European Journal of Medicinal Chemistry 45 (2010) 5602–5611

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

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

Anna Więckowska, Marek Bajda, Natalia Guzior, Barbara Malawska*

Department of Physicochemical Drug Analysis, Chair of Pharmaceutical Chemistry, Jagiellonian University Medical College, Medyczna 9 St. 30-688 Kraków, Poland

ARTICLE INFO

Article history:
Received 10 July 2009
Received in revised form
12 August 2010
Accepted 7 September 2010
Available online 17 September 2010

Keywords:
Acetylcholinesterase inhibitors
Butyrylcholinesterase inhibitors
Carbamate derivatives
Alzheimer's disease

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

* Corresponding author. Tel.: +48 12 62 05 450; fax: +48 12 6570 262.
E-mail address: mfmalaws@cyf-kr.edu.pl (B. Malawska).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.09.010

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

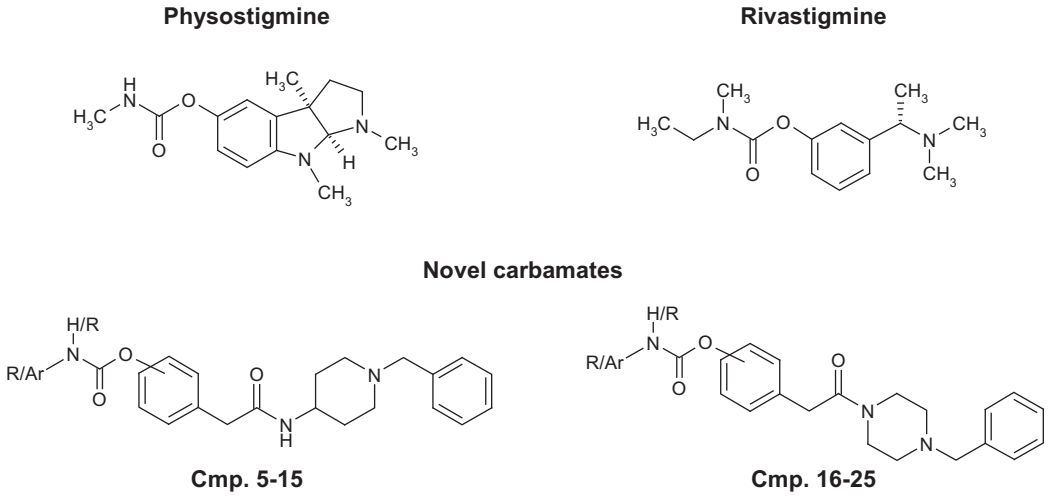
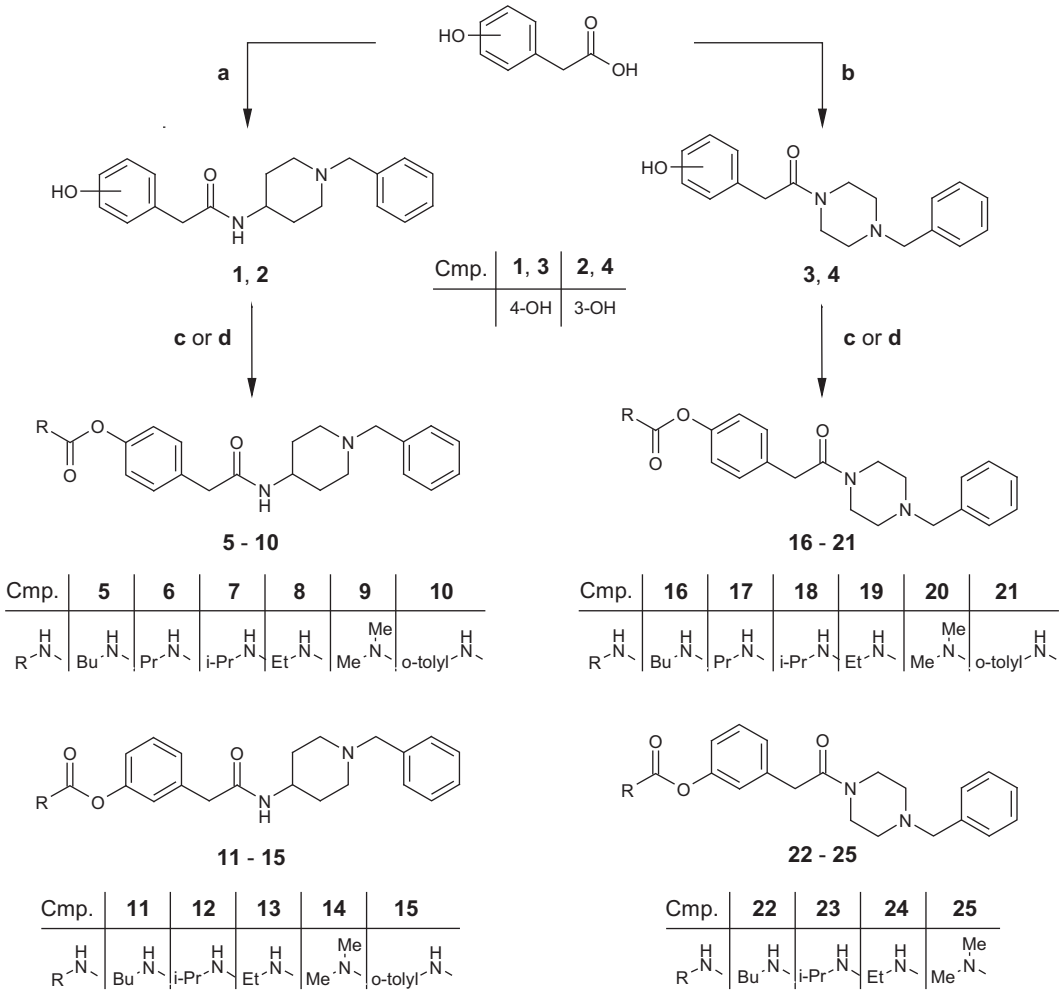


Fig. 1. Structures of cholinesterases inhibitors, physostigmine and rivastigmine, and new carbamate derivatives **5**–**25**.



Scheme 1. Synthesis of compounds **1**–**25**. Reagents and conditions: (a) 1-benzylpiperidin-4-ylamine, CDI/THF or EDC/CH_2Cl_2; (b) 1-benzylpiperazine, CDI/THF or EDC/CH_2Cl_2; (c) alkyl- or arylisocyanate, Et_3N; CH_2Cl_2, (d) dimethylcarbamoyl chloride, K_2CO_3, Me_2CO.

