fig. (2). Structures of huprines **25**–**28**.

Also, huprines **26**–**28** [Fig. (**2**)], formally derived from **16** by introduction of a methyl and a methoxy group or an oxo function at C13, were prepared through the same methodology developed for the synthesis of the lead compound **16** [44], starting from 9-methoxy-9-methylbicyclo[3.3.1]nonane-3,7-dione or from 9,9-dimethoxybicyclo[3.3.1]nonane-3,7-dione, readily available by double Michael condensation of the corresponding 4,4-disubstituted cyclohexa-2,5-dienones with dimethyl acetonedicarboxylate, followed by hydrolysis and decarboxylation of the resulting bicyclic diesters [42]. The AChE inhibitory activity of compounds **26**–**28** was significantly lower than that of **16** (21, 104, and 32-fold lower, respectively) [44].



Scheme 3. Synthetic procedure for huprine **33**.

Another huprine, designed by modification of the methylene bridge of **16**, was compound **33**. In this case, the structural variation consisted of the substitution of the C13-methylene by an *o*-phenylene bridge. The synthesis of the required enone **32** (Scheme 3) was first attempted through the general synthetic sequence developed for **16** starting from the known diketone **29**, but several attempts of fragmentation of the corresponding oxa-polycyclic mesylate were fruitless. These results prompted us to develop an alternative synthetic procedure, which involved monoacetalization of diketone **29**, followed by nucleophilic addition of methyllithium, mesylation of the resulting alcohol, elimination of the mesylate and hydrolysis of the acetal function, to afford the enone **32** [53]. Friedländer condensation of **32** with **15** led to the huprine **33**, which exhibited an AChE inhibitory activity 32-fold lower than that of the lead compound **16** [44].

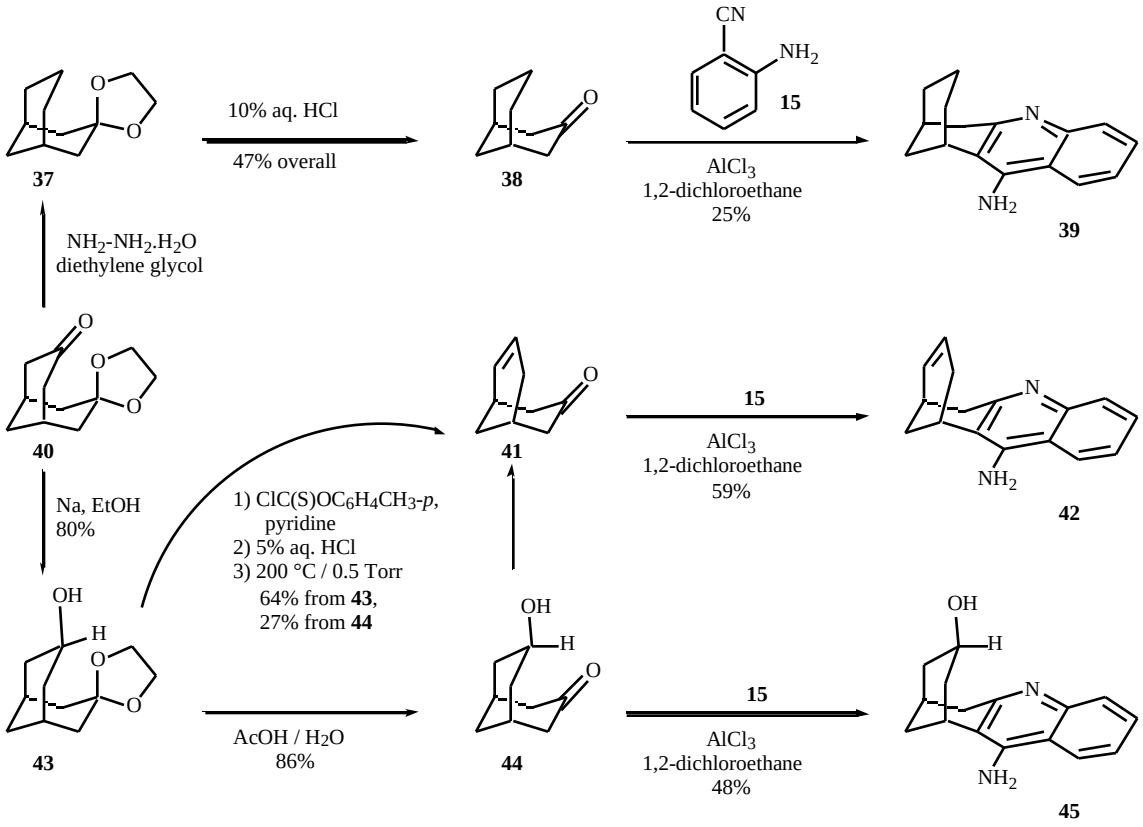
From the pharmacological data of these huprines modified at the methylene bridge, it became apparent that for an optimal activity this methylene bridge should be kept intact.

STRUCTURAL VARIATIONS AT THE THREE-CARBON UNSATURATED BRIDGE OF HUPRINES

Two compounds designed by substitution of the methyl group at position 9 of the lead compound **16** by another alkyl group or a hydrogen atom were prepared. The 9-ethyl-substituted analogue **34** [Fig. (**3**)] was easily synthesized in a similar way to that described for compound **16** [44]. The synthesis of the 9-unsubstituted analogue **42** (Scheme 4) was first attempted through the same methodology. However, fragmentation of the corresponding mesylate failed and enone **41** could not be obtained by this procedure. Alternatively, reduction of the known monoacetal **40** with sodium in ethanol gave the thermodynamically more stable *exo*-alcohol **43**, which was dehydrated by pyrolytic *syn*-elimination of the corresponding *O*-(*p*-tolyl)thiocarbonate, and, after acetal hydrolysis, enone **41** was obtained. Condensation of this enone with **15** gave the analogue **42** (Scheme 4) [44].

