Brief Articles

Synthesis, in Vitro Pharmacology, and Molecular Modeling of *syn*-Huprines as Acetylcholinesterase Inhibitors

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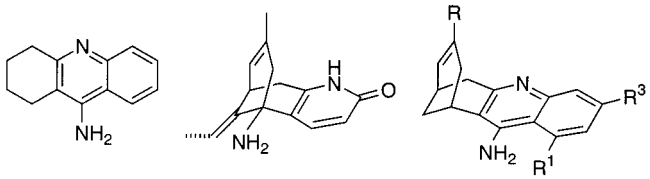
Two 12-amino-6,7,8,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline derivatives [9-Me(Et)] (*syn*-huprines) have been obtained by condensation of known 7-alkylbicyclo[3.3.1]non-6-en-3-ones with 2-(trifluoromethyl)aniline, followed by basic cyclization of the resulting imine, and chromatographic separation of the regioisomeric mixture of products, thus obtained. The new (±)-*syn*-huprines were shown to be slightly less active bovine or human acetylcholinesterase inhibitors than the corresponding *anti*-derivatives. Molecular modeling simulations allow us to explain the differences in inhibitory activity of these compounds on the basis of an inverse solvation effect.

Introduction

Since the approval of tacrine, **1** (Figure 1), by the FDA as the first drug for the cognitive enhancement in Alzheimer's disease (AD), several acetylcholinesterase (AChE) inhibitors have been marketed and important efforts are being made to develop new AChE inhibitors.[1−5] Huprines, **3**, are a new class of very potent, selective AChE inhibitors of potential interest for the symptomatic treatment of AD.[6−12] The most powerful compound, named huprine X [(−)-**3**, R = Et; R^1 = H; R^3 = Cl], exhibits one of the highest affinities reported for a reversible inhibitor, being ca. 40-fold more potent than donepezil, 180-fold more potent than (−)-huperzine A, and 1200-fold more potent than tacrine hydrochloride.[7] Huprines can be regarded as synthetic hybrids that combine the 4-aminoquinoline substructure of tacrine,[1] **1**, with the carbobicyclic substructure of (−)-huperzine A, (−)-**2**.[5] Accordingly, they should be able to interact simultaneously with several of the binding sites for tacrine and (−)-huperzine A, as they partially overlap within AChE.[13,14] Molecular modeling studies[8−10] have shown that the 4-aminoquinoline substructure occupies the same binding site as tacrine, i.e., stacked between the rings of Trp84 and Phe330, while the carbobicyclic subunit roughly occupies the same binding pocket as (−)-huperzine A.



Tacrine, **1** (−)-**Huperzine A**, (−)-**2** **Huprines**, (±)-**3**

Figure 1. Structures of huprines and their starting models.

More than 30 different huprines in racemic form have been synthesized by using Friedländer condensation of enones **4** with 2-aminobenzonitriles conveniently substituted at positions 4 and/or 6 in the presence of $AlCl_3$ as a Lewis acid catalyst (Scheme 1). This procedure has allowed us to explore the structure−activity relationships (SAR) of substituents attached to the aminoquinoline subunit and to position 9 of huprines.[6−10] However, in all cases only huprines having the heterocyclic ring and the endocyclic C═C double bond in an *anti*-arrangement were obtained. Since the unsaturated three-carbon bridge of huprines seems to be an essential feature for their AChE inhibitory activity,[12] it would be worthwhile to synthesize *syn*-huprines and to explore their pharmacologic profile as AChE inhibitors. Herein we describe (i) the first synthesis of two *syn*-huprines in racemic form, (ii) the AChE inhibitory activity of *syn*-huprines, and (iii) a discussion of the relative AChE inhibitory potency of a *syn*-huprine and its *anti*-regioisomer in light of the results provided by molecular modeling studies.