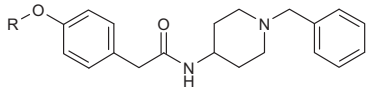
make principal interactions with the active site gorge of *Tc*AChE. Close to the bottom of the gorge the benzyl moiety creates hydrophobic interactions and π-π stacking with Phe330 and Trp84 residues (aromatic rings of amino acids are not situated directly over the phenyl ring but they are slightly shifted). The protonated piperidine nitrogen atom creates cation-π interactions with Tyr334 and an ionic bond with Asp72. These interactions resemble arrangement of donepezil in the catalytic center of AChE, however this inhibitor is not placed so deep in the gorge of the enzyme, therefore its interactions are weaker, which also explains lower activity of the compounds. The phenyl ring, to which the carbamoyl group is attached, stacks against the indole moiety of Trp279 in the peripheral binding site, in a classic parallel π-π interaction. The carbamoyl moiety remains outside the gorge and may probably not create significant interactions with Tyr70. Reverse arrangement, i.e. the carbamate group towards catalytic center is not preferred and characterized by higher energy values. Compound **20** represents *N*-benzylpiperazine derivatives, which were not active against AChE, and its interactions are different from those of *N*-benzylpiperidine series. The carbamoyl moiety of compound **20** is orientated towards the catalytic center. In this case such orientation is more favorable as regards energy. However, the distance between the carbamoyl group and the catalytic triad is too large to enable transfer to Ser200. The phenyl ring may create hydrophobic interactions with Phe330, Phe331 and Tyr334 residues but they are less significant due to non-parallel orientation and long distances between rings. The essential interaction is a hydrogen bond between the carbonyl group of piperazine amide and the Tyr121 residue. The Trp279 residue creates π-cation interaction with the protonated piperazine nitrogen atom. No interactions with the benzyl moiety are observed. These results show significant differences in types and energy of interactions between the compounds and the amino acids of the active site, which can explain differences in AChE inhibitory activity between two series of compounds.

We did not notice such divergency when docking to BuChE. Both series with *N*-benzylpiperidine (**5**–**15**) and *N*-benzylpiperazine (**16**–**25**) moieties display similar interactions within the active site of the enzyme, which are presented on example of the most active compound (**11**) (Fig. 3). The carbamoyl moiety of the ligand is orientated towards catalytic triad and its arrangement enables its transfer onto Ser198 residue. The *n*-butyl substituent creates hydrophobic interactions with Trp82 and additionally with Ile442, Met437 and Tyr440. Oxygen atoms of amide and carbamate moieties can create hydrogen bonds with water molecules located in the active center. The phenyl ring of the phenylacetamide (**11**) can interact with Thr120 while the benzyl moiety interacts with Pro285 via hydrophobic interactions.

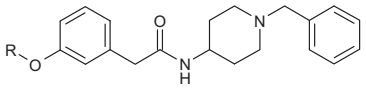
2.4. Kinetic studies of AChE and BuChE inhibition

The mechanism of AChE and BuChE inhibition was investigated in enzyme kinetic studies [23,31,32] using Ellman's test and compound **14**, the most potent, non-selective inhibitor of both

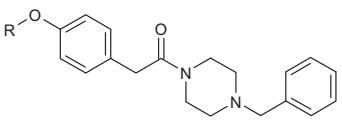
Table 1
Cholinesterases inhibitory activity of tested compounds **1**–**25** and reference compounds, tacrine and rivastigmine.



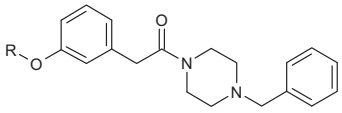
1, 5 - 10



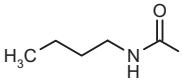
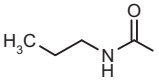
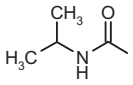
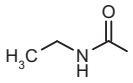
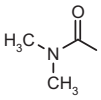
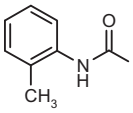
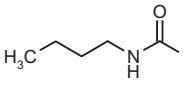
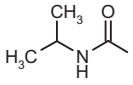
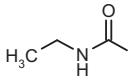
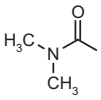
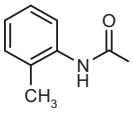
2, 11 - 15



3, 16 - 21

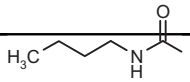
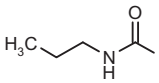
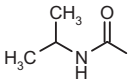
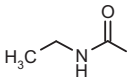
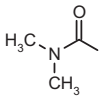
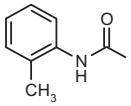
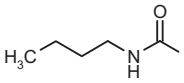
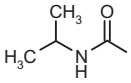
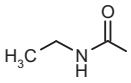
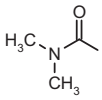


4, 22 - 25

Cmp.[a]	R	%I[b] AChE ± SEM	%I[b] BuChE ± SEM	BuChE pIC_{50} ± SEM
1		15.16 ± 0.12	45.63 ± 1.91	–
2		38.14 ± 0.39	49.82 ± 1.27	–
3		na	45.33 ± 0.91	–
4		na	31.48 ± 2.13	–
5		43.72 ± 1.31	63.37 ± 0.16	4.93 ± 0.36
6		33.81 ± 0.26	95.07 ± 0.13	5.03 ± 0.05
7		19.21 ± 0.98	63.15 ± 2.67	4.74 ± 0.03
8		23.43 ± 0.10	90.37 ± 1.39	4.97 ± 0.16
9		46.18 ± 0.73	89.76 ± 0.27	5.05 ± 0.02
10		24.69 ± 0.33	46.93 ± 0.76	–
11		39.86 ± 5.16	91.97 ± 0.33	5.24 ± 0.02
12		34.86 ± 1.04	72.22 ± 0.40	4.53 ± 0.04
13		28.53 ± 0.14	68.59 ± 0.41	4.43 ± 0.04
14		50.45 ± 0.49	82.13 ± 0.57	4.66 ± 0.02
15		nd	nd	nd

(*continued on next page*)

Table 1 (*continued*)

Cmp.[a]	R	%I[b] AChE ± SEM	%I[b] BuChE ± SEM	BuChE pIC_{50} ± SEM
16		na	83.09 ± 0.31	4.78 ± 0.02
17		na	88.26 ± 0.38	4.49 ± 0.06
18		na	72.18 ± 1.59	4.28 ± 0.03
19		na	83.37 ± 0.67	4.36 ± 0.05
20		na	32.47 ± 0.61	–
21		nd	nd	nd
22		na	92.70 ± 0.10	5.00 ± 0.01
23		na	77.73 ± 0.16	4.45 ± 0.02
24		na	62.49 ± 0.36	4.26 ± 0.03
25		na	78.95 ± 0.58	4.63 ± 0.03
		AChE pIC_{50}	BuChE pIC_{50}	
Tacrine		7.76 ± 0.02	8.31 ± 0.01	
Rivastigmine		5.38–6.03[c]	6.08–7.43[c]	

enzymes. Enzymes kinetics were analyzed by recording substrate–velocity curves in absence and presence of compound **14** or a reference–tacrine–reversible non-competitive inhibitor. Graphical analysis of Lineweaver–Burk plots of AChE activity (Fig. 4) showed increasing slopes (decreased V_{max}) with increasing inhibitor concentration. This pattern indicates non-competitive inhibition. Lineweaver–Burk plots of BuChE activity (Fig. 5) showed increasing slopes (lower V_{max}) and increasing intercepts (higher K_m), indicating mixed-type inhibition.