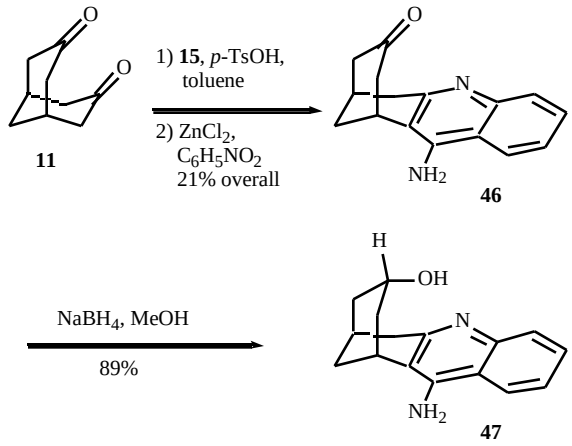
The 9-ethyl-substituted huprine **34** turned out to be 1.7-fold more active as AChE inhibitor than **16** (IC_{50} of 38.5 nM), while huprine **42** bearing a hydrogen atom at this position was 75-fold less active than **16** (IC_{50} of 4.89 μM), thus suggesting the convenience of a lipophilic group at position 9 for high AChE inhibitory activity [44].

Other huprines containing a saturated three-carbon bridge were prepared. Huprine **39** was prepared from the known ketone **38**, readily available by Wolff-Kishner reduction of monoacetal **40**, followed by hydrolysis of the acetal group (Scheme 4) [44]. Moreover, two saturated analogues bearing a hydroxyl group at position 9 were prepared. The *exo*-9-hydroxy-huprine **45** was prepared by reaction of the *exo*-hydroxy ketone **44** with **15** under standard conditions (Scheme 4) [44]. The *endo*-9-hydroxy-huprine **47** was prepared by initial formation of the monoimine derived from diketone **11** and 2-aminobenzonitrile, **15**, followed by



Scheme 4. Synthetic procedure for huprines **39**, **42** and **45**.

$ZnCl_2$-promoted cyclization, and $NaBH_4$ reduction of the intermediate ketone **46** (Scheme 5) [44]. The saturation of the three-carbon unsaturated bridge and the removal of the methyl group at position 9 led to a dramatic decrease of the AChE inhibitory activity, compound **39** being 643-fold less active than the lead compound **16**. The introduction of a hydroxyl group at position 9 of **39** improved the AChE inhibitory activity, although **45** and **47** were 25 and 66-fold less active than **16** [44].



Scheme 5. Synthetic procedure for huprine **47**.

Analogously, huprines **36** and **35** were prepared from diketone **29** or the corresponding monoketone, respectively, in a similar way to that described for compounds **47** and **39** [Fig. (**3**)]. Huprines **35** and **36** showed to be 40 and 234-fold less active than the lead compound **16** [44]. In view of these results, the alkyl-substituted three-carbon unsaturated bridge seemed to be an essential structural feature for a good AChE inhibitory activity in huprines.

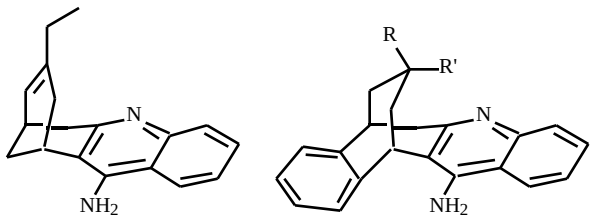


Fig. (3). Structures of huprines **34**−**36**.

STRUCTURAL VARIATIONS AT THE BENZENE RING OF THE 4-AMINOQUINOLINE SUBSTRUCTURE OF HUPRINES

A new huprine, **48**, designed by modification of the benzene ring of the 4-aminoquinoline moiety of the lead compound **16**, was also prepared. Compound **48**, in which the C1-C4 fragment of compound **16** had been replaced by a trimethylene bridge [Fig. (**4**)], was synthesized as described for **16**, but carrying out the Friedländer condensation with 2-aminocyclopent-1-enecarbonitrile. This modification led to a

clear decrease in the AChE inhibitory activity (IC_{50} of 5.58 μM), compound **48** being 86-fold less active than **16** [44].

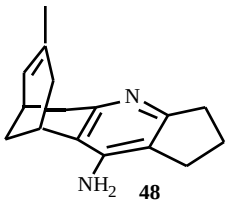


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Fig. (4). Structure of huprine **48**.