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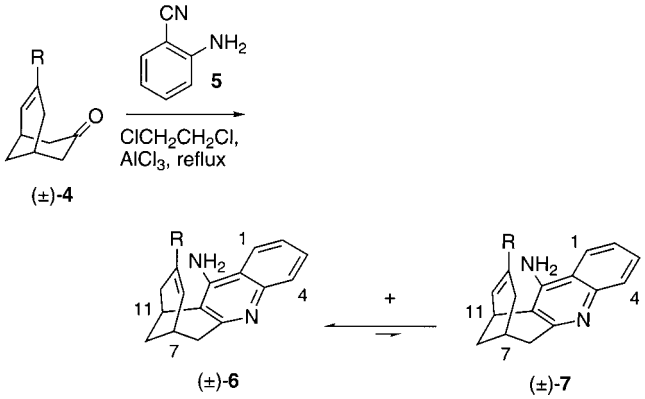
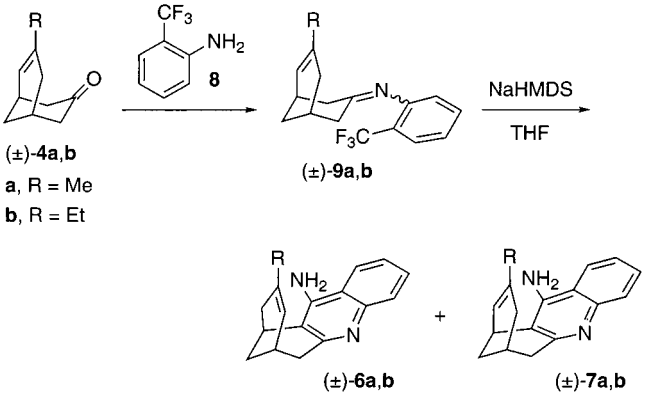
Scheme 1. A Possible Explanation of the Regioselectivity Observed in the Friedländer Reaction Leading to (±)-Huprines, (±)-**6**, Based on the Equilibration of Regioisomeric Derivatives (±)-**6** and (±)-**7**, through Acid-Promoted Carbon−Carbon Double Bond Migration



Scheme 2. Synthesis of (±)-*syn*-Huprines



Chemistry

The observed *anti*-regioselectivity of the Friedländer condensation leading to huprines could result from the equilibration under the acidic conditions of this reaction of a mixture of the hitherto not observed *syn*-huprines and the reasonably more stable and always isolated *anti*-regioisomeric huprines (Scheme 1). Therefore, an alternative procedure operating under nonequilibrating conditions would be required to synthesize *syn*-huprines. Recently, aza-analogues of tacrine were prepared by cyclization of 2-[2-(trifluoromethyl)phenylimino]pyrrolidine and related compounds on reaction with sodium hexamethyldisilazide (NaHMDS) in THF.[15] These reactions may be considered as a modification of the Friedländer reaction in which the 2-aminobenzonitrile is replaced by 2-(trifluoromethyl)aniline, the condensation step taking place under strongly basic conditions, through the chemistry of the anionically activated trifluoromethyl group.

Reaction of enones (±)-**4a** and (±)-**4b** with 2-(trifluoromethyl)aniline, **8** (1.4 equiv), in toluene under reflux for 2 days, followed by column chromatography of the resulting crude product, afforded in good yields imines (±)-**9a** and (±)-**9b** as mixtures of the (*E*)- and (*Z*)-stereoisomers, one of them being slightly more abundant (approximate ratio of 3:2) (Scheme 2), which were used in the cyclization step without further purification. Treatment of imine (±)-**9a** with NaHMDS (6 equiv) in THF from −78 °C to room temperature for 4.25 h afforded a crude product consisting mainly of a mixture of starting imine and the regioisomeric aminoquinolines (±)-**6a**/(±)-**7a**, in the approximate ratio of 70:30, from which the less polar *syn*-regioisomer (±)-**7a** could be isolated in pure form by silica gel column chromatography. The use of 4 equiv of NaHMDS led to lower yields of (±)-**6a** plus (±)-**7a**, while longer reaction times (15 h) led to higher yields of aminoquinolines [up to 61% total yield of (±)-**6a** and (±)-**7a**] with a lower ratio of the *syn*-regioisomer (±)-**7a**.

