Bioorganic & Medicinal Chemistry Letters 20 (2010) 6649–6652

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Berberine derivatives, with substituted amino groups linked at the 9-position, as inhibitors of acetylcholinesterase/butyrylcholinesterase

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ARTICLE INFO

Article history:
Received 4 June 2010
Revised 20 August 2010
Accepted 3 September 2010
Available online 15 September 2010

Dedicated to Professor Chan on the occasion of his 60th birthday

Keywords:
Berberine derivatives
Dual inhibitors
Acetylcholinesterase
Butyrylcholinesterase
Molecular modeling

ABSTRACT

Berberine derivatives with substituted amino groups linked at the 9-position using different carbon spacers were designed, synthesized, and biologically evaluated as inhibitors of acetylcholinesterase. Compound **10b**, with a cyclohexylamino group linked to berberine by a three carbon spacer, gave the most potent inhibitor activity with an IC_{50} of 0.020 μM for AChE. Kinetic studies revealed mixed inhibition of AChE, and molecular modeling simulations of the AChE–inhibitor complex confirmed that compounds bound to both the catalytic active site and the peripheral anionic site.

Alzheimer's disease (AD) is the most prevalent form of dementia affecting approximately 24 million people worldwide.[1] One possible approach to treating this disease is to restore the level of acetylcholine (ACh) by inhibiting acetylcholinesterase (AChE) with reversible inhibitors. The aim of AChE inhibitors is to improve the endogenous levels of ACh in the brain of AD patients, thereby increasing cholinergic neurotransmission.[2] By contrast, numerous studies in recent years found that butyrylcholinesterase (BuChE) levels are unchanged or even rise in advanced AD while AChE activity in certain brain regions decreased.[3–6] Therefore, these studies indicate that a balance of inhibition of both AChE and BuChE may be beneficial in treating the cognitive deficits seen in AD. Furthermore, it may not be an advantage to only have a selective inhibitor of AChE.[7–9] In addition, it has been reported that some peripheral anionic site (PAS) ligands, for example, propidium,[10] could inhibit the aggregation of beta-amyloid peptide induced by AChE, but is not involved in the catalytic active site (CAS) of AChE.[11,12] Given this observation, bis(7)-tacrine (**A** in Fig. 1),[13] and a series of hybrid compounds (e.g., **B** and **C** in Fig. 1[14,15]) were studied for their inhibitory activity of AChE and AChE induced Aβ aggregation.

In our earlier studies, we have designed and synthesized a series of novel molecules using berberine as the lead structure. Biological evaluation indicated that these compounds could be used as inhibitors of both AChE and BuChE. Among them, the compounds where berberine was linked with phenol by 4-carbon spacers (**D** in Fig. 1),[16] and the berberine linked with 3-methylpyridinium by a 2-carbon spacer (**E** in Fig. 1),[17] exhibited potent AChE inhibition with an IC_{50} of 0.097 μM and 0.048 μM, respectively. Herein, we report the design, synthesis and biological evaluation of another series of novel berberine derivatives which have an amino group linked at the 9-position using different carbon spacers.

The synthetic pathway of 9-substituted berberine derivatives **4–11** are shown in Scheme 1. Partial demethylation of berberine **1** at 190 °C under vacuum for 15 min gave berberrubine **2**, with a 68% yield.[18] Alkylations of berberrubine **2** with 1,2-dibromoethane and 1,3-dibromopropane in DMF afforded **3a** and **3b** with a 69% and 77% yield, respectively. Final products **4–10** were prepared by reactions **3a–b** with commercially available secondary amines (dimethylamine, pyrrolidine, etc.), which gave a 31–69% yield. All the compounds were characterized using analytical and NMR spectroscopic data (see Supplementary data).

