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Original article

Novel alkyl- and arylcarbamate derivatives with *N*-benzylpiperidine and *N*-benzylpiperazine moieties as cholinesterases inhibitors

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ABSTRACT

The study presents synthesis and biological activity of novel alkyl- and arylcarbamate derivatives with *N*-benzylpiperidine and *N*-benzylpiperazine moieties designed as cholinesterases inhibitors. These fragments turned out to determine compounds' selectivity between AChE and BuChE. Derivatives of *N*-benzylpiperazine (**16**–**25**) were selective BuChE inhibitors with 3-(2-(4-benzylpiperazin-1-yl)-2-oxoethyl)-phenyl butylcarbamate (**22**) being the most potent compound ($pIC_{50} = 5.00$) while a series of carbamate derivatives of *N*-benzylpiperidine (**5**–**14**) displayed non-selective BuChE/AChE inhibitory activity. Molecular modelling studies point out significant differences between orientations of these two groups of compounds in the active site of AChE, which can be an explanation of their different biological activity.

1. Introduction

Acetylcholinesterase inhibitors are still the most frequently used drugs in treatment of Alzheimer's disease (AD) [1,2]. Their introduction into the AD treatment arose from the *cholinergic hypothesis* of AD [3,4] blaming deficit in central cholinergic transmission for cognitive and non-cognitive symptoms observed in patients. As acetylcholinesterase (AChE) was thought to be the only enzyme responsible for the hydrolysis of acetylcholine in the central nervous system (CNS), it was the main target in search for anti-AD drugs. As a consequence, four AChE inhibitors belonging to different chemical groups have been approved for the symptomatic treatment of mild to moderate stages of AD: tacrine, donepezil, rivastigmine and galantamine. Recently, significant evidence pointing out the role of butyrylcholinesterase (BuChE) in the cholinergic system function has appeared [5]. In physiological conditions cholinesterase activity in the brain is mostly related to AChE. However, over the course of AD, AChE activity progressively decreases in certain brain regions, whereas BuChE activity increases and BuChE may then act as a compensatory mechanism for acetylcholine hydrolysis [6]. It has also been proved, that aside from their typical enzymatic function, cholinesterases display several non-classical activities like: regulation of cerebral blood flow and metabolism [7,8], modulatory effects on the amyloid cascade [9–11], glial proliferation, tau protein phosphorylation [12,13], and inflammatory processes [14], which are all important for the AD pathogenesis. Influence on the amyloid cascade is of special interest as it has been observed that in Alzheimer's disease AChE and BuChE are mostly located within neuritic plaques [15] and both seem to be associated with the formation of cytotoxic β-amyloid (Aβ) fibrills. In numerous experiments it has been shown that AChE not only initiates transformation of relatively inert Aβ into pathogenic plaques [9,11], but also it increases neurotoxicity of such aggregates [16–18].

Due to cholinergic hypothesis and numerous reports regarding multiple functions of cholinesterases in pathogenesis and development of AD, AChE and BuChE are very attractive targets for the development of anti-AD drugs. Recently developed cholinesterases inhibitors for the treatment of AD represent structural modifications of existing drugs and other compounds [19–25]. Taking into consideration that the role of BuChE in AD is not clearly understood [6,26], and relatively few selective inhibitors of this enzyme have been investigated [27–29], there is a constant need for new BuChE inhibitors, which can serve both as potential anti-AD drugs and pharmacological tools.

The presented study describes a synthesis and preliminary *in vitro* activity screening of 4 new series of potential cholinesterases inhibitors. The structures were designed as a combination of cholinesterases inhibitors pharmacophores, carbamates and arylalkylamines linked by a phenylacetamide fragment. Carbamate moieties, present in the structure of pseudoirreversible AChE inhibitors, rivastigmine and physostigmine, play a crucial role in

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their activity due to carbamylation of serine in the catalytic triad of the enzyme. *N*-benzylpiperidine, *N*-benzylpiperazine and amide fragments were introduced into structures of the new compounds to provide hydrophobic interactions and hydrogen bonds formation within cholinesterases active sites (Fig. 1).

2. Results and discussion

2.1. Chemistry

New carbamate derivatives (**5**–**25**) were synthesized via the route outlined in Scheme 1. In the first step, activation of the carboxylic groups of hydroxyphenylacetic acids using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) or 1,1′-carbonyldiimidazole (CDI) followed by the coupling with the 1-benzylpiperazine and 4-amino-1-benzylpiperidine lead to amides **1**–**4** in moderate yields (30–55%). In the next step the obtained phenols were transformed into the target compounds (**5**–**8**, **10**–**13**, **15**–**19**, **21**–**24**) in reactions with alkyl- and aryl isocyanates. Carbamoylation of compounds **1**–**4** with dimethylcarbamoyl chloride gave compounds **9**, **14**, **20** and **25**. Further purification by recrystallization or column chromatography afforded the desired carbamates in 44–99% yields.

