Bioorganic & Medicinal Chemistry Letters 21 (2011) 2384–2388

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis, biological assessment and molecular modeling of 14-aryl-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-13-amines

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ARTICLE INFO

Article history:
Received 18 January 2011
Revised 18 February 2011
Accepted 20 February 2011
Available online 14 March 2011

Keywords:
AChE
BuChE
Kinetic analysis
Inhibition mechanism
Docking analysis
Molecular dynamics simulation
Alzheimer's disease

ABSTRACT

The synthesis and pharmacological evaluation of racemic 14-aryl-10,11,12,14-tetrahydro-9*H*-benzo[5,6]-chromeno[2,3-*b*]quinolin-13-amines (**19**–**28**), prepared by Friedländer reaction of 3-amino-1-aryl-1*H*-benzo[*f*]chromene-2-carbonitriles (**10**–**18**) with suitable cycloalkanones is described. These molecules are potent, in the nanomolar range [IC_{50} (EeAChE) = 7–101 nM], and selective inhibitors of acetylcholinesterase (AChE). The most potent inhibitor, 4-(13-amino-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-14-yl)phenol (**20**) [IC_{50} (EeAChE) = 7 ± 2 nM] is four-fold more active than tacrine. Kinetic studies on compound **20** showed that this is a mixed-type inhibitor of EeAChE with a K_i of 5.00 nM. However, racemic **20** was unable to displace propidium iodide, suggesting that the inhibitor does not strongly bind to the peripheral anionic site (PAS) of AChE. Docking, molecular dynamics stimulations, and MM-GBSA calculations agree well with this behavior.

Alzheimer's disease (AD) is an age-related neurodegenerative process characterized by a progressive loss of memory, language skills, disorientation, attention, and depression.[1] Although the etiology of AD is still poorly understood, several factors such as amyloid-β (Aβ)[2] deposits, τ-protein aggregation, oxidative stress, or low levels of acetylcholine[3] are thought to play significant roles in the pathology of the disease.[4] In spite of the enormous research effort, an efficient strategy for the treatment of AD is still lacking. The cholinergic theory[1] suggests that the selective loss of cholinergic neurons in AD results in a deficit of acetylcholine (ACh) in specific regions of the brain that mediate learning and memory functions.[5] Thus, three acetylcholinesterase (AChE) inhibitors (donepezil, rivastigmine, and galanthamine) have been approved for commercial use, to improve AD symptoms by inhibiting AChE.[6a] A renewed interest for AChE inhibitors (AChEI) has been stimulated by the potential role of AChE in accelerating the formation of amyloid fibrils in the brain and forming stable complexes with Aβ.[7] This role involves the peripheral anionic site (PAS) of AChE, as noted by the fact that propidium iodide, a potent AChEI agent binding specifically to the PAS, affects Aβ aggregation in vitro, whereas other catalytic active site (CAS) inhibitors, such as tacrine, have not a similar effect.[8] It is known that during the development of AD, butyrylcholinesterase (BuChE) activity increases by 40–90%, and that high levels of BuChE are found to have a role in Aβ aggregation during the early stages of senile plaques formation.[6b] Consequently, compounds able to selectively inhibit BuChE are gaining interest for the treatment of AD.[6c] However, the multifactorial nature of AD supports new therapeutic strategies, based on the 'one molecule, multiple targets' paradigm.[9–20]

In this context, some years ago we embarked in a project aimed at the synthesis of a series of multipotent compounds designed to target AChE and neuronal Ca^{2+} modulation.[21] As a result, we have synthesized and evaluated a number of hybrid compounds, such as ethyl 5-amino-4-(4-methoxyphenyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrano[2,3-*b*]quinoline-3-carboxylate (**I**) (Chart 1),[21a] which combine the tetrahydroaminoquinoline moiety present in tacrine with a 4*H*-pyran bearing a substitution pattern similar to that found in the isosteric 1,4-dihydropyridines, well known calcium channel blockers. Compound **I** was less potent than

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doi:10.1016/j.bmcl.2011.02.094

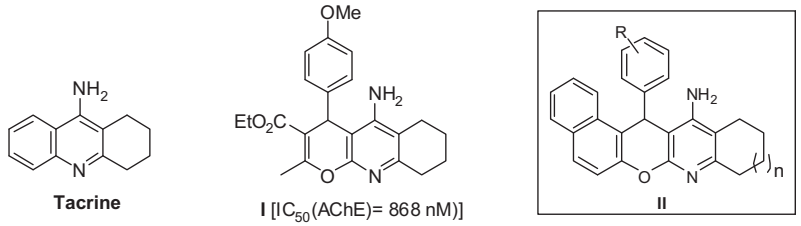


Chart 1. Structure of tacrine, the reference compound **I**, and 14-aryl-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-13-amines (**II**), the target molecules studied in this work.