The AChE and BuChE inhibitory effects of the berberine derivatives were determined using the spectroscopic method described by Ellman et al.[19] Results are summarized in Table 1. AChE (E.C. 3.1.1.7) was obtained from electric eel and BuChE (E.C. 3.1.1.8) was obtained from equine serum. It is interesting that eight of the twelve berberine derivatives where the amino group was linked with berberine using different carbon spacers exhibited better inhibitory activity for AChE than berberine. The carbon spacers seem to be very important for the inhibitory activities of these compounds. However, compounds **4a** and **4b**, which have

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doi:10.1016/j.bmcl.2010.09.013

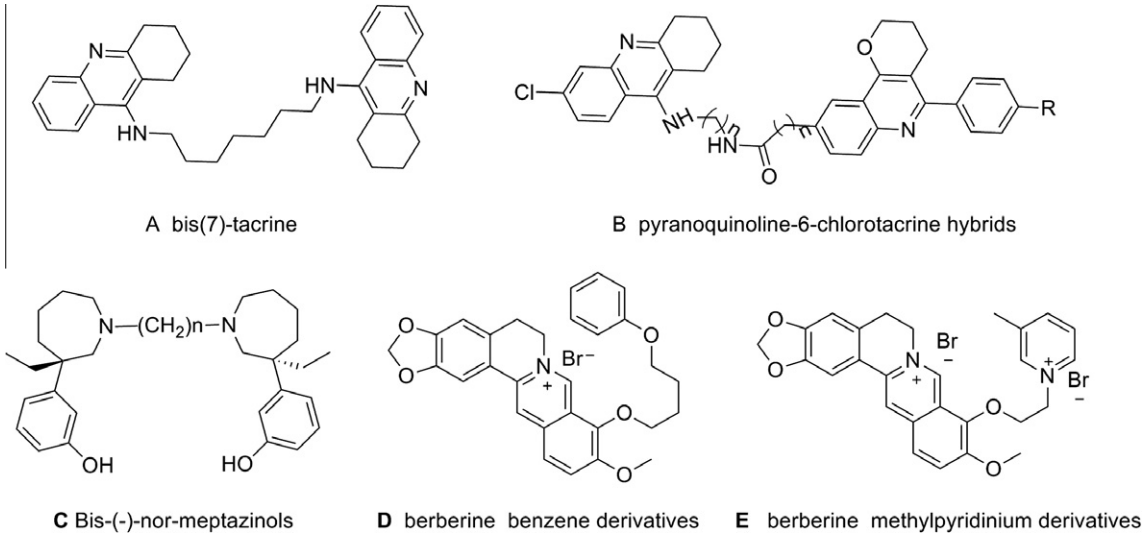
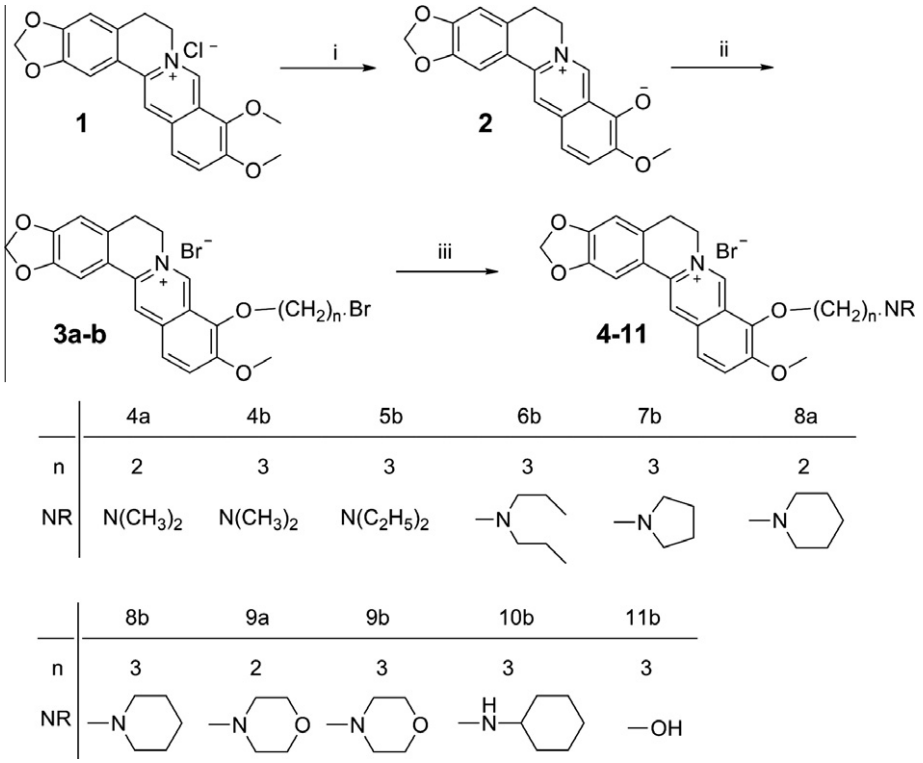


Figure 1. Structure of reported bifunctional AChE inhibitors.