SYNTHESIS OF THE SECOND GENERATION OF HUPRINES

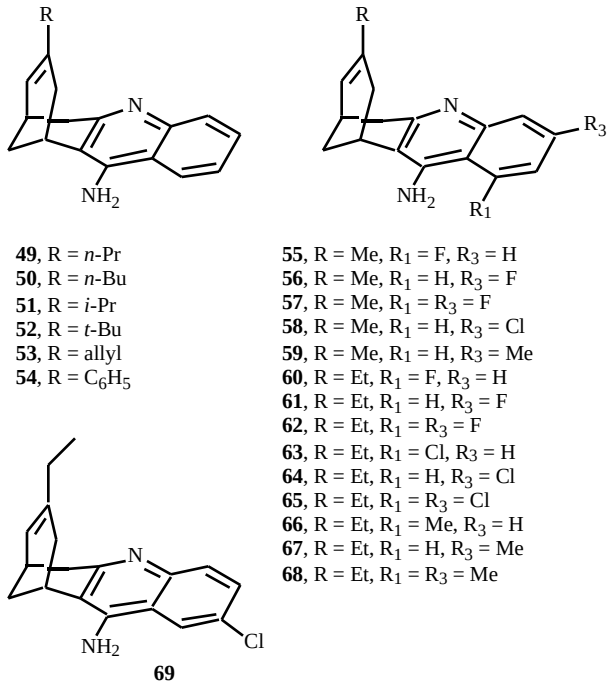
The results obtained with the primary structure–activity relationship exploration revealed that both the methylene bridge and the three-carbon unsaturated bridge of the lead compound **16** were essential structural features for a good AChE inhibitory activity. The sole structural variation of the lead structure that led to an improved activity was the substitution of the methyl group at position 9 by the more lipophilic ethyl group. Taking into account these results and some structure–activity relationship data reported for tacrine analogues, suggesting that the introduction of halogen atoms or methyl groups at positions 6 or 8 of these derivatives (corresponding to positions 3 or 1 in huprines) led to higher AChE inhibitory activities [54,55], the synthesis of a second generation of huprines was planned to develop new AChE inhibitors with increased potency and to complete the structure–activity relationship studies in this structural family. These new huprines were designed by substitution of the methyl group at position 9 of the lead compound **16** by more lipophilic lineal or branched alkyl groups, allyl or phenyl groups, and by introduction of halogen atoms or methyl groups on the benzene ring of the 4-aminoquinoline substructure.

STRUCTURAL VARIATIONS AT POSITION 9 OF HUPRINES

Six new huprines, **49**–**54**, bearing at position 9 a *n*-propyl, *n*-butyl, isopropyl, *tert*-butyl, allyl and phenyl group, respectively [Fig. (**5**)], were prepared by using the same methodology developed for the synthesis of compound **16**, using the required organometallic reagent in the first step of the synthetic sequence [43]. Huprines **49**–**54** showed to be 6.6, 4.3, 1.6, 4.1, 2.3, and 1.9-fold less active than the lead compound **16**, respectively [43]. Although these new compounds exhibited an interesting AChE inhibitory activity, the optimal activity in huprines involved the presence of a methyl or ethyl group at position 9.

STRUCTURAL VARIATIONS AT POSITIONS 1, 2 AND / OR 3 OF HUPRINES

In view of the above results, fifteen new huprines, **55**–**69**, substituted with a methyl or ethyl group at position 9 and with halogen atoms (fluorine, chlorine) or methyl groups at



49, R = *n*-Pr
50, R = *n*-Bu
51, R = *i*-Pr
52, R = *t*-Bu
53, R = allyl
54, R = C_6H_5

55, R = Me, R_1 = F, R_3 = H
56, R = Me, R_1 = H, R_3 = F
57, R = Me, R_1 = R_3 = F
58, R = Me, R_1 = H, R_3 = Cl
59, R = Me, R_1 = H, R_3 = Me
60, R = Et, R_1 = F, R_3 = H
61, R = Et, R_1 = H, R_3 = F
62, R = Et, R_1 = R_3 = F
63, R = Et, R_1 = Cl, R_3 = H
64, R = Et, R_1 = H, R_3 = Cl
65, R = Et, R_1 = R_3 = Cl
66, R = Et, R_1 = Me, R_3 = H
67, R = Et, R_1 = H, R_3 = Me
68, R = Et, R_1 = R_3 = Me

69

Fig. (5). Structures of huprines **49**–**69**.

positions 1, 2 and / or 3, were prepared by Friedländer condensation of the enone **14** or the corresponding 7-ethyl analogue with a conveniently substituted aminobenzonitrile [Fig. (**5**)]. All of the compounds substituted at positions 1 and / or 3 with fluorine, chlorine or methyl groups were much more active in inhibiting bovine AChE than tacrine hydrochloride (2.8–53-fold more active), than (–)-huperzine A (1.6–30-fold more active) and than the lead compound **16** (1.4–27-fold more active) [43,56,57]. As expected, the introduction of a substituent at position 2 led to a decreased inhibitory activity. Thus, compound **69**, substituted at position 2 with a chlorine atom, was 4-fold less active than the lead compound **16** [57].

Among the compounds substituted at positions 1 and / or 3 with fluorine, chlorine or methyl groups, there are no significant differences in the AChE inhibitory activities of the 9-methyl- and 9-ethyl-derivatives, being the last ones slightly more potent (1–1.5-fold more active).

Among the huprines monosubstituted at position 1 or 3, the optimal activity was exhibited by those substituted at position 3 (2.5–6.3-fold more active than the corresponding huprines substituted at position 1).