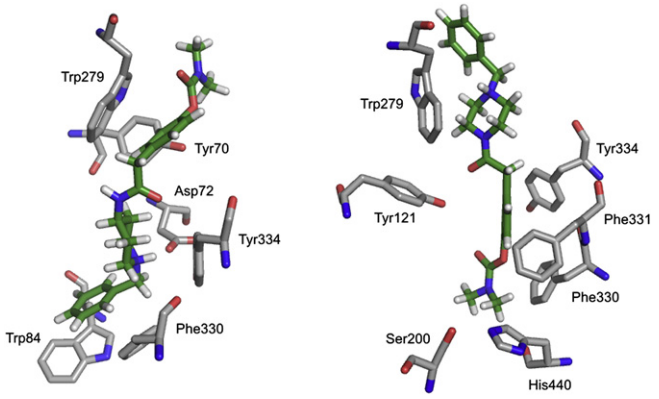


Fig. 2. Hypothetical binding mode of compound **9** (left) and **20** (right) in the AChE active site gorge.

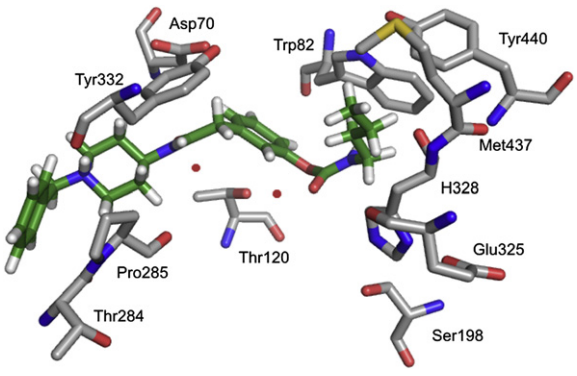


Fig. 3. Hypothetical binding mode of compound **11** in the BuChE active site gorge.

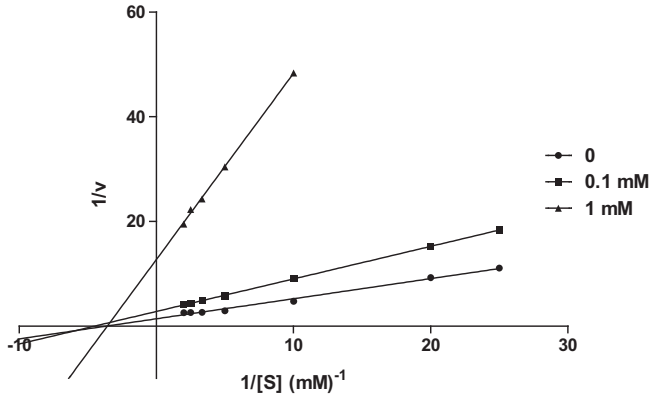


Fig. 4. Lineweaver–Burk plots resulting from substrate–velocity curves of AChE activity with different substrate concentrations (40–500 mM) in the absence and presence of compound **14** in concentrations of 0.1 mM and 1 mM.

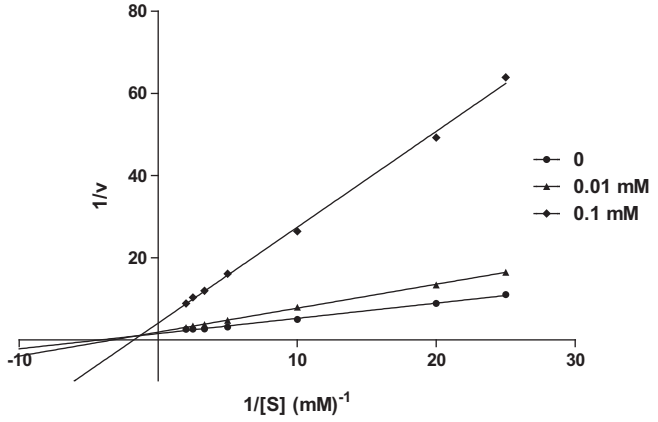


Fig. 5. Lineweaver–Burk plots resulting from substrate–velocity curves of BuChE activity with different substrate concentrations (40–500 mM) in the absence and presence of compound 14 in concentrations of 0.01 mM and 0.1 mM.

3. Conclusions

As AD progresses, acetylcholine regulation becomes increasingly dependent on BuChE, thus the enzyme is considered as one of the important factors implicated in the pathomechanism of AD. New selective inhibitors of the enzyme could serve as biological tools in research regarding physiological function of the enzyme and its role in AD and its treatment. This study has resulted in series of new cholinesterases inhibitors, both selective BuChE inhibitors (**16**–**20**, **22**–**25**) and non-selective AChE/BuChE inhibitors (**5**–**14**). Structures of compounds presented in this paper contain well-known cholinesterases inhibitors pharmacophores–alkyl- and arylcarbamates that improved the activity but were not crucial for it. *N*-Benzylpiperidine, *N*-benzylpiperazine and amide moieties turned out to be important for selectivity of the compounds. Molecular modelling studies suggest that a reason for selectivity of *N*-benzylpiperazine compounds may be the long distance between carbomoyl group and the catalytic site of AChE, which makes an interaction between them impossible. In the other series, the *N*-benzylpiperidine fragment is oriented towards the catalytic triad, creating interactions with amino acids of the active site. Preliminary kinetic studies performed for the most potent cholinesterases inhibitor obtained (**14**) indicated non-competitive type inhibition against AChE and mixed-type inhibition against (BuChE), which is in agreement with molecular modelling studies that showed that compound **14** can bind to the peripheral site of the enzyme. Overall, these results indicate that the novel carbamate derivatives of 1-(4-benzylpiperazin-1-yl)-2-(4- or 3-hydroxyphenyl)-ethanone are interesting structures for further research for selective and more potent BuChE inhibitors.

3.1. Experimental protocols

3.1.1. Chemistry

Reagents and solvents were purchased from common commercial suppliers and were used without further purification. Melting points were determined in open capillaries on an Electrothermal 9300 apparatus and are uncorrected. Merck silica gel 60 (70–230 mesh ASTM) was used for column chromatography. Analytical thin layer chromatography was performed on Merck TLC plates, silica gel 60 F_{254}. TLC visualization was achieved with a UV lamp or with ninhydrin solution. Nuclear magnetic resonance spectra (^{1}H NMR) were recorded with Varian Mercury VX 300 MHz spectrometer using $CDCl_3$ or DMSO-d_6. The chemical shifts (δ) are reported in parts per million (ppm) relative to the internal standard (TMS). Elemental analyses were performed on Vario EL III Elemental analyser. Mass spectra were recorded on MDX SCILEX API 2000 (Concord, ON, Canada) by using ESI method or on Finnigan MAT 95S (Bremen, Germany) by using EI method.

4. General procedure for the synthesis of compounds 1–4

Procedure A1. To a stirred solution of 3-hydroxyphenylacetic acid or 4-hydroxyphenylacetic acid (1 equiv.) and either 1-benzylpiperazine or 4-amino-1-benzylpiperidine (1 equiv.) in dichloromethane, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) (1.1 equiv.) was added at 0 °C. The reaction mixture was warmed to room temperature and stirred for 24 h. The solvent was evaporated and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 9.5:0.5) to afford the pure product.

Procedure A2. To the solution of 3-hydroxyphenylacetic acid or 4-hydroxyphenylacetic acid (1 equiv.) in anhydrous tetrahydrofuran (THF), 1,1′-carbonyldiimidazole (CDI) (1 equiv.) was added and the mixture was stirred at room temperature. After 3 h, 1-benzylpiperazine or 4-amino-1-benzylpiperidine (1 equiv.) was added and the resulting solution was stirred for 24 h. After evaporation under reduced pressure the crude product was purified by column chromatography (CH_2Cl_2/MeOH, 9.5:0.5) to give the pure product.