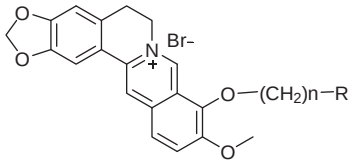
	4a	4b	5b	6b	7b	8a
n	2	3	3	3	3	2
NR	$N(CH_3)_2$	$N(CH_3)_2$	$N(C_2H_5)_2$	—N	—N	—N

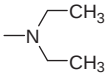
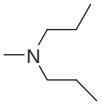
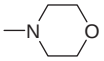
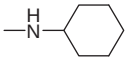
	8b	9a	9b	10b	11b
n	3	2	3	3	3
NR	—N	—N O	—N O	H —N	—OH

Scheme 1. 9-Substituted berberine derivatives **4**–**11**. Reagents and conditions: (i) 190 °C, 20–30 mm Hg, 15 min; (ii) $Br(CH_2)_nBr$, DMF, 1–4 h; (iii) amine, DMF, 80 °C.

a *N*,*N*-dimethyl amino group linked with berberine by two or three carbon spacers, were less potent inhibitors when compared with berberine (berberine, IC_{50} = 0.374 μM; compound **4a**, two carbon spacer, IC_{50} = 1.74 μM; compound **4b**, three carbon spacer, IC_{50} = 0.20 μM). When considering the optimal carbon spacer, the volume of the substituted amino groups is also important for inhibitory activity. Among the three aliphatic substituted amino groups, the *N*,*N*-diethyl amino group gave the best results (*N*,*N*-diethyl amino group, IC_{50} = 0.105 μM; *N*,*N*-dimethyl amino group, IC_{50} = 0.20 μM; *N*,*N*-dipropyl amino group, IC_{50} = 0.310 μM). Interestingly, cyclic substituted amino groups appeared to have better activity than the chain substituted amino groups. For example, compounds **7b** and **8b**, pyrrolidine and piperidine linked with berberine by three carbon spacers, gave IC_{50} values of 0.093 μM and 0.052 μM, respectively. Compounds **9a** and **9b**, morpholine linked with berberine by two and three carbon spacers, showed poor activity compared with the lead compound (IC_{50} = 1.80 μM for **9a**, and IC_{50} = 1.456 μM for **9b**). The most potent inhibitor was **10b** (IC_{50} value = 0.020 μM), which contained a cyclohexanamine linked with berberine by three carbon spacers and was 18-fold more potent than berberine. It was surprising that compound **11b,** which possessed a hydroxyl group at the end of the side chain, exhibited much weaker anti-AChE activity than that of the lead compound berberine (IC_{50} = 2.37 μM). This result

Table 1
In vitro inhibition IC_{50} (μM) and selectivity of compounds **1**, **4**–**13** on AChE and BuChE



Compound	R	*n*	IC_{50}[a](μM)		Selectivity for AChE[c]	IC_{50}[a](μM) BuChE/ACh[d]
			AChE/ACh[b]	BuChE/BuCh[c]		
1	Berberine		0.374 ± 0.024	18.2 ± 0.683	48.6	n.t.[e]
4a		2	1.74 ± 0.135	1.65 ± 0.106	0.95	0.936 ± 0.044
4b		3	0.201 ± 0.022	10.4 ± 0.578	51.7	14.2 ± 0.297
5b		3	0.105 ± 0.003	2.64 ± 0.258	25.1	3.46 ± 0.111
6b		3	0.310 ± 0.007	2.88 ± 0.155	9.3	1.48 ± 0.0.085
7b		3	0.093 ± 0.024	3.00 ± 0.116	32.3	3.42 ± 0.0.134
8a		2	0.287 ± 0.010	0.242 ± 0.005	0.84	0.060 ± 0.001
8b		3	0.052 ± 0.005	2.59 ± 0.180	48.8	2.97 ± 0.0.122
9a		2	1.80 ± 0.190	>30	>16.7	n.t.
9b		3	0.456 ± 0.059	>30	>43.8	n.t.
10b		3	0.020 ± 0.002	4.17 ± 0.391	208	1.35 ± 0.0.07
11b	–OH	3	2.37 ± 0.098	3.23 ± 0.197	1.36	2.10 ± 0.0.035
Tacrine			0.311 ± 0.009	0.041 ± 0.003	0.13	n.t.