The introduction of two fluorine atoms or methyl groups at positions 1 and 3 led to an essentially additive effect. Thus, the 1,3-difluoro huprines **57** (IC_{50} of 2.43 nM) and **62** (IC_{50} of 2.62 nM) were 13–18-fold more active than the corresponding huprines monosubstituted at position 1 and 2.8-3.5-fold more active than the corresponding compounds monosubstituted at position 3, while the dimethyl derivative **68** (IC_{50} of 3.59 nM) was 8.3 and 3.3-fold more active than the corresponding 1- or 3-methyl derivatives, respectively. In striking contrast with these results, the dichloro derivative **65** (IC_{50} of 39.6 nM) was clearly less active than both

monochloro derivatives **63** (IC_{50} of 16.2 nM) and **64** (IC_{50} of 2.77 nM).

Moreover, for a given location of the substituent on the benzene ring of the 4-aminoquinoline substructure, the optimal activity was displayed by the chloro derivatives (2.0−2.9-fold more active than the corresponding fluoro derivatives and 1.8−4.3-fold more active than the corresponding methyl derivatives).

In view of the high AChE inhibitory activity of these new huprines, their human AChE inhibitory activity was also tested. In general, the inhibitory activity toward human AChE was higher than that toward bovine AChE (1.1−2.0-fold more active toward human AChE than toward bovine AChE) [43,57]. This increase in inhibitory activity was much more important in the case of the 3-chloro-substituted huprines **58** (IC_{50} of 4.23 nM on bovine AChE, and 0.778 nM on human AChE) and **64** (IC_{50} of 2.77 nM on bovine AChE, and 0.750 nM on human AChE), which were 5.4 and 3.7-fold more active toward the human enzyme, respectively. Huprines **58** and **64** showed to be 171 and 177-fold more potent than tacrine hydrochloride as human AChE inhibitors.

Butyrylcholinesterase (BChE) is another cholinesterase which has a molecular structure closely related to that of AChE (53% identity of their amino acid sequences). BChE also hydrolyzes ACh, but unlike AChE it has a much broader substrate specificity and broader distribution. Thus, inhibition of BChE, which is abundant in human plasma, may be associated with increasing peripheral cholinergic effects. These new huprines displayed a high selectivity in inhibiting with more potency human AChE than human BChE (3.7−871-fold more active toward human AChE than toward human BChE). The most potent human AChE inhibitors, the 3-chloro-substituted huprines **58** and **64**, were quite selective, inhibiting human AChE with potency 303 and 21-fold higher than that for the inhibition of human BChE, respectively. Other very selective huprines, such as the 1,3-dihalo-derivatives **57**, **62**, and **65**, were 438, 110, and 871-fold more potent toward human AChE than toward human BChE, respectively.

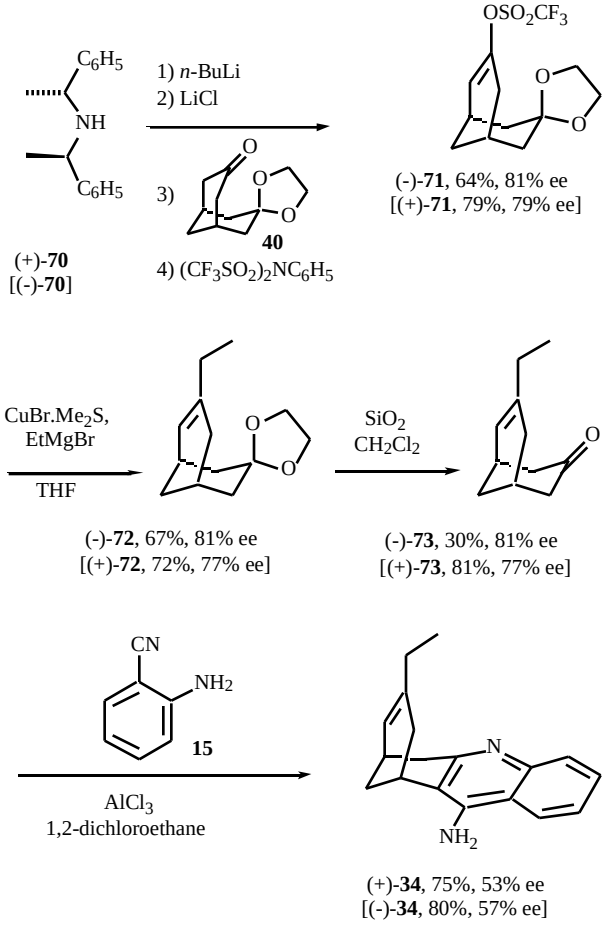
From the above structure−activity relationship exploration, it can be concluded that the effects on AChE inhibitory activity of structural variations carried out on the carbobicyclic substructure of huprines do not completely parallel those of structural variations carried out on the corresponding moiety of (−)-huperzine A. Thus, the substitution of the 9-methyl group of compound **16** or the 7-methyl group of (−)-huperzine A by larger groups leads to a drop in AChE inhibitory activity [25,43], except for the substitution of the methyl for an ethyl group in the case of huprines, which leads to an increased activity [44]. Recently, it has been carried out an attempt to rationalize the structural requirements of AChE inhibitors from literature data of molecular modeling and quantitative structure-activity relationship analyses corresponding to several chemical classes of reversible AChE inhibitors. It was concluded that the pre-requisites for the inhibitors to interact with AChE are hydrophobicity and the presence of an ionizable nitrogen [58]. Although hydrophobicity increases with the introduction of large substituents at this position, the decrease in the AChE inhibitory activity of compounds bearing substituents larger than ethyl group, may be due to steric clash between the inhibitor and the enzyme. Also, the three-carbon unsaturated bridge seems to be necessary for activity in both huprines and (−)-huperzine A. The low activity of huprines bearing a saturated bridge could be explained on the basis of differences in electrostatic potential about the three-carbon bridge, as it has been suggested for huperzine A analogues with similar structural variations [59]. The major differences between the effects on AChE inhitory activity of structural variations on the carbobicyclic substructure of huprines and (−)-huperzine A were observed for the effect of the ethylidene group at the methylene bridge, which seemed to be an essential structural feature for AChE inhibitory activity of (−)-huperzine A [25], while in the case of huprines the optimal activity was exhibited by compounds unsubstituted at the methylene bridge [44]. In order to ascertain the significance of the ethylidene appendage, as well as that of the ethyl group of the three-carbon unsaturated bridge, we carried out the synthesis of an 11-unsubstituted- and a 7-ethyl-substituted huperzine A analogues [60,61]. These compounds exhibited an AChE inhibitory activity 122 and 12-fold lower than that of (−)-huperzine A, thus suggesting that huprines and (−)-huperzine A do not have the same binding mode with the enzyme.

