European Journal of Medicinal Chemistry 46 (2011) 2170–2184

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Quinolizidinyl derivatives of bi- and tricyclic systems as potent inhibitors of acetyl- and butyrylcholinesterase with potential in Alzheimer's disease

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ARTICLE INFO

Article history:
Received 5 January 2011
Received in revised form
23 February 2011
Accepted 26 February 2011
Available online 5 March 2011

Keywords:
Acetylcholinesterase inhibitors
Butyrylcholinesterase inhibitors
Quinolizidine derivatives
Bi- and tricyclic systems derivatives
Single-entity multi-target agents

ABSTRACT

On the pattern of the potent and selective butyrylcholinesterase (BChE) inhibitors ethopropazine and Astra1397, sets of quinolizidinyl derivatives of bi- and tricyclic (hetero)aromatic systems were studied as dual, or BChE-selective inhibitors. All compounds exhibited activity against both cholinesterases, but inhibition of BChE was generally stronger, with submicromolar IC_{50} values for most of them (e.g. **15**: IC_{50} *versus* BChE = 0.15 μM; SI = 47). However, in a subset of quinolizidinyl derivatives of 6-hydroxycoumarin an inverted selectivity for acetylcholinesterase (AChE) was observed (e.g. **46**: IC_{50} *versus* AChE = 0.35 μM; SI = 0.06). Docking studies furnished a sound interpretation of the observed different enzyme activity. Several of the studied compounds have shown, in the past, additional pharmacological properties (as antagonism on presynaptic muscarinic autoreceptor; inhibition of enkephaline aminopeptidase and antipsychotic activity) of some relevance in Alzheimer's disease, and may, therefore, represent hits for the development of interesting single-entity multi-target drugs.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a gradual decline of cognitive processes, later associated with behavioral and psychiatric symptoms. The multifactorial pathogenesis of AD includes accumulation of aggregates of β-amyloid (Aβ) and tau protein and loss of cholinergic neurons, with consequent deficit of the neurotransmitter acetylcholine (ACh) [1].

The inhibition of acetylcholinesterase (AChE, EC 3.1.1.7), that is responsible for the breakdown of ACh, has proven a successful approach to relieve some cognitive and behavioral symptoms of AD [2,3]. In advancing AD, AChE levels in the brain are declining, but a progressive increase (up to 90%) of butyrylcholinesterase (BChE, EC. 3.1.1.8) is observed, which too is able, even if at lower rate, to hydrolyze Ach [4]. Selective BChE inhibitors have already been reported to increase the ACh levels in the brain, and, very interestingly, to also reduce the formation of abnormal amyloid [5–7]. Therefore the discovery of potent and highly selective BChE inhibitors and/or of dual AChE–BChE inhibitors, is an actively pursued goal in AD. Indeed, many research projects in the field have been focused on the identification of new ligands addressing multiple key targets through the so-called "single entity-multitarget ligand" or "multi-target directed ligand" approaches [8].

At present, cholinesterase inhibitors (Fig. 1) are the most commonly used drugs for the treatment of mild and moderate AD, despite no long-term efficacy has been proved. Donepezil [9] and galantamine [10] are highly active and specific AChE inhibitors, while rivastigmine [11] is a dual (and long lasting) inhibitor, which has been reported to co-inhibit AChE and BChE in human brain with equal potency [11c], in contrast with results on human erythrocytes and plasma enzymes [7] (Fig. 1). The first approved drug, tacrine [12], was recently withdrawn because of high incidence of hepatotoxicity, while clinical trials with eptastigmine [13] have been suspended due to adverse hematological effects.

Abbreviations: Aβ, β-amyloid; ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; ADME, absorption, distribution, metabolism and excretion; BChE, butyrylcholinesterase; ChE, cholinesterase; DTNB, 5,5-dithiobis(2-nitrobenzoic acid); HB, hydrogen bond; NMDA, N-methyl-d-aspartate; PAS, peripheral anionic site; SI, selectivity index; TcAChE, torpedo californica acetylcholinesterase.

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doi:10.1016/j.ejmech.2011.02.071