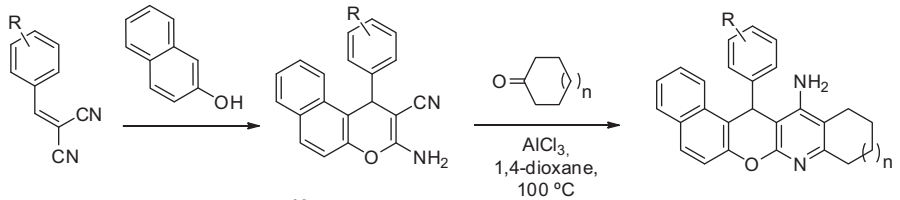
tacrine,[21b,c] but blocked voltage-dependent Ca^{2+} channels (50% inhibition),[21c] and Ca^{2+} uptake induced by nicotinic stimulation (90% inhibition) in bovine chromaffin cells, more efficiently than tacrine.[21f] With these precedents in mind,[21] and looking for more equipotent AChE versus BuChE inhibitory activities, increased neuroprotection and antioxidant capacities, we have now carried out the synthesis, the biological evaluation, and molecular modeling of a number of 14-aryl-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-13-amines (**II**), analogous derivatives of compound **I** (Chart 1), where we have incorporated an additional naphthalene fused ring, instead of the ethoxycarbonyl moiety. This structural modification would change presumably the physicochemical properties of the target compounds, favouring the binding to the active sites of the enzymes, by generating new favorable π–π or π–cation interactions. In this paper, we have shown that these compounds are easily available; behave as potent, in the nanomolar range, and selective, mixed-type AChE versus BuChE inhibitors.

The synthesis of the target molecules (**II**) (Chart 1) has been approached as shown in Scheme 1. Starting from the readily available 2-arylidenemalononitriles **1**–**9**, the reaction with 2-naphthol as described,[22] provided known compounds **10**,[23] **11**,[24] **12**,[25] **13**,[26] **14**,[27] **15**,[28] **16**,[29] and **18**[30] in good yields (Scheme 1). Intermediate **17** has been synthesized here for the first time from the corresponding precursor **8**.[31] Friedländer reaction[32] of 3-amino-1-aryl-1*H*-benzo[*f*]chromene-2-carbonitriles **10**–**18** with cyclohexanone or cyclopentanone, under the usual experimental conditions,[21] gave compounds **19–28** in good chemical yield[33,34] (Scheme 1). The synthesis of compound **28**[33a] and other tacrine analogs, such as 14-(3-nitrophenyl)-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-13-amine,13-(4-nitrophenyl)-9,10,11,13-tetrahydrobenzo[5,6]chromeno[2,3-*b*]cyclopenta[*e*]pyridin-12-amine, and 13-(4-chlorophenyl)-9,10,11,13-tetrahydrobenzo[5,6]chromeno[2,3-*b*]cyclopenta[*e*]pyridin-12-amine[33b] has been previously reported, but their pharmacological profile still remained unexplored.

Racemic 14-aryl-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-13-amines (**19–28**) were evaluated as inhibitors of AChE from *Electrophorus electricus* (Ee) and BuChE from horse serum, according to Ellman's protocol.[35] The IC_{50} values are shown in Table 1. From these results we can conclude that these tacrine analogs are potent and selective AChE inhibitors, in the nanomolar range, showing the best IC_{50} for compound **20** (7 nM, entry b). From the structure–activity relationship (SAR), some general trends were observed. The more electron-donating substituents in the aromatic ring at C14, the more potent AChE inhibitors the corresponding tacrines resulted. Thus, the strength of the inhibition increase in the order: **20** > **21** > **19** >> **28** for inhibitors bearing substituents at C-4′ such as OH, OMe, Me, and NO_2, respectively. Very interestingly, for the methoxy substituent, the good inhibitory activity is independent of the position of the substituent in the ring (compare inhibitors **21**, **22** with **23**), as well as the number of methoxy groups (two in inhibitor **26**, or three in compound **27**) incorporated in the ring.