On the contrary, the effects on AChE inhibitory activity of structural variations carried out on the 4-aminoquinoline substructure of huprines and the corresponding moiety of tacrine completely parallel. Thus, it has been reported that introduction of substituents at position 6 of tacrine (equivalent to position 3 of huprines) leads to an increased AChE inhibitory activity [54,55], being this activity optimal for 6-chlorotacrine [62]. Analogously, the optimal activity in huprines has been exhibited by the 3-chlorosubstituted huprines **58** and **64**. Although it is not known whether this chlorine substituent leads to favorable hydrophobic or electronic interactions with AChE [58], it has been recently hypothesized that the high activity exhibited by the 6-substituted tacrine analogues may be due to hydrophobic interaction between some enzyme residues and substituents at position 6 [62].

SYNTHESIS OF ENANTIOPURE HUPRINES

The chiral nature of the huprines and their superior inhibitory potency profile prompted us to develop a procedure for the obtention of both enantiomers of some of these compounds in order to determine possible differences in their AChE inhibitory activity. Initial attempts to access to the lead compound **16** in an enantiopure form, through synthetic sequences based on the enantioselective fragmentation of mesylate **13** in the presence of cinchonidine or on kinetic resolution of racemic enone **14** by enantioselective Sharpless dihydroxylation or by enantioselective Jacobsen epoxidation, were fruitless [63]. Also, the attempted resolution of a racemic mixture of compound **16** by recrystallization of the diastereomeric salts resulting from the treatment of this mixture with several enantiopure acids was fruitless. Finally, we developed an

asymmetric synthesis of the 9-ethyl huprine **34** (Scheme 6). Enantioselective deprotonation of monoacetal **40** with the lithium amide derived from enantiopure (+)-bis[(*R*)-1-phenylethyl]amine, (+)-**70**, followed by trapping of the enolate under non-racemizing conditions with *N*-phenyltriflimide, gave the enantioenriched enol triflate (−)-**71**. Reaction of enantioenriched (−)-**71** with an ethylcuprate reagent, followed by silica gel-promoted acetal hydrolysis and Friedländer condensation of the resulting enantioenriched enone (−)-**73** with 2-aminobenzonitrile, **15**, under standard conditions led to enantioenriched huprine (+)-**34** [63]. Similarly, starting from **40** and enantiopure (−)-bis[(*S*)-1-phenylethyl]amine, enantioenriched (−)-**34** was obtained [63]. The partial racemization observed in the last step of this sequence was initially ascribed to partial racemization of the starting enone by isomerization of the endocyclic carbon−carbon double bond. Another mechanistic explanation of this fact is currently being studied. It is worth noting that after recrystallization of the corresponding hydrochlorides of the final products, (+)-**34** and (−)-**34** were obtained with high ee's (≥ 99%).



Scheme 6. Enantioselective synthesis of huprines (+)- and (−)-**34**.

Alternatively, we developed an easier and more general procedure to obtain huprines in enantiopure form, based on the chromatographic resolution of their racemic mixtures by medium pressure liquid chromatography (MPLC), using microcrystalline cellulose triacetate as the chiral stationary phase. This procedure has been successfully applied to the separation of racemic huprines **16** and **34** [63], the 3-fluoro derivatives **56** and **61** [43,57], the 3-chloro compounds **58** and **64** [56,63], and the 3-methyl huprine **67** [43]. In all cases, the levorotatory enantiomer was the more active one (eutomer), exhibiting a bovine AChE inhibitory activity 7−424 fold higher than that of the dextrorotatory enantiomer and 1.1−3.7-fold higher than that of the racemic compound. It is worth noting the very high bovine AChE inhibitory activity of the levorotatory enantiomers of the 3-chlorosubstituted huprines **58** (IC_{50} of 1.15 nM) and **64** (IC_{50} of 1.30 nM), and their still higher inhibitory activity toward human AChE (IC_{50} of 0.318 nM and 0.323 nM, respectively), being thus these compounds 418 and 412-fold more active than tacrine hydrochloride. These results together with the high selectivity of compounds (−)-**58** and (−)-**64** in inhibiting human AChE *vs* human BChE (777 and 492-fold, respectively, more potent toward human AChE than toward human BChE) attracted our attention to both compounds as the most promising huprines for a potential symptomatic treatment of AD. Additional pharmacological and biochemical studies on the so-called huprine Y and huprine X, compounds (−)-**58** and (−)-**64**, respectively, have shown that both compounds act as *tight-binding* reversible AChE inhibitors, able to cross the blood−brain barrier [57], and to bind to the human AChE with an inhibition constant (K_I) around 30 pM, indicating that they bind to the enzyme with one of the highest affinities yet reported [56]. The affinity of these compounds for human AChE is around 1200-fold higher than that of tacrine salt, 180-fold higher than that of (−)-huperzine A and 40-fold higher than that of donepezil, the most selective AChE inhibitor currently approved for therapeutic use.

