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Bioorganic & Medicinal Chemistry Letters 17 (2007) 3765–3768
Bioorganic & Medicinal Chemistry Letters

Derivatives of oxoisoaporphine alkaloids: A novel class of selective acetylcholinesterase inhibitors

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Received 26 January 2007; revised 31 March 2007; accepted 5 April 2007
Available online 10 April 2007

Abstract—A series of 9-aminoalkanamido-1-azabenzanthrones derviatives (**3a**–**i** Ar–NHCO$(CH_2)_n$$NR^1R^2$) and their quaternary methiodide salts (**4a**–**g** Ar–NHCO$(CH_2)_n$$N^+(CH_3)R^1R^2I^-$) were designed and synthesized as acetylcholinesterase (AChE) or butyrylcholinesterase (BuChE) inhibitors. The synthetic compounds exhibited high AChE inhibitory activity with IC_{50} values in the nanomolar range and high selectivity for AChE over BuChE (45- to 1980-fold). The structure–activity relationships (SARs) were discussed.

Acetylcholinesterase (EC 3.1.1.7, AChE) is a hydrolase that catalyzes the hydrolysis of neurotransmitter acetylcholine and is present in the most prominent constituents of central cholinergic pathways. AChE plays a crucial role in central and peripheral nervous systems. Terminating the impulse transmission at cholinergic synapses, rapid hydrolysis by AChE into acetylcholine (ACh) is the vital function of AChE. Controlling the inhibition of the AChE enzyme activity can be used for treatment of diseases associated with ACh depletion, such as Alzheimer's disease,[1] myasthenia gravis,[2] and glaucoma.[3]

The comprehensive study of the AChE/inhibitor complexes by X-ray crystallography had indicated that AChE possessed a narrow gorge with two separate ligand binding sites, an acylation site (active site) and a peripheral site which was also called peripheral anionic site (PAS). Ligands which either occupied the active site or the PAS could inhibit the AChE activity, such as tacrine[4] (Fig. 1a) and propidium[5] (Fig. 1b).

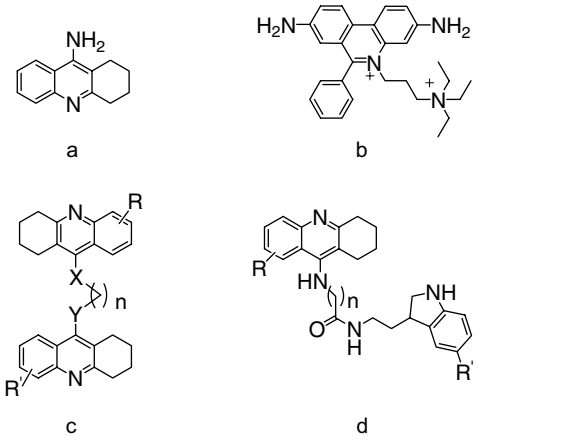

Figure 1. Chemical structures of some alkaloid inhibitors of AChE.

Recent study showed that AChE could also play a key role in accelerating senile amyloid β-peptide (Aβ) plaque deposition.[6] It was likely that AChE interacted with Aβ and promoted amyloid fibril formation through a pool of amino acids located in the proximity of PAS.[7]

Moreover, a peculiar structural difference between acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) was the lack of the PAS moiety in BuChE,[8]

Keywords: Oxoisoaporphine alkaloids; Synthesis; Acetylcholinesterase inhibitors; Butyrylcholinesterase.
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doi:10.1016/j.bmcl.2007.04.015

that seems to prevent the interaction of BuChE with Aβ, resulting ineffective on Aβ aggregation.[9] Actually, an inhibitor of AChE that strongly interacted with the PAS, that would be showing high AChE/BuChE selectivity, and that would exhibit inhibitory effect against AChE-induced Aβ aggregation is required. It implied that AChE inhibitors that were recognized at the PAS or interacted with both the catalytic site and PAS might exert a dual pharmacological effect,[10] which combined the enhancement of the cholinergic neurotransmission and the reduction in the pro-aggregating action of AChE, thus opening the way to a new promising therapeutic approach to Alzheimer's disease (AD). Based on the strategy, a number of studies had been performed, which included that of tacrine-related homo- and heterobivalent ligands (Fig. 1c),[11] and tacrine-melatonin hybrids (Fig. 1d).[12]