4.1. N-(1-Benzylpiperidin-4-yl)-2-(4-hydroxyphenyl)-acetamide (1)

Procedure: A2; *Reagents*: 4-hydroxyphenylacetic acid (1.52 g, 10 mmol), THF (30 mL), CDI (1.62 g, 10 mmol), 4-amino-1-benzylpiperidine (1.90 g, 10 mmol); pale yellow solid; yield: 40%; mp: 215–217 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.24–1.37 (m, 2H), 1.81–1.84 (m, 2H), 2.07–2.15 (m, 2H), 2.83 (d, J= 11.82 Hz, 2H), 3.46 (s, 2H), 3.49 (s, 2H), 3.73–3.86 (m, 1H), 5.29 (d, J= 8.28 Hz, 1H), 6.63–6.68 (m, 2H), 6.97–7.01 (m, 2H), 7.23–7.28 (m, 5H), OH signal not detected; ESI-MS (*m/z*) 325.4 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 266–268 °C; Anal. calcd. for $C_{20}H_{25}N_2O_2Cl \cdot H_2O$: C, 63.40; H, 7.18; N, 7.39. Found: C, 62.92; H, 7.36; N, 7.60.

4.2. N-(1-Benzylpiperidin-4-yl)-2-(3-hydroxyphenyl)-acetamide (2)

Procedure: A1; *Reagents*: 3-hydroxyphenylacetic acid (0.76 g, 5 mmol), CH_2Cl_2 (50 mL), EDC (1.05 g, 5.5 mmol), 4-amino-1-benzylpiperidine (0.95 g, 5 mmol); white solid; yield: 55.5%; mp:

137–140 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.33–1.46 (m, 2H), 1.79–1.84 (m, 2H), 2.11–2.20 (m, 2H), 2.88 (d, *J*= 11.83 Hz, 2H), 3.44 (s, 2H), 3.54 (s, 2H), 3.73–3.86 (m, 1H), 5.50 (d, *J* = 8.12 Hz, 1H), 6.59–6.70 (m, 3H), 7.13 (t, *J*= 7.78 Hz, 1H), 7.27–7.30 (m, 5H), OH signal not detected; ESI-MS (*m/z*) 325.7 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: decomposition > 234 °C; Anal. calcd. for $C_{20}H_{25}N_2O_2Cl \cdot H_2O$: C, 63.40; H, 7.18; N, 7.39. Found: C, 63.22; H, 7.68; N, 6.91.

4.3. 1-(4-Benzylpiperazin-1-yl)-2-(4-hydroxyphenyl)-ethanone (3)

Procedure: A2; *Reagents*: 4-hydroxyphenylacetic acid (1.52 g, 10 mmol), THF (30 mL), CDI (1.62 g, 10 mmol), 1-benzylpiperazine (1.76 g, 10 mmol); white solid; yield: 30%; mp: 151–153 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.30 (t, *J* = 4.95 Hz, 2H), 2.43 (t, *J* = 5.07 Hz, 2H), 3.45 (t, *J* = 5.00 Hz, 2H), 3.48 (s, 2H), 3.63 (s, 2H), 3.66 (t, *J* = 5.06 Hz, 2H), 6.65–6.70 (m, 2H), 6.99 (d, *J* = 8.50 Hz, 2H), 7.22–7.34 (m, 5H), OH signal not detected; EI-MS (*m/z*) 310,1 $[M]^+$. The HCl salt was prepared in ethanol; mp: 245–247 °C; Anal. calcd. for $C_{19}H_{23}N_2O_2Cl \cdot H_2O$: C, 62.55; H, 6.91; N, 7.68. Found: C, 62.30; H, 7.05; N, 7.80.

4.4. 1-(4-Benzylpiperazin-1-yl)-2-(3-hydroxyphenyl)-ethanone (4)

Procedure: A1; *Reagents:* 3-hydroxyphenylacetic acid (0.76 g, 5 mmol), CH_2Cl_2 (50 mL), EDC (1.05 g, 5.5 mmol), 1-benzylpiperazine (0.88 g, 5 mmol); white solid; yield: 45%; mp: 122–125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.25 (t, *J* = 5.00 Hz, 2H), 2.41 (t, *J* = 5.10 Hz, 2H), 3.42–3.46 (m, 4H), 3.64–3.67 (m, 4H), 6.63–6.66 (m, 1H), 6.69–6.73 (m, 1H), 6.85–6.87 (m, 1H), 7.09–7.14 (m, 1H), 7.21–7.33 (m, 5H), OH signal not detected; ESI-MS (*m/z*) 311.7 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 194–198 °C; Anal. calcd. for $C_{19}H_{23}N_2O_2Cl$: C, 65.79; H, 6.68; N, 8.08. Found: C, 65.79; H, 7.05; N, 8.11.

5. General procedure for the synthesis of compounds 5–25

Procedure K1. A solution of compound **1**, **2**, **3** or **4** (1 equiv.), isocyanate (1 equiv.), and triethylamine (Et_3N) in CH_2Cl_2 was allowed to stir at room temperature overnight. The reaction mixture was concentrated *in vacuo* and the resulting residue was washed with diethyl ether and if necessary purified via column chromatography or recrystallization.

Procedure K2. A mixture of compound 1, 2, 3 or 4 (1 equiv.), dimethylcarbamoyl chloride (1 equiv.) and K_2CO_3 (3 equiv.) in acetone was stirred at room temperature for 24 h. The mixture was filtered and the organic solvent was evaporated *in vacuo*. The resulting residue was purified by recrystallization or column chromatography.

5.1. 4-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl butylcarbamate (5)

Procedure: K1; *Reagents:* comp. **1** (0.32 g, 1 mmol), butyl isocyanate (0.10 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); white solid; yield: 83%; mp: 188–190 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.95 (t, *J* = 7.23 Hz, 3H), 1.31–1.45 (m, 4H), 1.51–1.60 (m, 2H), 1.80–1.85 (m, 2H), 2.03–2.12 (m, 2H), 2.72 (d, *J* = 11.71 Hz, 2H), 3.27 (dd, *J* = 6.89, 13.08 Hz, 2H), 3.45 (s, 2H), 3.51 (s, 2H), 3.71–3.84 (m, 1H), 5.03–5.07 (m, 1H), 5.28 (d, *J* = 7.89 Hz, 1H), 7.08–7.11 (m, 2H), 7.19–7.22 (m, 2H), 7.24–7.32 (m, 5H); ESI-MS (*m/z*) 424.6 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 185–188 °C; Anal. calcd. for $C_{25}H_{34}N_3O_3Cl \cdot H_2O$: C, 62.82; H, 7.59; N, 8.79. Found: C, 63.49; H, 7.63; N, 9.23.