X-Ray diffraction analysis from a monocrystal of compound (-)-**58** as a salt with *o*-iodobenzoic acid revealed its absolute configuration [63]. Taking into account the same sign and the close values for the specific rotations displayed by the other levorotatory huprines, together with their inhibition activity profile, the same absolute configuration was assigned to all the levorotatory huprines, and vice versa for their enantiomers. The absolute configuration of the levorotatory enantiomer of huprine **16** is shown in Figure 6 together with that of (−)-huperzine A.

Taking advantage of these crystallographic data and those reported for the crystallographic structures of the complexes of AChE of *Torpedo californica* with tacrine [64] and (−)-huperzine A [65], a molecular modeling study of the interaction of these huprines with the enzyme was carried out [43,57,66]. The results of this study provided a basis to suggest that these compounds act as truly tacrine−huperzine A hybrids, but in a different manner from that we had initially assumed. The 4-aminoquinoline substructure of the (−)-huprines occupies the same position of the corresponding substructure in tacrine, thus sharing all of the features that modulate the binding of tacrine to AChE [Fig. (**6**)]. On the other hand, the three-carbon unsaturated bridge of the huprines occupies roughly the same position of the corresponding moiety in (−)-huperzine A, while the methylene bridges of the (−)-huprines and (−)-huperzine A are positioned in opposite directions, as well as their heterocyclic rings [Fig. (**6**)].

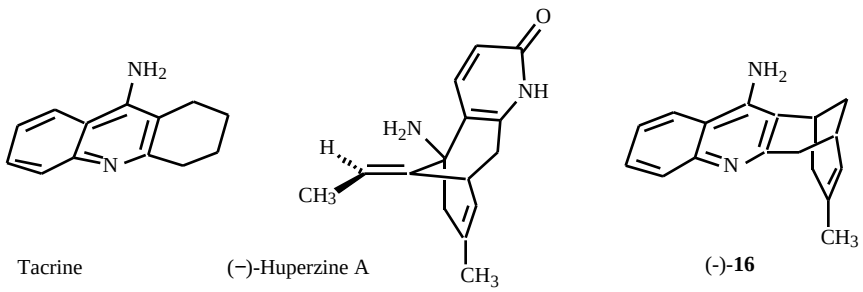


Fig. (6). Approximate relative orientation of tacrine, (−)-huperzine A and huprine (−)-**16** in the active site of *Torpedo californica* AChE (*Tc*AChE). The complex huprine (−)-**16**-*Tc*AChE was modeled starting from the published X-ray data for the complexes of tacrine-*Tc*AChE and (−)-huperzine A-*Tc*AChE.

FUTURE PROSPECTS

Structural variations of the lead compound of the structural family of huprines have led to several derivatives with very increased AChE potency and AChE *vs* BChE selectivity, some of them displaying affinities for AChE which are among the highest yet reported. In spite of the fact that the definite validation of the binding model proposed from the molecular modeling studies has to await a 3D X-ray structure of a complex AChE−(−)-huprine, it represents a very valuable tool to enable rational design of new huprines of still improved inhibition activity profile. The synthesis of new rationally designed huprines is currently being studied.

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LIST OF ABBREVIATIONS

Aβ = β-amyloid peptide

AβPP = β-amyloid precursor proteine

ACh = Acetylcholine

AChE = Acetylcholinesterase

AD = Alzheimer's Disease

BChE = Butyrylcholinesterase

FDA = Food and Drug Administration

GABA = γ-aminobutyric acid

IC_{50} = Concentration that inhibits 50% of the AChE or BChE activity

MPLC = Medium pressure liquid chromatography

*Tc*AChE = *Torpedo Californica* Acetylcholinesterase

REFERENCES

[1] Launer, L.J.; Fratiglioni, L.; Andersen, K.; Breteler, M.M.B.; Copeland, R.J.M.; Dartigues, J.-F.; Lobo, A.; Martinez-Lage, J.; Soininen, H.; Hofman, A. In *Alzheimer's Disease and Related Disorders: Etiology, Pathogenesis and Therapeutics*; Iqbal, K.; Swaab, D.F.; Winblad, B.; Wisniewski, H.M. Eds.; John Wiley & Sons, New York, **1999**, p. 9.

[2] Hasegawaa, K.; Aoba, A. In *Anti-Dementia Agents*; Academic Press, New York, **1994**, p. 13.

[3] Leonard, B.E. *Hum. Psychopharmacol.*, **1998**, *13*, 83.

[4] Cacabelos, R.; Alvarez, A.; Lombardi, V.; Fernández-Novoa, L.; Corzo, L.; Pérez, P.; Laredo, M.; Pichel, V.; Hernández, A.; Varela, M.; Figueroa, J.; Prous, J., Jr.; Windisch, M.; Vigo, C. *Drugs Today*, **2000**, *36*, 415.

[5] Martin, J.B. *New Engl. J. Med.*, **1999**, *340*, 1970.