Besides the electronic modulation of the aromatic ring, one should focus on the ability of the aromatic substituents to form hydrogen bonds, most of them as hydrogen bond acceptors. Methoxy substituents are described to accept hydrogen bonds, but more weakly than hydroxy groups, because of the steric hindrance promoted by the methyl. Thus, the increase in the number of substituents, although being hydrogen bond acceptors, is not improving the binding, as the steric hindrance becomes a limiting factor. Regarding the size of the fused cycloalkyl ring, for the same type and number of substituents in the aromatic ring at C14, inhibitor **24** (IC_{50} = 101 nM, entry f) is four-fold more active than the related inhibitor **25** bearing a cyclopentyl instead of cyclohexyl ring. Finally, and comparing with tacrine, the most potent 4-(13-amino-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-14-yl)phenol (**20**) is four-fold more active for the AChE inhibition, but lacks completely of BuChE inhibitory activity (Table 1).

The mechanism involved in the AChE inhibition by compounds **19**–**28** was investigated using compound **20**, the most potent AChE



1 R = 4-Me
2 R = 4-OH
3 R = 4-OMe
4 R = 3-OMe
5 R = 2-OMe
6 R = 4-OH-3-OMe

7 R = 3,4-di-OMe
8 R = 3,4,5-tri-OMe
9 R = 4-NO_2

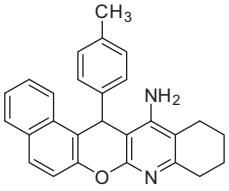
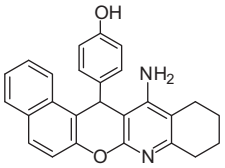
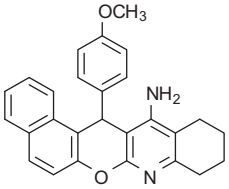
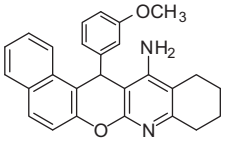
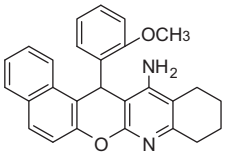
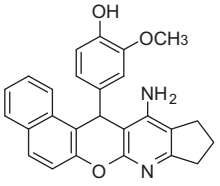
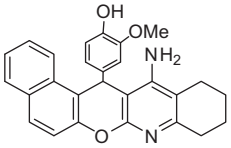
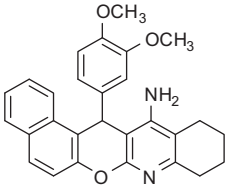
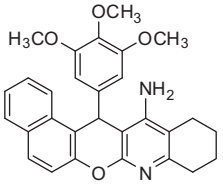
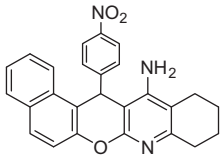
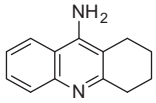
10 R = 4-Me
11 R = 4-OH
12 R = 4-OMe
13 R = 3-OMe
14 R = 2-OMe
15 R = 4-OH-3-OMe

16 R = 3,4-di-OMe
17 R = 3,4,5-tri-OMe
18 R = 4-NO_2

19 R = 4-Me, n = 1 (67%)
20 R = 4-OH, n = 1 (63%)
21 R = 4-OMe, n = 1 (70%)
22 R = 3-OMe, n = 1 (87%)
23 R = 2-OMe, n = 1 (68%)
24 R = 4-OH-3′-OMe, n = 0 (25%)
25 R = 4-OH-3′-OMe, n = 1 (75%)
26 R = 3,4′-di-OMe, n = 1 (71%)
27 R = 3,4,5-tri-OMe, n = 1 (66%)
28 R = 4-NO_2, n = 1 (55%)

Scheme 1. Synthesis of 14-aryl-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-13-amines (**19**–**28**).

Table 1
Inhibition of AChE from *Electrophorus electricus* (EeAChE) and horse serum butyrylcholinesterase (eqBuChE) by tacrine and compounds **19**–**28**.[a]

Entry	Product	Structure	IC_{50} (nM)	
			EeAChE	eqBuChE
a	**19**		43 ± 2	>10,000
b	**20**		7 ± 2	>10,000
c	**21**		18 ± 1	>10,000
d	**22**		16 ± 3	>10,000
e	**23**		20 ± 1	>10,000
f	**24**		101 ± 9	>10,000
g	**25**		28 ± 1	>10,000
h	**26**		26 ± 2	>10,000
i	**27**		26 ± 2	>10,000
j	**28**		170 ± 30	>10,000
k	Tacrine		27 ± 2	5.2 ± 0.2

[a] Data are expressed as means ± s.e.m. of at least three different experiments in quadruplicate.

inhibitor here described. The type of inhibition was elucidated from the analysis of Lineweaver–Burk reciprocal plots (Fig. 1). The increasing slopes and intercepts in *y*-axis (lower V_{max} and higher K_m) with higher inhibitory concentration suggests a mixed-type inhibition (K_i = 5.00 nM).