5.2. 4-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl propylcarbamate (6)

Procedure: K1; *Reagents:* comp. **1** (0.320 g, 1 mmol), propyl isocyanate (0.085 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); white solid; yield: 81%; mp: 184–186 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.97 (t, *J* = 7.40 Hz, 3H), 1.23–1.41 (m, 2H), 1.54–1.66 (m, 2H), 1.80–1.85 (m, 2H), 2.03–2.11 (m, 2H), 2.71 (d, *J* = 11.66 Hz, 2H), 3.23 (dd, *J* = 6.63, 13.53 Hz, 2H), 3.45 (s, 2H), 3.51 (s, 2H), 3.74–3.83 (m, 1H), 5.05–5.08 (m, 1H), 5.27 (d, *J* = 7.64 Hz, 1H), 7.08–7.11 (m, 2H), 7.19–7.22 (m, 2H), 7.25–7.32 (m, 5H); ESI-MS (*m/z*) 410.5 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 205–207 °C; Anal. calcd. for $C_{24}H_{32}N_3O_3Cl$: C, 64.63; H, 7.23; N, 9.42. Found: C, 64.51; H, 7.75; N, 9.46.

5.3. 4-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl isopropylcarbamate (7)

Procedure: K1; *Reagents:* comp. **1** (0.320 g, 1 mmol), isopropyl isocyanate (0.085 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); white solid; yield: 83%; mp: 197–199 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.24 (d, *J* = 6.55 Hz, 6H), 1.28–1.39 (m, 2H), 1.80–1.85 (m, 2H), 2.03–2.13 (m, 2H), 2.71 (d, *J* = 11.73 Hz, 2H), 3.45 (s, 2H), 3.51 (s, 2H), 3.73–3.83 (m, 1H), 3.84–3.95 (m, 1H), 4.88 (d, *J* = 6.78 Hz, 1H), 5.24 (d, *J* = 7.70 Hz, 1H), 7.09–7.11 (m, 2H), 7.20–7.23 (m, 2H), 7.27–7.32 (m, 5H); ESI-MS (*m/z*) 410.5 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 216–219 °C; Anal. calcd. for $C_{24}H_{32}N_3O_3Cl \cdot H_2O$: C, 62.13; H, 7.39; N, 9.06. Found: C, 62.78; H, 7.50; N, 9.09.

5.4. 4-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl ethylcarbamate (8)

Procedure: K1; *Reagents:* comp. **1** (0.320 g, 1 mmol), ethyl isocyanate (0.071 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); white solid; yield: 99%; mp: 174–176 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.21 (t, *J* = 7.25 Hz, 3H), 1.30–1.42 (m, 2H), 1.80–1.85 (m, 2H), 2.05–2.14 (m, 2H), 2.74 (d, *J* = 11.60 Hz, 2H), 3.27–3.36 (m, 2H), 3.47 (s, 2H), 3.51 (s, 2H), 3.72–3.84 (m, 1H), 5.03–5.07 (m, 1H), 5.32 (d, *J* = 7.23 Hz, 1H, amide-N*H*), 7.08–7.11 (m, 2H), 7.20–7.23 (m, 2H), 7.28–7.32 (m, 5H); ESI-MS (*m/z*) 396.5 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 181–185 °C; Anal. calcd. for $C_{23}H_{30}N_3O_3Cl$: C, 63.95; H, 7.00; N, 9.73. Found: C, 63.55; H, 7.25; N, 9.70.

5.5. 4-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl dimethylcarbamate (9)

Procedure: K2; *Reagents:* comp. **1** (0.320 g, 1 mmol), dimethylcarbamoyl chloride (0.107 g, 1 mmol), K_2CO_3 (0.410 g, 3 mmol), acetone (10 mL); *Purification:* recrystallization from EtOAc; white solid; yield: 89%; mp: 126–128 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.27–1.40 (m, 2H), 1.79–1.83 (m, 2H), 2.03–2.11 (m, 2H), 2.73 (d, *J* = 11.77 Hz, 2H), 3.01 (s, 3H), 3.10 (s, 3H), 3.45 (s, 2H), 3.51 (s, 2H), 3.71–3.84 (m, 1H), 5.32 (d, *J* = 7.92 Hz, 1H), 7.06–7.09 (m, 2H), 7.19–7.23 (m, 2H), 7.24–7.33 (m, 5H); ESI-MS (*m/z*) 396.5 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 218–222 °C; Anal. calcd. for $C_{23}H_{30}N_3O_3Cl \cdot 3H_2O$: C, 56.84; H, 7.47; N, 8.65. Found: C, 57.17; H, 7.16; N, 8.43.

5.6. 4-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl o-tolylcarbamate (10)

Procedure: K1; *Reagents:* comp. **1** (0.32 g, 1 mmol), *o*-tolyl isocyanate (0.13 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); *Purification:* column chromatography ($CHCl_3$/MeOH, 9:1); white solid;

yield: 50%; mp: 178–181 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.30–1.43 (m, 2H), 1.81–1.87 (m, 2H), 2.05–2.13 (m, 2H), 2.34 (s, 3H), 2.74 (d, J = 11.77 Hz, 2H), 3.47 (s, 2H), 3.53 (s, 2H), 3.70–3.86 (m, 1H), 5.29 (d, J = 7.92 Hz, 1H), 6.72–6.81 (m, 1H), 7.05–7.10 (m, 1H), 7.17–7.32 (m, 11H), 7.78–7.89 (m, 1H); ESI-MS (*m/z*) 458.4 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 205–208 °C; Anal. calcd. for $C_{28}H_{32}N_3O_3Cl \cdot H_2O$: C, 65.68; H, 6.69; N, 8.21. Found: C, 65.54; H, 7.15; N, 7.87.

5.7. 3-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl butylcarbamate (11)

Procedure: K1; *Reagents:* comp. **2** (0.32 g, 1 mmol), butyl isocyanate (0.10 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); *Purification*: recrystallization from EtOAc; white solid; yield: 90%; mp: 127–128 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.95 (t, J = 7.25 Hz, 3H), 1.31–1.45 (m, 4H), 1.50–1.60 (m, 2H), 1.81–1.85 (m, 2H), 2.06–2.13 (m, 2H), 2.74 (d, J = 11.61 Hz, 2H), 3.26 (dd, J = 6.95, 13.06 Hz, 2H), 3.47 (s, 2H), 3.52 (s, 2H), 3.71–3.83 (m, 1H), 5.08 (t, J = 5.12 Hz, 1H), 5.40 (d, J = 7.41 Hz, 1H), 7.02–7.06 (m, 3H), 7.20–7.33 (m, 6H); ESI-MS (*m/z*) 425.1 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 205–206 °C; Anal. calcd. for $C_{25}H_{34}N_3O_3Cl \cdot H_2O$: C, 62.82; H, 7.59; N, 8.79. Found: C, 63.11; H, 7.75; N, 8.72.

5.8. 3-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl isopropylcarbamate (12)

Procedure: K1; *Reagents:* comp. **2** (0.320 g, 1 mmol), isopropyl isocyanate (0.085 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); *Purification*: recrystallization from EtOAc; white solid; yield: 80%; mp: 153–155 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.23 (d, J = 6.54 Hz, 6H), 1.32–1.45 (m, 2H), 1.81–1.86 (m, 2H), 2.07–2.14 (m, 2H), 2.75 (d, J = 11.62 Hz, 2H), 3.48 (s, 2H), 3.52 (s, 2H), 3.72–3.93 (m, 2H), 4.91 (d, J = 6.98 Hz, 1H), 5.40 (d, J = 7.41 Hz, 1H), 7.03–7.06 (m, 3H), 7.23–7.33 (m, 6H); ESI-MS (*m/z*) 410.3 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 178–182 °C; Anal. calcd. for $C_{24}H_{32}N_3O_3Cl \cdot H_2O$: C, 62.13; H, 7.39; N, 9.06. Found: C, 61.61; H, 7.42; N, 8.74.