[6] Coughlan, C.M.; Breen, K.C. *Pharmacol. Therapeut.*, **2000**, *86*, 111.

[7] Haass, C.; Schlossmacher, M.G.; Hung, A.Y.; Vigo-Pelfrey, C.; Mellon, A.; Ostaszewski, B.L.; Lieberburg, I.; Koo, E.H.; Schenk, D.; Teplow, D.B.; Selkoe, D. *Nature*, **1992**, *359*, 322.

[8] Shoji, M.; Golde, T.E.; Ghiso, J.; Cheung, T.T.; Estus, S.; Shaffer, L.M.; Cai, X.D.; McKay, D.M.; Tintner, R.; Frangione, B.; Younkin, S.G. *Science*, **1992**, *258*, 126.

[9] Hardy, J. *Trends Neurosci.*, **1997**, *20*, 154.

[10] Selkoe, D.J. *Science*, **1997**, *275*, 630.

[11] Schenk, D.; Barbour, R.; Dunn, W.; Gordon, G.; Grajeda, H.; Guido, T.; Hu, K.; Huang, J.; Johnson-Wood, K.; Khan, K.; Kholodenko, D.; Lee, M.; Liao, Z.; Lieberburg, I.; Motter, R.; Mutter, L.; Soriano, F.; Shopp, G.; Vasquez, N.; Vandevert, C.; Walker, S.; Wogulis, M.;

Yednock, T., Games, D.; Seubert, P. *Nature*, **1999**, *400*, 173.

[12] Gualtieri, F.; Deu, S.; Manetti, D.; Romanelli, M.N. *Farmacologie*, **1995**, *50*, 489.

[13] Bartus, R.T.; Dean, R.L.; Beer, B.; Lippa, A.S. *Science*, **1982**, *217*, 408.

[14] McGeer, P.L. *Can. J. Physiol. Pharmacol.*, **1984**, *62*, 741.

[15] Sugimoto, H.; Yamanishi, Y.; Iimura, Y.; Kawakami, Y. *Curr. Med. Chem.*, **2000**, *7*, 303.

[16] Brufani, M.; Filocamo, L.; Lappa, S.; Magg, A. *Drugs Future*, **1997**, *22*, 397.

[17] Alvarez, A.; Alarcon, R.; Opazo, C.; Campos, E.O.; Munoz, F.J.; Calderon, F.H.; Dajas, F.; Gentry, M.K.; Doctor, B.P.; DeMello, F.G.; Inestrosa, N.C. *J. Neurosci.*, **1998**, *18*, 3213.

[18] Davis, M. *New Engl. J. Med.*, **1992**, *327*, 1253.

[19] Watkins, P.B.; Zimmerman, H.J.; Knapp, M.J.; Gracon, S.I.; Lewis, K.W. *JAMA*, **1994**, *271*, 992.

[20] Sugimoto, H.; Iimura, Y.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.*, **1995**, *38*, 4821.

[21] Rainer, M. *CNS Drugs*, **1997**, *7*, 89.

[22] Prous, J.; Rabasseda, X.; Castañer, J. *Drugs Future*, **1996**, *19*, 656.

[23] Cummings, J.L.; Cyrus, P.A. Biever, F.; Mas, J.; Orazem, J.; Gulanski, B. *Neurology*, **1998**, *50*, 1214.

[24] Morris, J.C.; Cyrus, P.A.; Orazem, J.; Biever, F.; Ruzicka, B.B.; Gulanski, B. *Neurology*, **1998**, *50*, 1222.

[25] Kozikowski, A.P.; Tückmantel, W. *Acc. Chem. Res.*, **1999**, *32*, 641.

[26] Tang, X.C.; He, X.C.; Bai, D.L. *Drugs Future*, **1999**, *24*, 647.

[27] Bai, D.L.; Tang, X.C.; He, X.C. *Curr. Med. Chem.*, **2000**, *7*, 355.

[28] He, X.C.; Wang, Z.Y.; Li, Y.L.; Xu, Z.R.; Bai, D.L. *Chinese Chem. Lett.*, **1993**, *4*, 597.

[29] Wu, B.G.; Zhen, W.P.; Bo, Y.X.; He, X.C.; Bai, D.L. *Chinese Chem. Lett.*, **1995**, *6*, 193.

[30] Shibanoki, S.; Ishii, Y.; Kubo, T.; Kogure, M.; Asai, S.; Ishikawa, K. *Pharmacol. Biochem. Behav.*, **1991**, *39*, 499.

[31] Ishii, Y.; Sumi, T. *Neuropharmacology*, **1992**, *31*, 61.

[32] McClelan, K.J.; Benfield, P. *CNS Drugs*, **1998**, *9*, 69.

[33] Ishihara, Y.; Hirai, K.; Miyamoto, M.; Goto, G. *J. Med. Chem.*, **1994**, *37*, 2292.

[34] Pang, Y.-P.; Quiram, P.; Jelacic, T.; Hong, F.; Brimijoin, S. *J. Biol. Chem.*, **1996**, *271*, 23646.

[35] Carlier, P.R.; Han, Y.F.; Chow, E.S.-H.; Li, C P.-L.; Wang, H.; Lieu, T.X.; Wong, H.S.; Pang, Y.-P. *Bioorg. Med. Chem.*, **1999**, *7*, 351.

[36] Carlier, P.R.; Chow, E.S.-H.; Han, Y.; Liu, J.; El Yazal, J.; Pang, Y.-P. *J. Med. Chem.*, **1999**, *42*, 4225.