Similarly, from imine (±)-**9b**, after reaction with 8 equiv of NaHMDS for 6 h and silica gel column chromatography of the resulting crude product, pure (±)-**7b** was obtained. The new *syn*-huprines (±)-**7a** and (±)-**7b** were fully characterized as hydrochlorides through their spectroscopic data and elemental analyses (C, H, N, Cl). Structural assignment of (±)-**7a** and (±)-**7b** and differentiation from the corresponding *anti*-regioisomers, (±)-**6a** and (±)-**6b**, was straightforward on the basis of $^1H/^1H$ and $^1H/^{13}C$ COSY experiments (HMQC sequence).

Pharmacology

To evaluate the potential interest of *syn*-huprines for the treatment of AD, the AChE inhibitory activity of (±)-**7a**·HCl and (±)-**7b**·HCl was determined by the method of Ellman et al.[16] on AChE from bovine and human erythrocytes. To establish their selectivity, the butyrylcholinesterase (BChE) inhibitory activity was also assayed by the same method on human serum BChE.

Table 1 summarizes the data for the *syn*-huprines (±)-**7a**·HCl and (±)-**7b**·HCl, as well as for tacrine·HCl, (−)-huperzine A, and huprines (±)-**6a**·HCl and (±)-**6b**·HCl as reference compounds. *syn*-Huprines (±)-**7a**·HCl and (±)-**7b**·HCl are less potent than (±)-**6a**·HCl and (±)-**6b**·HCl, respectively, as bovine (7.9 and 4.3-fold, respectively) and human (8.4 and 2.0-fold, respectively) AChE inhibitors. However, in contrast to tacrine·HCl and (−)-huperzine A, which are less active toward human than bovine AChE, *syn*-huprines (±)-**7a**·HCl and (±)-**7b**·HCl, as well as huprines (±)-**6a**·HCl and (±)-**6b**·HCl, are slightly more potent toward human AChE. Particularly, *syn*-huprine (±)-**7b**·HCl is 2.7- and 3.4-fold more potent than tacrine·HCl and (−)-huperzine A against human AChE. Regarding the BChE inhibitory activity, *syn*-huprines (±)-**7a**·HCl and (±)-**7b**·HCl are comparable to huprines (±)-**6a**·HCl and (±)-**6b**·HCl.

The results in Table 1 allow us to conclude that *syn*-huprines, though less active than their *anti*-regioisomeric huprines, have significant AChE inhibitory activity. On the basis of our previous SAR studies,[6−10] attachment of a chlorine atom at position 3 of *syn*-huprines could further enhance the binding affinity for human AChE relative to both tacrine and (−)-huperzine A.

Molecular Modeling Studies

The unexpected difference in AChE inhibitory activity between the *syn*-huprines and their *anti*-regioisomers led us to study the relationship between the *anti* → *syn* migration of the C=C double bond and the change in inhibitory potency. This information can be valuable to

Table 1. Pharmacological Data of Tacrine·HCl, (−)-Huperzine A, and the Hydrochlorides of the Huprines (±)-**6a**,**b** and Their Regioisomeric (±)-*syn*-Huprines (±)-**7a**,**b**[a]

compound	IC_{50} (nM) bovine AChE	IC_{50} (nM) human AChE	IC_{50} (nM) human BChE	IC_{50} bovine AChE/IC_{50} human AChE	IC_{50} human BChE/IC_{50} human AChE
(−)-tacrine·HCl	130 ± 10	205 ± 18	44 ± 17	0.63	0.21
(−)-huperzine A	74.0 ± 5.5	260 ± 18	>10000	0.28	>38
(±)-**6a**·HCl	65 ± 15	51.6 ± 5.4	126 ± 21	1.26	2.44
(±)-**6b**·HCl	38.5 ± 4.0	37.8 ± 3.7	79.3 ± 9.7	1.02	2.10
(±)-**7a**·HCl	511 ± 40	436 ± 68	148 ± 15.3	1.17	0.34
(±)-**7b**·HCl	165 ± 21	76.9 ± 6.4	63.0 ± 17.1	2.14	0.82

[a] Values are expressed as mean ± standard error of the mean of at least four experiments. IC_{50} inhibitory concentration (nM) of AChE (from bovine or human erithrocytes) or BChE (from human serum) activity.