5.9. 3-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl ethylcarbamate (13)

Procedure: K1; *Reagents:* comp. **2** (0.320 g, 1 mmol), ethyl isocyanate (0.071 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); *Purification*: recrystallization from EtOAc; white solid; yield: 88%; mp: 125–128 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.21 (t, J = 7.24 Hz, 3H), 1.41–1.50 (m, 2H), 1.83–1.87 (m, 2H), 2.12–2.19 (m, 2H), 2.79 (d, J = 11.67 Hz, 2H), 3.25–3.34 (m, 2H), 3.52 (s, 2H), 3.53 (s, 2H), 3.73–3.85 (m, 1H), 5.08–5.14 (m, 1H), 5.52 (d, J = 6.89 Hz, 1H), 7.02–7.07 (m, 3H), 7.22–7.33 (m, 6H); ESI-MS (*m/z*) 396.1 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 180–183 °C; Anal. calcd. for $C_{23}H_{30}N_3O_3Cl \cdot 2H_2O$: C, 59.03; H, 7.32; N, 8.98. Found: C, 59.10; H, 7.32; N, 8.53.

5.10. 3-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl dimethylcarbamate (14)

Procedure: K2; *Reagents:* comp. **2** (0.320 g, 1 mmol), dimethylcarbamoyl chloride (0.107 g, 1 mmol), K_2CO_3 (0.410 g, 3 mmol), acetone (10 mL); *Purification*: recrystallization from EtOAc; white solid; yield: 81%; mp: 85–88 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.30–1.43 (m, 2H), 1.78–1.83 (m, 2H), 2.03–2.12 (m, 2H), 2.73 (d, J = 11.83 Hz, 2H), 2.98 (s, 3H), 3.07 (s, 3H), 3.45 (s, 2H), 3.50 (s, 2H), 3.68–3.82 (m, 1H), 5.48 (d, J = 7.82 Hz, 1H), 6.99–7.05 (m, 3H), 7.22–7.32 (m, 6H); ESI-MS (*m/z*) 396.7 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 190–193 °C; Anal. calcd. for $C_{23}H_{30}N_3O_3Cl \cdot 2H_2O$: C, 59.03; H, 7.32; N, 8.98. Found: C, 59.71; H, 7.49; N, 8.93.

5.11. 3-(2-(1-Benzylpiperidin-4-ylamino)-2-oxoethyl)-phenyl o-tolylcarbamate (15)

Procedure: K1; *Reagents:* comp. **2** (0.32 g, 1 mmol), *o*-tolyl isocyanate (0.13 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); *Purification:* column chromatography ($CHCl_3$/MeOH, 9:1); white solid; yield: 44%; mp: 151–154 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.29–1.43 (m, 2H), 1.81–1.85 (m, 2H), 2.04–2.12 (m, 2H), 2.33 (s, 3H), 2.73 (d, J = 11.74 Hz, 2H), 3.46 (s, 2H), 3.54 (s, 2H), 3.72–3.86 (m, 1H), 5.43 (d, J = 7.52 Hz, 1H), 6.78–6.87 (m, 1H), 7.05–7.38 (m, 12H), 7.77–7.89 (m, 1H); ESI-MS (*m/z*) 459 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 172–175 °C; Anal. calcd. for $C_{28}H_{32}N_3O_3Cl \cdot 5H_2O$: C, 57.58; H, 7.25; N, 7.19. Found: C, 57.17; H, 6.72; N, 7.12.

5.12. 4-(2-(4-Benzylpiperazin-1-yl)-2-oxoethyl)-phenyl butylcarbamate (16)

Procedure: K1; *Reagents:* comp. **3** (0.31 g, 1 mmol), butyl isocyanate (0.10 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); white solid; yield: 85%; mp: 111–113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.94 (t, J = 7.25 Hz, 3H), 1.33–1.45(m, 2H), 1.50–1.60 (m, 2H), 2.27 (t, J = 4.92 Hz, 2H), 2.40 (t, J = 5.09 Hz, 2H), 3.26 (dd, J = 13.07, 6.89 Hz, 2H), 3.43 (t, J = 4.99 Hz, 2H), 3.47 (s, 2H), 3.64 (t, J = 5.06 Hz, 2H), 3.69 (s, 2H), 5.02 (t, J = 5.05 Hz, 1H), 7.05–7.08 (m, 2H), 7.19–7.23 (m, 2H), 7.24–7.33 (m, 5H); ESI-MS (*m/z*) 410.5 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 215–218 °C; Anal. calcd. for $C_{24}H_{32}N_3O_3Cl$: C, 64.63; H, 7.23; N, 9.42. Found: C, 64.21; H, 7.27; N, 9.35.

5.13. 4-(2-(4-Benzylpiperazin-1-yl)-2-oxoethyl)-phenyl propylcarbamate (17)

Procedure: K1; *Reagents:* comp. **3** (0.310 g, 1 mmol), propyl isocyanate (0.085 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); white solid; yield: 80%; mp: 130–132 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.97 (t, J = 7.40 Hz, 3H), 1.53–1.65 (m, 2H), 2.26–2.29 (m, 2H), 2.39–2.42 (m, 2H), 3.23 (dd, J = 6.72, 13.44, Hz, 2H), 3.42–3.46 (m, 2H), 3.48 (s, 2H), 3.62–3.66(m, 2H), 3.69 (s, 2H), 5.04 (t, J = 5.29 Hz, 1H), 7.05–7.08 (m, 2H), 7.19–7.22 (m, 2H), 7.24–7.34 (m, 5H); EI-MS (*m/z*) 395.2 $[M]^+$. The HCl salt was prepared in ethanol; mp: 235–238 °C; Anal. calcd. for $C_{23}H_{30}N_3O_3Cl$: C, 63.95; H, 7.00; N, 9.73. Found: C, 63.88; H, 7.11; N, 9.80.

5.14. 4-(2-(4-Benzylpiperazin-1-yl)-2-oxoethyl)-phenyl isopropylcarbamate (18)

Procedure: K1; *Reagents:* comp. **3** (0.310 g, 1 mmol), isopropyl isocyanate (0.085 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); white solid; yield: 70%; mp: 127–129 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.23 (d, J = 6.55 Hz, 6H), 2.27 (t, J = 4.99 Hz, 2H), 2.40 (t, J = 5.11 Hz, 2H), 3.42 (t, J = 5.03 Hz, 2H), 3.46 (s, 2H), 3.64 (t, J = 5.07 Hz, 2H), 3.69 (s, 2H), 3.80–3.93 (m, 1H), 4.90 (d, J = 7.23 Hz, 1H), 7.05–7.08 (m, 2H), 7.18–7.22 (m, 2H), 7.26–7.33 (m, 5H); ESI-MS (*m/z*) 396.1 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 239–243 °C; Anal. calcd. for $C_{23}H_{30}N_3O_3Cl$: C, 63.95; H, 7.00; N, 9.73. Found: C, 63.51; H, 7.03; N, 9.64.