[37] Carlier, P.R.; Du, D.-M.; Han, Y.; Liu, J.; Pang, Y.-P. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 2335.

[38] Carlier, P.R.; Du, D.-M.; Han, Y.-F.; Liu, J.; Perola, E.; Williams, I.D.; Pang, Y.-P. *Angew. Chem., Int. Ed.*, **2000**, *39*, 1775.

[39] Mary, A.; Renko, D.Z.; Guillou, C.; Thal, C. *Bioorg. Med. Chem.*, **1998**, *6*, 1835.

[40] Kimoto, K.; Imagawa, T.; Kawanisi, M. *Bull. Chem. Soc. Jpn.*, **1972**, *45*, 3698.

[41] Liu, J.-H.; Kovacic, P. *J. Org. Chem.*, **1973**, *38*, 3462.

[42] Camps, P.; El Achab, R.; Font-Bardia, M.; Görbig, D.; Morral, J.; Muñoz-Torrero, D.; Solans, X.; Simon, M. *Tetrahedron*, **1996**, *52*, 5867.

[43] Camps, P.; El Achab, R.; Görbig, D.M.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J.E.; Vivas, N.M.; Barril, X.; Orozco, M.; Luque, F.J. *J. Med. Chem.*, **1999**, *42*, 3227.

[44] Badia, A.; Baños, J.E.; Camps, P.; Contreras, J.; Görbig, D.M.; Muñoz-Torrero, D.; Simon, M.; Vivas, N.M. *Bioorg. Med. Chem.*, **1998**, *6*, 427.

[45] Ellman, G.L.; Courtney, K.D.; Andres, B. Jr.; Featherstone, R.M. *Biochem. Pharmacol.*, **1961**, *7*, 88.

[46] Kozikowski, A.P.; Campiani, G.; Tückmantel, W. *Heterocycles* **1994**, *39*, 101.

[47] Camps, P.; Contreras, J.; Font-Bardia, M.; Solans, X. *Synth. Commun.*, **1996**, *26*, 9.

[48] Barton, D.H.R.; Crich, D.; Motherwell, W.B. *Tetrahedron*, **1985**, *41*, 3901.

[49] Barton, D.H.R.; Zard, S.Z. *Pure Appl. Chem.*, **1986**, *58*, 675.

[50] Crich, D. *Aldrichimica Acta*, **1987**, *20*, 35.

[51] Barton, D.H.R. *Tetrahedron*, **1992**, *48*, 2529.

[52] Barton, D.H.R. *Pure Appl. Chem.*, **1994**, *66*, 1943.

[53] Camps, P.; Görbig, D.; Muñoz-Torrero, V.; Pérez, F. *Coll. Czech. Chem. Commun.*, **1997**, *62*, 1585.

[54] Kawakami, H.; Ohuchi, R.; Kitano, M.; Ono, K. Patent EPO 0 268 871 A1, Sumitomo Pharmaceuticals Co. Ltd., 1987.

[55] Gregor, V.E.; Emmerling, M.R.; Lee, C.; Moore, C.J. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 861.

[56] Camps, P.; Cusack, B.; Mallender, W.D.; El Achab, R.; Morral, J.; Muñoz-Torrero, D.; Rosenberry, T.L. *Mol. Pharmacol.*, **2000**, *57*, 409.

[57] Camps, P.; El Achab, R.; Morral, J.; Muñoz-Torrero, D.; Badia, A.; Baños, J.E.; Vivas, N.M.; Barril, X.; Orozco, M.; Luque, F.J. *J. Med. Chem.*, **2000**, *43*, 4657-4666.

[58] Kaur, J.; Zhang, M.-Q. *Curr. Med. Chem.*, **2000**, *7*, 273.

[59] Kahn, M.; Nakanishi, H.; Chrusciel, R.A.; Fitzpatrick, D.; Johnson, M.E. *J. Med. Chem.*, **1991**, *34*, 3399.

[60] Camps, P.; Contreras, J.; Morral, J.; Muñoz-Torrero, D.; Font-Bardia, M.; Solans, X. *Tetrahedron*, **1999**, *55*, 8481.

[61] Camps, P.; Contreras, J.; El Achab, R.; Morral, J.; Muñoz-Torrero, D.; Font-Bardia, M.; Solans, X.; Badia, A.; Vivas, N.M. *Tetrahedron*, **2000**, *56*, 4541.

[62] Recanatini, M.; Cavalli, A.; Belluti, F.; Piazzi, L.; Rampa, A.; Bisi, A.; Gobbi, S.; Valenti, P.; Andrisano, V.; Bartolini, M.; Cavrini, V. *J. Med. Chem.*, **2000**, *43*, 2007.

[63] Camps, P.; Contreras, J.; Font-Bardia. M.; Morral, J.; Muñoz-Torrero, D.; Solans, X. *Tetrahedron: Asymmetry*, **1998**, *9*, 835.

[64] Harel, M.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P.; Silman, I.; Sussman, J.L. *Proc. Natl. Acad. Sci. U.S.A.*, **1993**, *90*, 9031.

[65] Raves, M.L.; Harel, M.; Pang, Y.-P.; Silman, I.; Kozikowski, A.P.; Sussman, J.L. *Nature Struct. Biol.*, **1997**, *4*, 57.

[66] Barril, X.; Orozco, M.; Luque, F.J. *J. Med. Chem.*, **1999**, *42*, 5110.