[a] Mean ± SD of at least three independent measurements.
[b] Acetylcholine substrate for evaluation of antiacetylcholinesterase activity.
[c] Butyrylcholine substrate for evaluation of antibutyrylcholinesterase activity.
[d] Acetylcholine substrate for evaluation of antibutyrylcholinesterase activity.
[e] n.t. = not tested.

indicated that the group at the end of the molecule influenced inhibitory activity.

In vitro BuChE inhibition was also determined using the same method. Most of these compounds demonstrated higher inhibitory potency against BuChE than the lead compound berberine. Compound **8a**, which gave an IC_{50} value of 0.287 μM for AChE, was the most potent inhibitor for BuChE with an IC_{50} value of 0.242 μM. Because BuChE preferentially acts on BuCh, but also hydrolyzes ACh, BuChE inhibition was also evaluated in the presence of ACh as the enzyme substrate. The IC_{50} values showed similar trends as those when BuCh was employed as the enzyme substrate.

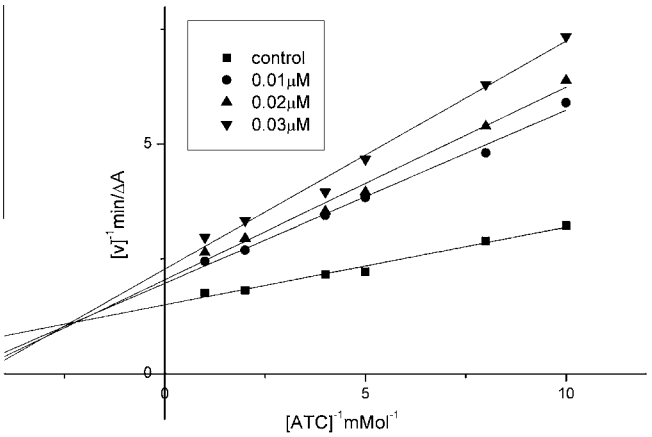


Figure 2. Steady-state inhibition by **10b** of AChE hydrolysis of ACh. Reciprocal plots of initial velocity and substrate concentration showing mixed-type inhibition for **10b** on AChE.

The mechanism of inhibition of AChE was investigated using the derivative **10b**, one of the most potent AChE inhibitors. Steady-state inhibition data of compound **10b** for AChE is shown in Figure 2. Lineweaver–Burk reciprocal plots revealed that there was an increasing slope and an increasing intercept at higher inhibitor concentrations, indicating mixed inhibition. The similar binding mode of compounds **10b**, E and D[16,17] was not surprising because all of these compounds had similar structures with minor differences only in the terminal group at the 9-position.

To investigate the possible mode of interaction of the compounds with *T. californica* enzyme (TcAChE), some of the test compounds were docked to the AChE active site gorge using the CDOCKER program in Discovery studio 2.1 software, based on the structure of the complex of TcAChE (PDB entry 2CMF). The most probable conformations of the ligands were chosen based on the docked energy value. As an example, the position of compound **10b** in the binding site with respect to the key residues is shown in Figure 3. Even though it behaved differently in the binding model compared with the original ligand of the X-ray structure, bis(5)-tacrine, compound **10b** could interact with both CAS and PAS which was consistent with the results from kinetic studies (see