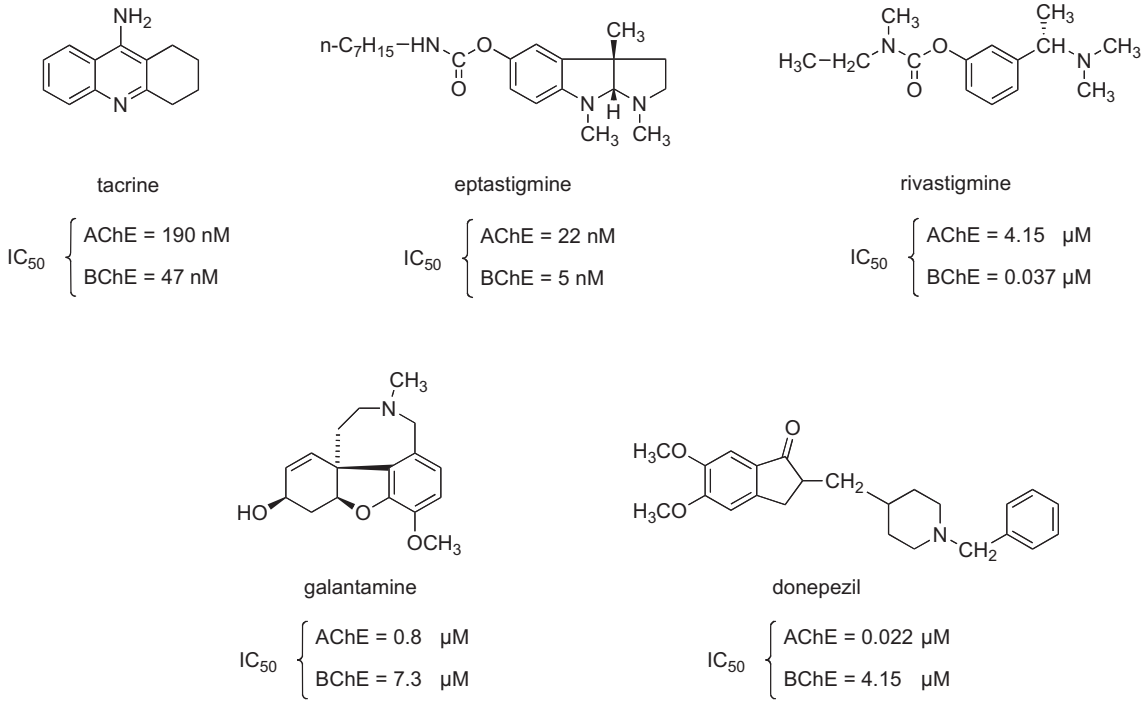

Fig. 1. Cholinesterase inhibitors used in AD therapy. IC_{50} for human erythrocyte and plasma enzymes.

An alternative symptomatic treatment of moderate-to-advanced AD may be made with memantine [14], an N-methyl-D-aspartate (NMDA) receptor antagonist, while there is no approved treatment with proven disease-modifying effects [2b].

Potent and highly selective BChE inhibitors are represented by some physostigmine derivatives and analogs, as phenethylcymserine [15] and [4-(4-morpholinyl)butyl]carbamic acid 3-(1,1-dimethyl-2-dimethylaminoethyl)phenyl ester [16] (MF 8622), and also by some phenothiazine derivatives as ethopropazine [17], N-[(2-diethylamino)propionyl]phenothiazine [18] (ASTRA1397; **28**) and 10-(9-anthrylcarbonyl)phenothiazine [19] (Fig. 2). The last compound is the most potent (K_i = 3.5 nM) among a large set of 10-aroyl-, 10-(ω-aryl)alkanoyl- and 10-alkanoylphenothiazines studied by Darvesh and coll. [19,20] and, interestingly, is devoid of any basic group and exhibits a log P value as high as 6.31. According to the authors, enzyme selectivity and inhibitory potency might be related to the larger active site gorge of BChE in respect to AChE, while the two aromatic rings of phenothiazine provided the binding to BChE through π–π interactions with two aromatic side chains of residues F329 and Y332.

Thus, in order to achieve novel cholinesterase inhibitors, either dual or, even better, selective for BChE and to gain insight on the structural determinants of high BChE affinity and selectivity we have investigated a number of derivatives of phenothiazine and other related tricyclic systems. Moreover, to better exploit the key role of the tricyclic systems in ligand binding, some additional compounds derived from diphenylamine and diphenylmethane and from bicyclic heterocycles as coumarin, were also considered. Indeed coumarin moiety has been demonstrated to be compatible with high anticholinesterase potency [21,22].

Most of the tested compounds are characterized by the presence of a quinolizidine ring, a basic moiety that is simultaneously bulky and high lipophilic, linked to the aromatic moieties through spacers of different length and flexibility. It is worth noting that even simple derivatives of quinolizidine nucleus, as the *epi*-lupinine esters of 4-hydroxycinnamic and ferulic acids [23] and dicarboxylic acid esters of lupinine [24], are endowed with some anticholinesterase activity. Glutaric acid ester of lupinine exhibited K_i = 199 and 14800 nM *versus* AChE and BChE, respectively; quaternarization reduced the potency *versus* AChE while increased that for BChE, thus shifting the ratio of the corresponding K_i values from 74 to 4.3.

Some well known tricyclic drugs, as the antiparkinson ethopropazine [17,25] and methixene [26], the antipsychotic triflupromazine [27] and periciazine [28] as well as **28** [18,29] and the antimalarial quinacrine [30], have been included in our biological screening. Three of them which have been already proven to be endowed with anticholinesterase action (i.e., ethopropazine, **28** and quinacrine) served also as reference compounds.

On the whole 48 compounds were evaluated for their inhibitory activity on AChE and BChE from bovine erythrocytes and equine serum, respectively. Their structures and inhibition data are collected in Table 1.

2. Chemistry

Most of the studied compounds have been already described by some of us [31–42] and investigated for different pharmacological aims, and were now purposely reprepared according to the references cited for each of them in Table 1. Ethopropazine (**1**), periciazine (**3**) and methixene (**14**) were recovered and thoroughly purified from commercially available drugs (Parsidol®, Neuleptil® and Tremaril®, respectively), while quinacrine (**40**) was purchased from Sigma–Aldrich. The remaining compounds **4**, **16**, **21**, **24**, **25**, **33**, **42** and **45**–**48**, in Table 1 were obtained as follows.

Compound **16** was obtained by reacting the previously described **15** [36] with methyl iodide.

The quinuclidinyl phenothiazine **4**, though described in a patent [43a], has been now prepared with minor modifications by reacting phenothiazine with 3-quinuclidinyl tosylate [43b] obtaining a product with a higher melting point. The phenothiazine derivative **33** was prepared by reacting phenothiazine with 2-bromopropionyl bromide, followed by thiolupinine [(1*R*,9a*R*)