5.15. 4-(2-(4-Benzylpiperazin-1-yl)-2-oxoethyl)-phenyl ethylcarbamate (19)

Procedure: K1; *Reagents:* comp. **3** (0.310 g, 1 mmol), ethyl isocyanate (0.071 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); white solid; yield: 97%; mp: 108–112 °C; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.21 (t, J = 7.24 Hz, 3H), 2.27 (t, J = 4.97 Hz, 2H), 2.40 (t, J = 5.10 Hz, 2H), 3.25–3.34 (m, 2H), 3.43(t, J = 5.02 Hz, 2H), 3.47 (s, 2H), 3.64 (t, J = 5.05 Hz, 2H), 3.69 (s, 2H), 5.07–5.10 (m, 1H), 7.04–7.07 (m, 2H), 7.18–7.21 (m, 2H), 7.24–7.33 (m, 5H); ESI-MS (*m/z)* 381.3 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 218–220 °C; Anal. calcd. for $C_{22}H_{28}N_3O_3Cl$: C, 63.23; H, 6.75; N, 10.05. Found: C, 62.88; H, 6.75; N, 10.01.

5.16. 4-(2-(4-Benzylpiperazin-1-yl)-2-oxoethyl)-phenyl dimethylcarbamate (20)

Procedure: K2; *Reagents:* comp. **3** (0.310 g, 1 mmol), dimethylcarbamoyl chloride (0.107 g, 1 mmol), K_2CO_3 (0.410 g, 3 mmol), acetone (10 mL); *Purification*: recrystallization from EtOAc; white solid; yield: 89%; mp: 81–84 °C; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.26 (t, J = 4.99 Hz, 2H), 2.39 (t, J = 5.09 Hz, 2H), 2.99 (s, 3H), 3.08 (s, 3H), 3.41 (t, J = 4.91 Hz, 2H), 3.45 (s, 2H), 3.62 (t, J = 5.00 Hz, 2H), 3.68 (s, 2H), 7.01–7.06 (m, 2H), 7.18–7.21 (m, 2H), 7.24–7.32 (m, 5H); ESI-MS (*m/z*) 381.3 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 237–240 °C; Anal. calcd. for $C_{22}H_{28}N_3O_3Cl$: C, 63.23; H, 6.75; N, 10.05. Found: C, 63.10; H, 6.98; N, 10.17.

5.17. 4-(2-(4-Benzylpiperazin-1-yl)-2-oxoethyl)-phenyl o-tolylcarbamate (21)

Procedure: K1; *Reagents:* comp. **3** (0.31 g, 1 mmol), *o*-tolyl isocyanate (0.13 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); *Purification*: column chromatography (AcOEt/MeOH, 10:0.5); oil; yield: 47%; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.29 (t, J = 5.03 Hz, 2H), 2.34 (s, 3H), 2.41 (t, J = 5.13 Hz, 2H), 3.45 (t, J = 5.00 Hz, 2H), 3.48 (s, 2H), 3.65(t, J = 5.15 Hz, 2H), 3.72 (s, 2H), 7.06–7.34 (m, 13H), 7.80–7.87 (m, 1H); ESI-MS (*m/z)* 444.6 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 200–206 °C; Anal. calcd. for $C_{27}H_{30}N_3O_3Cl \cdot 0.5H_2O$: C, 66.25; H, 6.33; N, 8.58. Found: C, 66.41; H, 6.84; N, 8.91.

5.18. 3-(2-(4-Benzylpiperazin-1-yl)-2-oxoethyl)-phenyl butylcarbamate (22)

Procedure: K1; *Reagents:* comp. **4** (0.31 g, 1 mmol), butyl isocyanate (0.10 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); oil; yield: 88%; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.94 (t, J = 7.28 Hz, 3H), 1.32–1.45 (m, 2H), 1.50–1.60 (m, 2H), 2.27 (t, J = 4.97 Hz, 2H), 2.40 (t, J = 5.08 Hz, 2H), 3.25 (dd, J = 6.96, 13.05 Hz, 2H), 3.39–3.43 (m, 2H), 3.46 (s, 2H), 3.62–3.65 (m, 2H), 3.71 (s, 2H), 5.05 (t, J = 5.39 Hz, 1H), 6.99–7.05 (m, 3H) 7.22–7.33 (m, 6H); ESI-MS (*m/z)* 410.8 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 203–206 °C; Anal. calcd. for $C_{24}H_{32}N_3O_3Cl \cdot H_2O$: C, 62.13; H, 7.39; N, 9.06. Found: C, 62.77; H, 7.29; N, 9.23.

5.19. 3-(2-(4-Benzylpiperazin-1-yl)-2-oxoethyl)-phenyl isopropylcarbamate (23)

Procedure: K1; *Reagents:* comp. **4** (0.310 g, 1 mmol), isopropyl isocyanate (0.085 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); *Purification*: recrystallization from EtOAc; white solid; yield: 81%; mp: 166–168 °C; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.23 (d, J = 6.55 Hz, 6H), 2.27 (t, J = 4.96 Hz, 2H), 2.40 (t, J = 5.10 Hz, 2H), 3.42 (t, J = 4.99 Hz, 2H), 3.46(s, 2H), 3.64 (t, J = 5.05 Hz, 2H), 3.71 (s, 2H), 3.80–3.95 (m, 1H), 4.88 (d, J = 6.74 Hz, 1H), 7.00–7.06 (m, 3H), 7.20–7.35 (m, 6H); ESI-MS (*m/z)* 396.6 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 210–215 °C; Anal. calcd. for $C_{23}H_{30}N_3O_3Cl$: C, 63.95; H, 7.00; N, 9.73. Found: C, 63.36; H, 6.87; N, 9.53.

5.20. 3-(2-(4-Benzylpiperazin-1-yl)-2-oxoethyl)-phenyl ethylcarbamate (24)

Procedure: K1; *Reagents:* comp. **4** (0.310 g, 1 mmol), ethyl isocyanate (0.071 g, 1 mmol), Et_3N (5 drops), CH_2Cl_2 (10 mL); oil; yield: 99%; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.21 (t, J = 7.25 Hz, 3H), 2.27(t, J = 4.97 Hz, 2H), 2.40 (t, J = 5.11 Hz, 2H), 3.24–3.34 (m, 2H), 3.42(t, J = 5.02 Hz, 2H), 3.46 (s, 2H), 3.64 (t, J = 5.06 Hz, 2H), 3.71 (s, 2H), 5.03–5.06 (m, 1H), 7.00–7.06 (m, 3H), 7.21–7.33 (m, 6H); ESI-MS (*m/z)* 382.3 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 188–191 °C; Anal. calcd. for $C_{22}H_{28}N_3O_3Cl \cdot H_2O$: C, 60.61; H, 6.94; N, 9.64. Found: C, 61.10; H, 6.83; N, 9.42.