2.2. Biological activity

Inhibitory potencies of the newly synthesized compounds against AChE from electric eel and BuChE from horse serum were evaluated by the spectroscopic method of Ellman et al. [30]. This test is based on the reaction of 5,5′-dithio-bis-(2-nitrobenzoic) acid, known as DTNB or Ellman's reagent, with the sulfhydryl group of acetylthiocholine or butyrylthiocholine which results in formation of a yellow-coloured product, i.e. 2-nitro-5-thiobenzoic acid. Changes of absorbance recorded at 412 nm determine the activity of tested compounds.

The assays were performed at a 100 μM concentration of the potential inhibitors. The activity measured is given in percentage of enzyme inhibition and as pIC_{50} values. pIC_{50} values were only determined for compounds with more than 60% inhibitory activity.

As shown in Table 1 almost all carbamate derivatives displayed moderate or good BuChE inhibitory activities ranging from $pIC_{50} = 4.26$ to 5.24 (62.49–91.97%). Only compounds **10** and **20** showed activities lower than 50%. Similar activities against BuChE appear for both *N*-benzylpiperidine and *N*-benzylpiperazine series as well as for *meta*- or *para*- substituted analogues. The results suggest that the larger and more lipophilic butyl substituent seems to be beneficial for the activity of the compounds (**11**, **22**) while other structural changes do not have such a strong influence. Interestingly, among the tested compounds only the *N*-benzylpiperidine series (**5**–**14**) displayed activity against AChE. Inhibitory potencies of compounds in this group were ranging from 19.21 to 50.45% at 100 μM concentration, which makes them weak AChE inhibitors with the most potent compounds bearing a small dimethyl substituent in the carbamate group, **9** ($pIC_{50} = 4.00$) and **14** ($pIC_{50} = 3.98$). The same tendency was noticed for intermediates **1**–**4**, lacking carbamoyl moieties, although their activities were lower (Table 1). These results point out the importance of the *N*-benzylpiperazine and *N*-benzylpiperidine fragments for activity and selectivity of these two groups of compounds, whereas carbamoyl groups, though able to modify activity, seem not to be crucial.

2.3. Molecular modelling

In order to explain possible interactions between the newly synthesized compounds and the active site of acetylcholinesterase and butyrylcholinesterase, molecular modelling studies were performed. Using Glide 4.5 (Schrödinger LLC, 1999–2007), compounds (**5**–**25**) were docked to the active site of AChE derived from complex of the enzyme with donepezil (PDB code: 1EVE) and to BuChE derived from complex of the enzyme with butyrate (PDB code: 2J4C). Water molecules were deleted beyond the radius of 5 Å from reference ligand (donepezil or butyrate, respectively) by Protein Preparation Wizard and then AChE or BuChE was used to prepare the Receptor Grid. All ligands were optimized with OPLS-2005 method by LigPrep. Docking was carried out on all derivatives resulting in 10 poses of each ligand, sorted by increasing scoring function.

Molecular modelling and docking studies showed significant differences between orientations of compounds of *N*-benzylpiperidine (**5**–**15**) and *N*-benzylpiperazine (**16**–**25**) series in the active site of AChE. Types of interactions for selected representatives of each series (**9**, **20**) are presented in Fig. 2 (visualized by PyMOL program). Compound **9** orientates along the active site gorge, extending from the active site at the bottom near Trp84 (*N*-benzylpiperidine fragment), to the peripheral binding site at the top near Trp279 (carbamoyl fragment). Three major functional moieties of this molecule, i.e. the benzyl group, the piperidine nitrogen atom and the phenyl ring

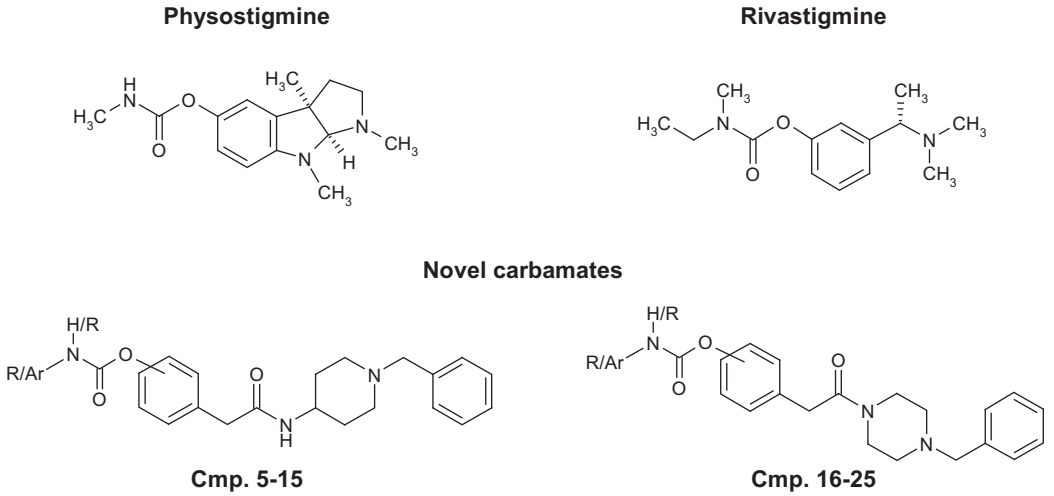

Fig. 1. Structures of cholinesterases inhibitors, physostigmine and rivastigmine, and new carbamate derivatives **5**–**25**.