Oxoisoaporphine alkaloids[13,14] were isolated from the rhizome of *Menispermum dauricum* DC. (Menispermaceae) which were widely present in the People's Republic of China. The rhizomes of the plant were used in traditional Chinese medicine and are officially listed in the Chinese Pharmacopoeia as an analgesic and antipyretic. Oxoisoaporphine alkaloids possessed a 1-azabenzanthrone moiety in their structures. Based on the structural information of AChE and AChE inhibitors, the planar 1-azabenzanthrone moiety with an ammonium group might bind to PAS by π–π stacking and electronic interaction. Introduction of a side chain with terminal amines or ammonium groups in the 1-azabenzanthrone could greatly improve AChE/BuChE selectivity and water-solubility of the alkaloids.

In view of the above reasons, a series of oxoisoaporphine derivatives (**3a**–**i** and **4a**–**g** in Scheme 1) with different basic side chain (n = 1, 2, and 3) at 9-position of 1-azabenzanthrone were designed and synthesized, and their anti-AChE and BuChE activities were tested. The structure-activity relationships (SARs) were also discussed.

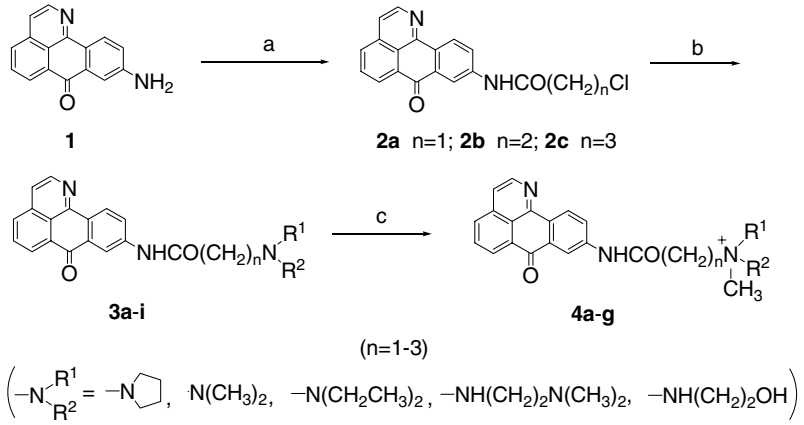
Target compounds **3a**–**i** and **4a**–**g** were synthesized as shown in Scheme 1. Reaction of 9-nitro-1-azabenzanthrone[15,16] with Na_2S led to 9-amino-1-azabenzanthrone **1** in 95% yield. The ω-haloalkanamides **2a**–**c** were prepared in quantitative yield by acylation of **1** with the appropriate acid halide. Subsequent aminolysis of **2a**–**c** by reflux treatment with the appropriate secondary amines or primary amines gave compounds **3a**–**i**. Finally, the corresponding quaternary methiodide salts **4a**–**g** were obtained by treatment with CH_3I in $CHCl_3$.

Inhibitory activities toward AChE and BuChE in vitro of synthetic compounds were determined according to the modified Ellman method[17] using commercially available galanthamine as the reference standard. AChE[18] from *electric eel* and BuChE from *equine serum* were purchased from Sigma Corporation.

The IC_{50} values for AChE and BuChE inhibition are summarized in Table 1. All the synthesized compounds demonstrated much higher inhibitory potency against AChE than the lead compound **1**, inhibitory activity with IC_{50} values in the nanomolar range, and high selectivity for AChE over BuChE. This result indicated that introduction of the amino group side chains could increase the inhibitory capacity and selectivity.