Next, a propidium competition assay was carried out on compound **20**. Propidium has been demonstrated in previous studies to be a selective ligand for the PAS of AChE.[36a] Propidium exhibits an increase in fluorescence on binding to PAS, making it as a useful probe for non-competitive ligands.[36b] Under the usual experimental conditions (Supplementary data), from 100 nM to 30 μM,[36c] racemic **20** did not show a significant ability to displace propidium from the PAS of AChE, suggesting that this mixed-type inhibitor does not bind strongly to the PAS of AChE. Although this inhibitor has some resemblance to tacrine, a comparison with a mixed-type inhibitor such as donepezil seems appropriate, as this drug is known to occupy the PAS, but shows a very low (22%) aggregation inhibitory potency.[8]

To shed some light on this aspect we have performed docking, MD simulations, and binding free energy estimations (MM-GBSA) of the (*R*)- and (*S*)-enantiomers for compound **20**. The docking box encompassed the entire catalytic channel (see Fig. S1, Supplementary data) so both the PAS and the CAS have been targeted. The most probable docking solutions (poses) for both enantiomers were found at the PAS (no single solution was found at the CAS) and highly equivalent from a structural point of view, what has been translated into very similar, although moderate, binding energies (see Table S2, Supplementary data). MD trajectories for both poses, although differently, turned out to be stable enough

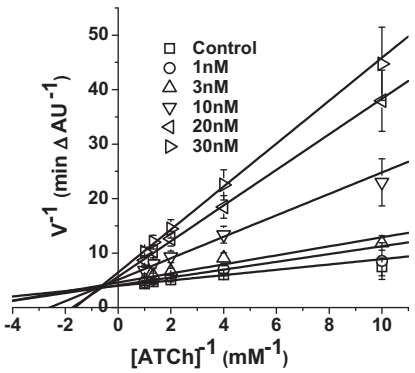


Figure 1. Steady-state inhibition of acetylcholinesterase (AChE) hydrolysis of acetylthiocholine (ATCh) by compound **20**. Lineweaver–Burk reciprocal plots of initial velocity and substrate concentrations are presented. Lines were derived from a weighted least-squares analysis of data points.

as compared to the initial structures (see Figs. S2 and S3, Supplementary data) or in terms of residue fluctuations (see Figs. S4 and S5, Supplementary data). The MM-GBSA analysis showed the same energetic behavior as the docking solutions: (*R*)-**20** and (*S*)-**20** presented the same interactions energies (see Table S3, Supplementary data). Besides, and as it was anticipated from the SAR analysis, the same type of stabilizing interactions were also found during the trajectories, namely the π–π staking and hydrogen bonding (see Figs. S6 and S7, Supplementary data). Therefore, these results are in good agreement with the experimental observations in the sense that both enantiomers interact in the same way (equivalent pose), and with the same, moderate, energy, at the PAS, giving rise to a common interaction pattern that could explain the behavior of the racemic specie. To go a step further, and bearing in mind that the MM-GBSA model does not take into account an entropic term, we decided to perform this calculation for both enantiomers. The results (see Table S3, Supplementary data) showed a negative contribution to the total free energy in the case of the (*S*)-**20** enantiomer, whereas the opposite becomes true for the (*R*)-**20** enantiomer, with a difference around 8 kcal/mol favouring the (*S*)-enantiomer. Looking at the average structures obtained from the last, stable, part of the trajectory and comparing them with the initial ones (see Fig. S8, Supplementary data), it can be seen that this effect could be associated to a higher deformation occurring at the binding site when trying to accommodate the (*R*)-enantiomer, as compared to the spatial requirements of the (*S*)-enantiomer. This would mean that the observed activity would be due to just one of the enantiomers.

The neuroprotective profile of compounds **19**–**28** has also been evaluated in the MTT reduction method in SH-SY5Y cells, showing that these products were modest neuroprotective agents, with values lower than 17%.