5.21. 3-(2-(4-Benzylpiperazin-1-yl)-2-oxoethyl)-phenyl dimethylcarbamate (25)

Procedure: K2; *Reagents:* comp. **4** (0.310 g, 1 mmol), dimethylcarbamoyl chloride (0.107 g, 1 mmol), K_2CO_3 (0.410 g, 3 mmol), acetone (10 mL); *Purification*: column chromatography (CH_2Cl_2/MeOH, 9.5:0.5); oil; yield: 84%; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.27 (t, J = 5.03 Hz, 2H), 2.40 (t, J = 5.10 Hz, 2H), 2.99 (s, 3H), 3.07 (s, 3H), 3.42 (t, J = 5.04 Hz, 2H), 3.46 (s, 2H), 3.64 (t, J = 5.05 Hz, 2H), 3.71 (s, 2H), 6.98–7.05 (m, 3H), 7.20–7.32 (m, 6H); ESI-MS (*m/z)* 382.7 $[M + H]^+$. The HCl salt was prepared in ethanol; mp: 218–220 °C; Anal. calcd. for $C_{22}H_{28}N_3O_3Cl$: C, 63.23; H, 6.75; N, 10.05. Found: C, 63.52; H, 7.02; N, 10.14.

5.22. AChE/BuChE inhibition activity

AChE and BuChE inhibitory activities were evaluated by spectrophotometrical Ellman's method [30] using AChE from electric eel and BuChE from horse serum (2.5 units/1 mL). The reaction took place in a final volume of 3.32 mL of 100 mM phosphate buffer, pH 8.0, containing 0.25 unit of AChE or BuChE, 0.3 mmol 5,5′-dithio-bis (2-nitrobenzoic) acid (DTNB, Ellman's reagent) and 0.45 mmol acetylthiocholine or butyrylthiocholine as substrates. The tested compounds were preincubated with the enzyme for 5 min at 20 °C before starting the reaction by adding a substrate. Enzyme activity was determined by measuring the absorbance at 412 nm during 5 min with the Perkin Elmer Lambda 12. As a reference, sample without inhibitor was used (100% enzyme activity). Each compound was assayed at 6 concentrations in triplicate. The reaction rates were compared and the percent of inhibition due to the presence of the test compounds was calculated. Data from concentration-inhibition experiments were calculated by nonlinear regression analysis using the GraphPad Prism program (GraphPad Prism Software Inc. 2005), which gave estimates of IC_{50} (the compound concentration producing 50% of enzyme inhibition). DNTB, acetylthiocholine, butyrylthiocholine and the enzymes were purchased from Sigma–Aldrich.

5.23. Kinetic studies of AChE and BuChE inhibition

Kinetic studies were performed using Ellman's method for compound **14**, which was the most potent inhibitor of both AChE and BuChE. The test was carried out without the inhibitor and in 0.1 mM and 1 mM concentration of the inhibitor for AChE and 0.01 mM and 0.1 mM concentration for BuChE. Substrates of the reaction, acetylthiocholine and butyrylthiocholine, were used in following concentrations: 40, 50, 100, 200, 300, 400 and 500 mM. The obtained data were used to create substrate–velocity curves,

which were transformed in GraphPad Prism program to Lineweaver–Burk plots (Figs. 4 and 5).

Acknowledgments

This work was supported by funds from grant of Ministry of Science and High Education no 3254/P01/2006/31 and the project of Jagiellonian University Collegium Medicum no K/ZDZ/000723. We thank Dr. Krzysztof Więckowski for critical review of this article and Dr. Malte Behrends for linguistic advice.

References

[1] A. Lleo, S.M. Greenberg, J.H. Growdon, Ann. Rev. Med. 57 (2006) 513–533.
[2] U. Holzgrabe, P. Kapková, V. Alptüzün, J. Scheiber, E. Kugelmann, Expert Opin. Ther. Targets 11 (2007) 161–179.
[3] P. Davies, A.J. Maloney, Lancet 2 (1976) 1403.
[4] R.T. Bartus, R.L. Dean III, B. Beer, A.S. Lippa, Science 217 (1982) 408–414.
[5] M. Mesulam, A. Guillozet, P. Shaw, B. Quinn, Neurobiol. Dis. 9 (2002) 88–93.
[6] E. Giacobini, Neurochem. Res. 28 (2003) 515–522.
[7] P. Kasa, H. Papp, P. Kasa Jr., I. Torok, Neuroscience 101 (2000) 89–100.
[8] D.P. Geaney, N. Soper, B.J. Shepstone, P.J. Cowen, Lancet 335 (1990) 1484–1487.
[9] N.C. Inestrosa, A. Alvarez, C.A. Perez, R.D. Moreno, M. Vicente, C. Linker, et al., Neuron 16 (1996) 881–891.
[10] N.C. Inestrosa, A. Alvarez, F. Calderon, Mol. Psychiatr. 1 (1996) 359–361.
[11] T. Rees, P.I. Hammond, H. Soreq, S. Younkin, S. Brimijoin, Neurobiol. Aging 24 (2003) 777–787.
[12] E. Stefanova, K. Blennow, O. Almkvist, E. Hellstrom-Lindahl, A. Nordberg, Neurosci. Lett. 338 (2003) 159–163.
[13] E. Hellstrom-Lindahl, Eur. J. Pharmacol. 393 (2000) 255–263.
[14] M. Reale, C. Iarlori, F. Gambi, C. Feliciani, A. Salone, L. Toma, et al., J. Neuroimmunol. 148 (2004) 162–171.
[15] S. Darvesh, D.A. Hopkins, C. Geula, Nat. Rev. Neurosci. 4 (2003) 131–138.
[16] F.J. Munoz, N.C. Inestrosa, FEBS Lett. 450 (1999) 205–209.
[17] A. Alvarez, R. Alarcon, C. Opazo, E.O. Campos, F.J. Munoz, F.H. Calderon, et al., J. Neurosci. 18 (1998) 3213–3223.
[18] M. Meyer-Luehmann, T.L. Spires-Jones, C. Prada, M. Garcia-Alloza, C.A. deRozkalne, J. Koenigsknecht-Talboo, et al., Nature 451 (2008) 720–724.
[19] A. Musiał, M. Bajda, B. Malawska, Curr. Med. Chem. 14 (2007) 2654–2679.
[20] Y. Shen, J. Zhang, R. Sheng, X. Dong, Q. He, B. Jang, et al., J. Enzym. Inhib. Med. Chem. 24 (2009) 372–380.
[21] R. Sheng, Y. Xu, C. Hu, J. Zhang, X. Lin, J. Li, et al., Eur. J. Med. Chem. 44 (2009) 7–17.
[22] F. Belluti, L. Piazzi, A. Bisi, S. Gobbi, M. Bartolini, A. Cavalli, et al., Eur. J. Med. Chem. 44 (2009) 1341–1348.
[23] H. Tang, Y.-B. Wei, C. Zhang, F.-X. Ning, W. Qiao, S.-L. Huang, et al., Eur. J. Med. Chem. 44 (2009) 2523–2532.
[24] J.C. Verheijen, K.A. Wiig, S. Du, S.L. Connors, A.N. Martin, J.P. Ferreira, et al., Bioorg. Med. Chem. Lett. 19 (2009) 3243–3246.
[25] P. Jia, R. Sheng, J. Zhang, L. Fang, Q. He, B. Yang, et al., Eur. J. Med. Chem. 44 (2009) 772–784.
[26] R.M. Lane, S.G. Potkin, A. Enz, