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Novel aromatic–polyamine conjugates as cholinesterase inhibitors with notable selectivity toward butyrylcholinesterase

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ABSTRACT

Three types of aromatic–polyamine conjugates (**6a**–**6s**) were designed, synthesized and evaluated as potential inhibitors for cholinesterases (ChEs). The results showed that anthraquinone–polyamine conjugates (AQPCs) exhibited the most potent acetylcholinesterase (AChE) inhibitory activity with IC_{50} values from 1.50 to 11.13 μM. Anthracene–polyamine conjugates (APCs) showed a surprising selectivity (from 76- to 3125-fold) and were most potent at inhibiting butyrylcholinesterase (BChE), with IC_{50} values from 0.016 to 0.657 μM. A Lineweaver–Burk plot and molecular modeling studies indicated that the representative compounds, **6l** and **6k**, targeted both the catalytic active site (CAS) and the peripheral anionic site (PAS) of ChEs. Furthermore, APCs did not affect HepG2 cell viability at the concentration of 100 μM. Consequently, these polyamine conjugates could be thoroughly and systematically studied for the treatment of AD.

1. Introduction

Alzheimer's disease (AD) is characterized by dementia, cognitive impairment, and memory loss and is one of the most common diseases in elderly people[1,2] Current treatment of AD focuses on acetylcholinesterase (AChE) inhibitors, such as tacrine, donepezil, rivastigmine and galantamine. However, the potential effectiveness of such inhibitors in clinical use is often complicated by their associated side effects. For example, clinical studies have shown that the AChE inhibitor tacrine causes hepatotoxicity.[3] Since AD is a multi-pathogenic illness, a current drug-discovery strategy is to develop novel anti-Alzheimer agents with multiple potencies such as inhibition of both AChE and butyrylcholinesterase (BChE).[4]

Two major ChEs, AChE and BChE, are involved in the hydrolysis and regulation of acetylcholine in vertebrates. Various cholinergic drugs, primarily inhibitors of AChE also function as BChE inhibitors.[5] The use of agents with enhanced selectivity for BChE including cymserine and MF-8622 indicated potential therapeutic benefit of inhibiting BChE in AD and related dementias. BChE specific inhibition is unlikely to be associated with adverse events and may show efficacy without remarkable side effects.[6] Therefore BChE may be considered as an important target for novel drug development to treat AD. In the future, the development of specific BChE inhibitors and the continued use of cholinesterase inhibitors may lead to improved clinical outcomes.[7]

Polyamines such as putrescine, spermidine and spermine (Fig. 1) are aliphatic molecules with amine groups distributed along their structure and are the most common natural products. These compounds have always been regarded by medicinal chemists as a universal template[8] and many research groups have chosen to focus their investigations on the polyamine metabolic cycle or the design of polyamine modified drugs.[9–12] Our group has chosen to focus on the development of polyamine conjugates as potential drugs for the treatment of cancer and AD for many years.[13–16] Our research has found that quinoline–polyamine conjugates exhibit potent ChEs inhibitory activity and that polyamines occupy the gorge of AChE.[17] Therefore, to further explore the anti-Alzheimer potential of the polyamine conjugates, we designed and synthesized three types of aromatic–polyamine conjugates and screened them for their ability to inhibit ChEs.

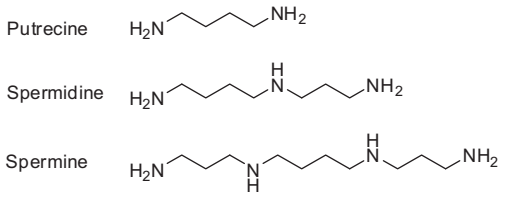

Figure 1. Chemical structures of putrecine, spermidine and spermine.

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2. Results and discussion

2.1. Chemistry

It is report that many aromatic polycyclic such anthrarobin and anthraquinone have shown diverse activity depending on the functional groups attached[18,19] In this paper we chose three kinds of aromatic polycyclic building blocks (naphthalene, anthracene and anthraquinone) that are coupled to diverse polyamine motifs (Fig. 2) to evaluate their inhibition ability to ChEs. The synthesis of compound **2** was shown in Supplementary data, polyamine skeletons **4e**–**4j** were synthesized in our lab[16] and the target compounds were synthesized according to Scheme 1. Intermediates **1a**, **2a**, **3a** were obtained by the condensation of chloroacetyl chloride with **1**, **2** and **3** as the starting material. The reaction of **1a**–**3a** with amines **4a**–**4j** gave the intermediates **5a**–**5s**, and their Boc groups in the polyamine skeleton were subsequently removed with 4 M HCl at room temperature to provide hydrochloride salt target compounds. The structures of the target compounds were confirmed by ^{1}H NMR, ESI-MS and elemental analysis.

2.2. In vitro inhibition studies on AChE and BChE

To determine the potential effectiveness of the target compounds **6a**–**6s** for the treatment of AD, their ChEs inhibitory activity was determined by the method described by Ellman et al,[20] using tacrine as a reference compound. The IC_{50} values for ChEs inhibition and the selectivity index (SI) are summarized as shown in Table 1. Due to their poor solubility, compounds **6f** and **6g** were not measured. Generally, most of the synthetic compounds tested (**6b**–**6d**, **6h**–**6o**) showed inhibitory selectivity for BChE over AChE. Anthraquinone–polyamine conjugates (AQPCs) (**6p**–**6s**) inhibited AChE better than naphthalene–polyamine conjugates (NPCs) and anthracene–polyamine conjugates (APCs), while APCs **6h**–**6o** exhibited the most potent inhibition of BChE.

Compounds **6l**, **6q** and **6r** were the most potent inhibitors of AChE and had an IC_{50} value of 2.74, 1.50 and 2.63 μM, respectively, while compounds **6k** and **6l**, the most potent inhibitors of BChE, had IC_{50} value of 0.016 and 0.023 μM, respectively, which were much lower than that of tacrine (IC_{50} = 0.037 μM). Moreover, a compound's ability to inhibit enzyme activity was directly proportional to the chain length of its polyamine moiety such that four (**6k**) and six (**6l**) methylene groups was the optimal chain length between two nitrogen atoms associated with the most potent inhibition of BChE. When the distance between these two nitrogen atoms is too long or too short, the compound is unable to optimally inhibit BChE.

All of the synthetic anthracene conjugates showed high selectivity for BChE over AChE and the ratio of BChE/AChE selectivity ranged from 76- to 3125-fold. The selectivity ratios of compounds were dependent on their inhibitory potential against BChE. The compounds showed higher inhibitory potential against BChE that would possess higher BChE/AChE selectivity ratios. Compounds **6k** showed the highest inhibitory activity (IC_{50} = 16 nM) and also had the highest selectivity ratio (3125-fold). This result indicated that the anthracene conjugates could favor binding to BChE.

2.3. Kinetic characterization of ChEs inhibition

Graphical analysis of steady state inhibition of the most potent compounds for AChE and BChE, APCs **6l** and **6k**, were investigated to determine the kinetics of ChEs inhibition as shown in Figure 3.[21] The Lineweaver–Burk plots showed both increasing slopes and increasing intercepts for higher inhibitor concentration. The pattern indicated a mixed-type inhibition, hence this kinetic study suggested that compound **6l** bound to both catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE. A similar interaction was found between **6k** and BChE.

2.4. Molecular modeling study

To investigate the interaction mode of compound **6l** with *Tc*AChE (PDB code: 1ACJ), molecular modeling was carried out by AUTODOCK 4.0 package with PyMOL program as shown in Figure 4.[22,23] The docking result demonstrated that compound **6l** exhibited multiple binding modes with AChE. In the **6l**–*Tc*AChE complex, compound **6l** occupied the entire enzymatic CAS, midgorge and PAS. The anthracene moiety was bound to CAS, displaying a classic π–π stacking interaction between Trp84 and Phe330. At the PAS, the end protonated nitrogen atom of the polyamine

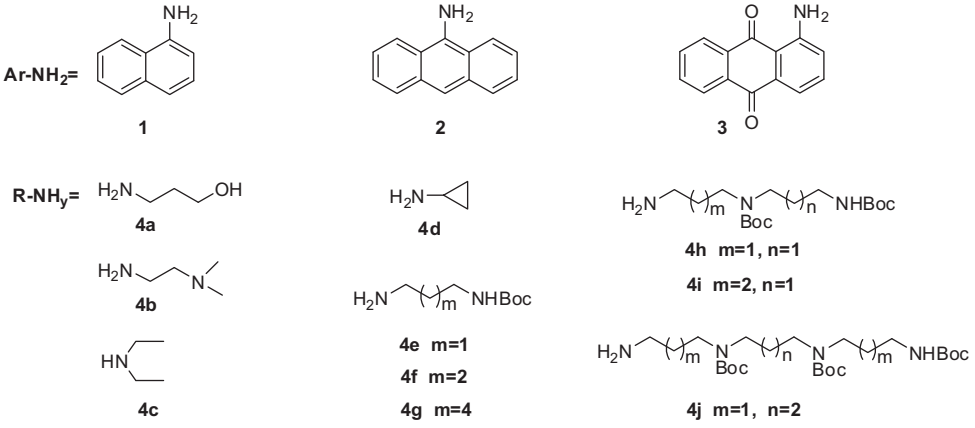
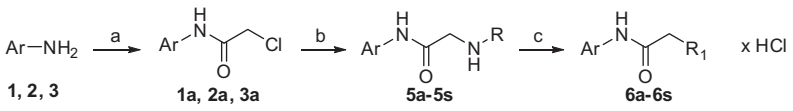


Figure 2. Building blocks of the designed library.



Scheme 1. Reagents and conditions: $ClCOCH_2Cl$, THF, Et_3 N, rt, 6 h for **1a** and **2a**; $ClCOCH_2Cl$, DMF, pyridine, rt, 24 h for **3a**; (b) 1.0 equiv of R–NH_y, EtOH, Et_3N, reflux, 6 h for **5a**–**5o**; 1.0 equiv of R–NH_2, CH_3CN, K_2CO_3, KI, rt, 24 h for **5p**–**5s**; (c) 4 M HCl, EtOH, rt, overnight.