In addition, and in order to explore the potential of the selected compounds as putative voltage-dependent calcium channels antagonist, changes in cytosolic Ca^{2+} signals ($[Ca^{2+}]_c$) elicited by depolarizing solutions (70 mM K^+) were evaluated in bovine chromaffin cell populations. In these experiments, the application of K^+ (70 mM) elicited a sharp increase in $[Ca^{2+}]_c$ that reached a plateau and then tended to slowly decline along the 40 s recording. Incubation of the cells with compounds (at concentrations between 0.3 and 100 μM) did not promoted any significant change K^+-induced in $[Ca^{2+}]_c$ signal even at the highest concentration tested (100 μM). These results suggest that these compounds do not behave as voltage-dependent calcium channel antagonists.

Finally, a series of theoretical calculations (see Supplementary data) allowed us to describe the ADME (Absorption, Distribution, Metabolism, and Excretion) properties of compounds **19**–**28** within the organism. All these four criteria influence the drug levels, its kinetics and exposure to the tissues, and hence the performance and pharmacological activity of a drug. Lipinski's rule of five[37] (RO5) is a rule of thumb to evaluate the drug likeness of a molecule based on some molecular descriptors representing ADME properties (see Table S1, Supplementary data). All the compounds fulfill molecular weigh, and the number of hydrogen donors and acceptors, whereas a log *P*o/w <5 is met by only four of them (**20**, **24**, **25** and **28**). The solubility (log *S*) of organic molecules in water has a significant impact on many ADME-related properties like uptake, distribution, transport, and eventually bioavailability. Only compounds **20** (log *S* = −6.3) and **24** (log *S* = −6.1) present solubility values within the limits (−6.5–0.5), while the rest of the compounds show values between −6.5 and −7.3, being in the limits of aqueous solubility. The most used parameter for Blood Brain (BB) barrier penetration is log BB. The log BB of many prescribed CNS drugs is >−0.5 and compounds with log BB <−1.0 penetrate poorly into the brain, yet some commercial CNS drugs have log BB <−1.0.[38] Compounds **19**–**28** present acceptable log BB values', not being smaller than −1.0, in particular compound **20** presents a log BB value of −0.53.

To sum up, in this manuscript we have reported the synthesis and pharmacological analysis of 14-aryl-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-13-amines, prepared by Friedländer reaction of 3-amino-1-aryl-1*H*-benzo[*f*]chromene-2-carbonitriles with suitable cycloalkanones. The biological evaluation showed that these molecules are potent and selective inhibitors of AChE, in the nanomolar range, the most potent inhibitor being 4-(13-amino-10,11,12,14-tetrahydro-9*H*-benzo[5,6]chromeno[2,3-*b*]quinolin-14-yl)phenol (**20**) [IC_{50} (EeAChE) = 7 ± 2 nM], a mixed-type inhibitor for EeAChE with a K_i of 5.00 nM. From these results, as well as the theoretical physicochemical properties, we conclude that compound **20** can be considered as an attractive molecule on a key pharmacological receptor playing a key role in the progress of Alzheimer's disease, that deserve further analyzes. In fact, and according to the docking and molecular dynamics predictions (Supplementary data), the resolution of the racemic compound **20**, as well as their biological evaluation, are being investigated in our laboratories, and the results reported in due course.

Acknowledgments

F.C. acknowledges the Ministry of Higher Education, Scientific Research and Technology in Tunisia for financial support. A.S. thanks CSIC for a I3P-post-doc contract. J.M.C. thanks MICINN (SAF2006-08764-C02-01; SAF2009-07271), and Comunidad de Madrid (S/SAL-0275-2006) for financial support. C.R.S. thanks Fundación CIEN (ISCIII, MICINN) and Fundación Teófilo Hernando for financial support. The authors thankfully acknowledge the computer resources, technical expertise and assistance provided by the Barcelona Supercomputing Center—Centro Nacional de Supercomputación. A.M. thanks Comunidad de Madrid for BIPPED project financial support. J.M.C. thanks Dr. M. Villarroya, and Prof. L. Gandía (Instituto Teófilo Hernando, Madrid, UAM, Madrid, Spain) for the neuroprotective tests, and for the Ca^{+2} analyzes, respectively.

Supplementary data

Supplementary data (synthesis of compounds **17**, **19**–**28**, the pharmacological, the molecular modeling methods and their theoretical physicochemical properties) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.094.

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