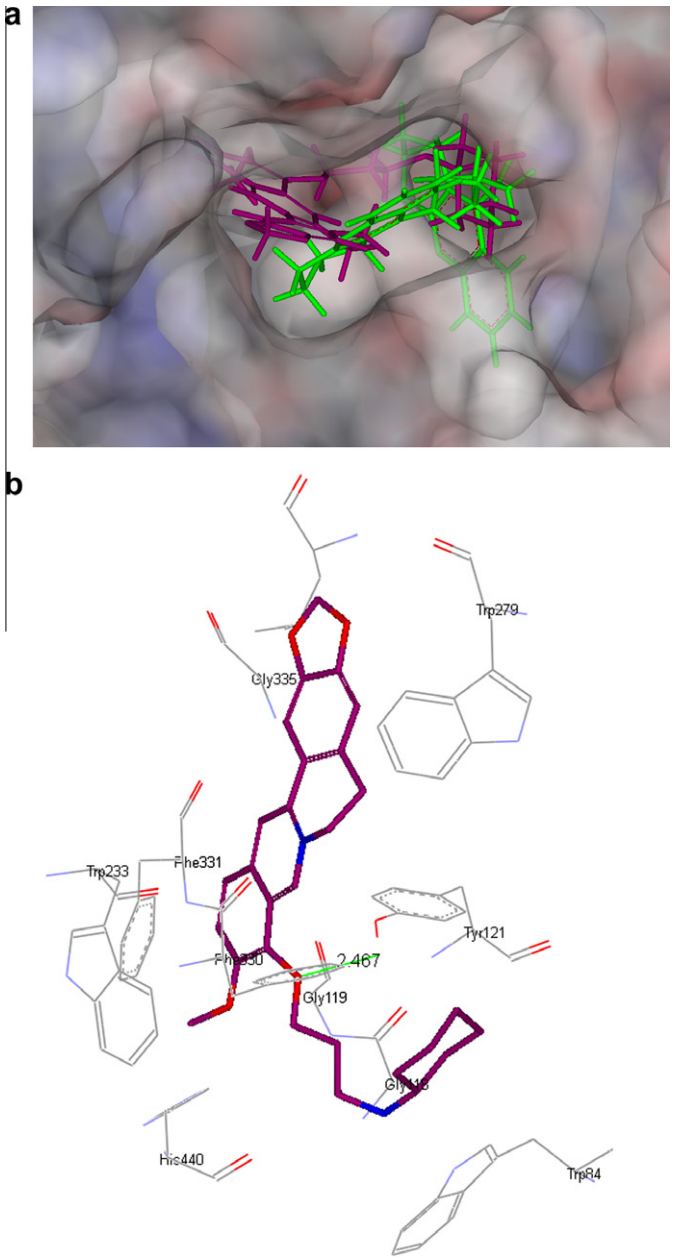


Figure 3. Docking models of the compound–enzyme complex. (a): stereoviews looking down the gorge of TcAChE binding with **10b** (colored purple) and the original ligand of the X-ray structure bis(5)-tacrine (colored green). (b) Representation of compound **10b** docked into the binding site of AChE highlighting the protein residues that form the main interactions with the inhibitor. Compound **10b** is shown in purple. Hydrogen-bonding interaction between ligand and residues Tyr121 is shown with the green line.

Fig. 3a). As shown in Figure 3b, the berberine moiety was firmly bound to the peripheral sites of AChE by its B ring which formed a tight connection with the electron-rich indole ring of Trp279 (average distance between rings of 4.2 Å). A classic parallel cation–π stacking of the quaternary nitrogen of berberine occurred with the aromatic rings of Tyr121, Phe330 and Phe331 (average distance of 5.0 Å, 5.1 Å and 5.8 Å, respectively), which snaked along the gorge of the enzyme active site. It is interesting that the 9-oxygen atom of berberine forms a direct hydrogen-bond contact with the backbone OH group of Tyr121 (average OH distance of 2.4 Å), which may contribute significantly to the inhibition activity of compound **10b**. Near the bottom of the gorge, the cyclohexylamine moiety of compound **10b** might interact with Phe330 and Trp84, as protonated amino groups at physiological pH, via hydrophobic interactions to form a cation–π interaction with these aromatic residues.

In conclusion, a series of berberine derivatives, with various amino groups linked at the 9-position of berberine with different carbon spacers, were designed, synthesized, and biologically evaluated as inhibitors of AChE and BuChE. All these berberine derivatives were potent inhibitors of AChE, with IC_{50} values ranging from micromolar to sub-micromolar. Among them, compound **10b** with a cyclohexylamino group linked to berberine by a three carbon spacer, gave the most potent inhibitor activity with an IC_{50} value of 0.020 μM against AChE. In addition, all these compounds showed moderate to potent activity towards BuChE. Kinetic studies indicated that these compounds exhibited a mixed type inhibition for both the CAS and the PAS, which were suggested by molecular modeling simulations of the AChE–inhibitor complex. Studies into the inhibitory activity of aggregation of beta-amyloid peptide induced by AChE are in progress.

Acknowledgments

We thank the Natural Science Foundation of China (20972198) and the Ministry of Science and Technology of China (No. 2009ZX09501-017) for financial support of this study.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.013.

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