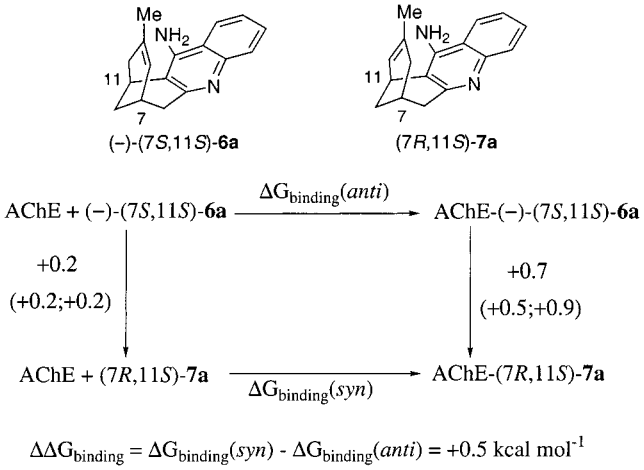


Figure 2. Thermodynamic cycle used in free energy calculations in water and in the AChE enzyme to determine relative binding affinities between (7*R*,11*S*)-**7a** and (−)-(7*S*,11*S*)-**6a**. The results for the forward and reverse mutations are given in parentheses. Values are in kcal mol^{-1}.

design chemical modifications that improve the pharmacological profile of *syn*-huprines relative to their *anti*-regioisomers. To this end, thermodynamic integration (TI) calculations coupled with molecular dynamics (MD) were performed to rationalize the difference in binding affinity between *syn*-huprines and their *anti*-regioisomers (see Supporting Information). The change in binding free energy between the more active enantiomer (eutomer) of huprine **6a**, (−)-(7*S*,11*S*)-**6a**, and the corresponding product of C=C double bond isomerization, the *syn*-huprine (7*R*,11*S*)-**7a**, both in protonated form, was determined from the thermodynamic cycle shown in Figure 2. The results predict that the conversion of huprine (−)-(7*S*,11*S*)-**6a** to the *syn*-huprine (7*R*,11*S*)-**7a** decreases the binding affinity for the enzyme by 0.5 kcal mol^{-1}, which is in agreement with the experimental data (see above and Table 1).

Combination of the free energy change for the *anti* → *syn* mutation in water (+0.2 kcal mol^{-1}; Figure 2) with the free energy difference for the *anti* → *syn* mutation in the gas phase (−0.1 kcal mol^{-1}) indicate that the *anti*-regioisomer hydrates better than the *syn*-huprine by 0.3 kcal mol^{-1}. This result is confirmed by self-consistent reaction field calculations[17] (see Supporting Information), which indicate that the *anti*-regioisomer is better hydrated than the *syn*-huprine by around 0.6 kcal mol^{-1}. The preferential hydration of the *anti*-regioisomer can be understood from comparison of molecular interaction potential (MIP) maps[18] (see Supporting Information), which point out the lower affinity of the NH_2 group of the 4-aminoquinoline subunit to interact with a water molecule in *syn*-huprine compared to its *anti*-regioisomer. Such a difference in the affinity for a water molecule can be related to the closer proximity of the C=C double bond to the amino group in the *syn*-huprine. Thus, whereas the MIP minimum located around the >NH group of the 4-aminoquinoline unit in *syn*-huprine is nearly identical to that in the *anti*-regioisomer (−66.5 and −66.3 kcal mol^{-1}, respectively), the minima around the NH_2 group are slightly lower (in absolute values) in the *syn*-huprine compared to its *anti*-regioisomer, especially in the region closer to the carbobicyclic ring (−60.5 and −62.0 kcal mol^{-1}, respectively).