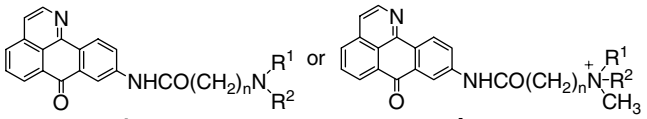
According to the data shown in Table 1 and Figure 2 the synthesized cationic compounds with quaternary nitrogen showed higher inhibitory effects on AChE (**4a** and **4b** with AChE inhibitory activity at 1.08 and 1.06 nM, respectively), comparing with the corresponding non-quaternary nitrogen compounds (**3a** and **3b** with AChE inhibitory activity at 6.18 and 2.47 nM, respectively). Similar tendency was also found in compounds **3c**–**g** and their corresponding quaternary methiodide salts **4c**–**g**. Structural resemblance of quaternary functionality between the synthesized cationic compounds and ACh would be responsible for competition for the binding site of AChE.

The structure of terminal groups of side chain has also effects on their inhibitory activities (Fig. 3). High inhibitory potency was found to be associated with pyrrolidine at the end of side chain (**3a**–**c** and **4a**–**c**).



Scheme 1. Synthesis of oxoisoaporphine derivatives. Reagents and conditions: (a) $ClCO(CH_2)_nCl$/reflux; (b) R_2NH/EtOH/KI/reflux; (c) CH_3I/$CHCl_3$/rt/24 h.

Table 1. In vitro inhibition and selectivity of compounds **1**, **3a–i**, **4a–g** and Galanthamine on AChE and BuChE



3a-i **4a-g**

Compound	$-N\begin{matrix}R_1\\R_2\end{matrix}$	*n*	Yield (%)	IC_{50}[a](nM) for AChE	IC_{50}[b](nM) for BuChE	Selectivity for AChE/BuChE[c]
1			88	26900 ± 1430	$>10^5$	
3a		1	87	6.18 ± 1.33	3490 ± 52	560
3b	—N	2	88	2.47 ± 0.15	2410 ± 27	980
3c		3	20	12.08 ± 0.74	3610 ± 61	300
4a		1	90	1.08 ± 0.11	1690 ± 23	1565
4b	—N+	2	88	1.06 ± 0.17	1960 ± 25	1850
4c		3	65	2.51 ± 0.26	3230 ± 57	1290
3d		1	80	96.46 ± 3.7	4370 ± 91	45
3e	$-N(CH_3)_2$	2	70	33.87 ± 0.53	1950 ± 22	58
3f		3	15	30.51 ± 0.93	4400 ± 63	144
4d		1	85	14.95 ± 1.13	1310 ± 20	88
4e	$-N^+(CH_3)_3$	2	87	4.81 ± 0.36	2820 ± 23	590
4f		3	67	4.79 ± 0.44	2370 ± 31	490
3g	$-N(CH_2CH_3)_2$	2	78	11.59 ± 1.28	6060 ± 68	523
4g	$-N^+(CH_3)(CH_2CH_3)_2$	2	85	2.62 ± 0.19	5190 ± 84	1980
3h	$-NH(CH_2)_2N(CH_3)_2$	2	70	119.9 ± 3.5	5370 ± 78	45
3i	$-NH(CH_2)_2OH$	2	90	55.1 ± 1.9	5340 ± 88	97
Galanthamine				550 ± 8.74	14400 ± 120	26

[a] IC_{50}, 50% inhibitory concentration (means ± SEM of three experiments) of AChE.
[b] IC_{50}, 50% inhibitory concentration (means ± SEM of three experiments) of BuChE.
[c] Selectivity for AChE = IC_{50} (BuChE)/IC_{50} (AChE).