Very often, a reduction in the desolvation penalty of a drug leads to an increase in the binding free energy. For the *syn*-huprine and its *anti*-regioisomer, the situation is just the opposite, since the compound with the strongest binding affinity (i.e., *anti*-regioisomer) has also the better hydration. (i.e., the largest desolvation penalty). Therefore, the better binding affinity of huprine (−)-(7*S*,11*S*)-**6a** must stem from stronger drug−enzyme interactions than for the *syn*-huprine (7*R*,11*S*)-**7a**. This is shown by combination of the free energy change for the *anti* → *syn* mutation in the protein (+0.7 kcal mol^{-1}; see Figure 2) and in the gas phase (−0.1 kcal mol^{-1}), which indicates that the protein stabilizes preferentially the binding of the *anti*-regiosomer, (−)-(7*S*,11*S*)-**6a**, by around 0.8 kcal mol^{-1}.

Inspection of the snapshots collected during the last 500 ps of the 2 ns MD simulations for the complexes *Tc*AChE-(−)-(7*S*,11*S*)-**6a** and *Tc*AChE-(7*R*,11*S*)-**7a** indicate that the only remarkable structural alteration in the interaction pattern between *syn*-huprine (7*R*,-11*S*)-**7a** and its *anti*-regioisomer (−)-(7*S*,11*S*)-**6a** with the AChE binding site is the disruption of the highly hydrated environment of the NH_2 group of huprine. Thus, whereas the two N−H bonds of the amino group in (−)-(7*S*,11*S*)-**6a** form hydrogen-bonds to water molecules in around 90% and 99% of the structures examined, they are hydrogen-bonded to water molecules only in around 80% and 85% in the *syn*-regioisomer (7*R*,-11*S*)-**7a**. Since the binding of huprine is mediated by water bridges that link the amino group to several residues of the binding site, such as Asp72, Tyr121, and Ser122, the disruption of the network of water-mediated bridges leads to the reduced binding affinity of the *syn*-huprine.

Conclusion

For the first time, the regioisomeric (±)-*syn*-huprines (±)-**7a**,**b** have been obtained through a protocol that implies: (i) condensation of ketones (±)-**4a**,**b** with

2-(trifluoromethyl)aniline, (ii) treatment of the intermediate stereoisomeric mixture of (*E*)- and (*Z*)-imines with excess sodium hexamethyldisilazide, and (iii) separation of the thus formed regioisomeric mixture of *syn*- and *anti*-derivatives, (±)-**7a**,**b**/(±)-**6a**,**b**. The AChE inhibitory activity of (±)-**7a**,**b**·HCl toward AChE from bovine and human erythrocytes was shown to be slightly lower than that of the corresponding *anti*-regioisomers, huprines (±)-**6a**,**b**·HCl, although (±)-**7b**·HCl showed higher activity toward human AChE than tacrine·HCl and (−)-huperzine A. The reduction in the binding affinity related to the *anti* → *syn* isomerization in huprines stems from an inverse solvation mechanism, which reveals the structural features of the interaction of huprines with the binding site of AChE and particularly the important role of water molecules in mediating the binding of these AChE inhibitors. The new procedure of obtaining (±)-*syn*-huprines, (±)-**7a**,**b**, opens the way to a new kind of AChE inhibitors, whose development can take advantage of the known structure−activity relationships in the *anti*-regioisomeric series (huprines).

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Supporting Information Available: Experimental part (chemistry, pharmacology, and molecular modeling with additional references and a figure of MIP maps for the interaction of (−)-(7*S*,11*S*)-**6a** and (7*R*,11*S*)-**7a** with the TIP3P water oxygen atom). This material is available free of charge via the Internet at http://pubs.acs.org.

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