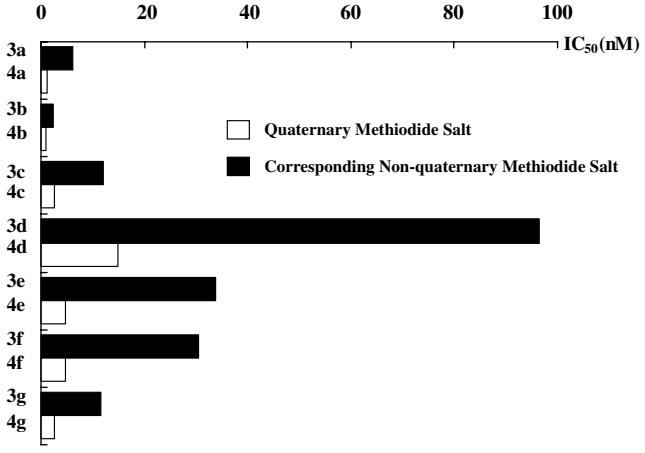


Figure 2. In vitro effects of cationic derivatives on AChE.

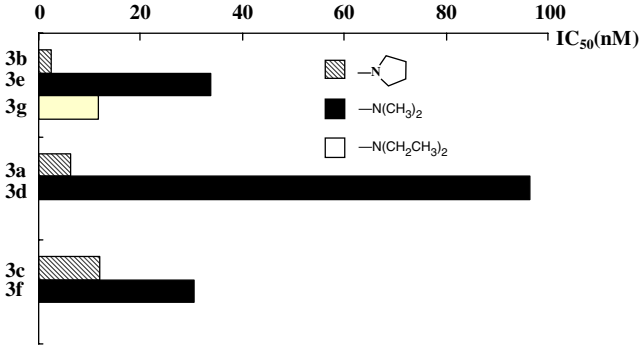


Figure 3. Effects of structure of terminal groups on AChE.

Dimethylamine derivatives showed less potency (**3d–f** and **4d–f**).

The effects on AChE inhibition of synthetic compounds with variation of chain length (*n* = 1, 2, or 3) are shown in Table 1. It had less influence on activity than the functional group structure.

All the synthetic compounds showed high selectivity for AChE over BuChE. The range of AChE/BuChE selectivity ratios was from 45- to 1980-fold. The selectivity ratios of compounds were dependent on their inhibitory potential against AChE. The compounds showed higher inhibitory potential against AChE that would possess higher AChE/BuChE selectivity ratios. Compounds **4b** showed the highest inhibitory activity (IC_{50} 1.06 nM) and also had highest selectivity ratios (1850-fold). This result indicated that the synthetic compounds could favor binding to PAS and have a strong binding affinity with PAS of AChE.

In conclusion, a novel class of synthetic 9-aminoalkanamido-1-azabenzanthrone derivatives were designed

and synthesized. All the synthetic compounds showed high AChE inhibitory activity with IC_{50} values in the nanomolar range. The synthesized cationic compounds with quaternary nitrogen showed higher inhibitory effects on AChE. The compounds with pyrrolidine at the end of side chain possessed higher inhibitory activity. But the compounds with variation of chain length showed less influence on inhibitory activity. Moreover, the synthetic compounds also showed high selectivity for AChE over BuChE (45- to 1980-fold). The selectivity ratios of compounds were dependent on their inhibitory potential against AChE, which resulted from the binding of compounds with PAS of AchE. We hope that the work could be beneficent for development of potential AChE inhibitors with higher inhibitory activity and good selectivity for AChE over BuChE in the future.

Acknowledgments

We thank the Natural Science Foundation of China (Grant 20472117), the NSFC/RGC Joint Research Scheme (Grants 20710006, N_PolyU 508/06), the Science Foundation of Guangzhou (2006Z2-E402), and the Hong Kong Polytechnic University Area of Strategic Development Fund for financial support of this study.

Supplementary data

Supplementary data (such as synthetical procedure, structural analysis data, and in vitro AChE and BuChE assay method) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.04.015.

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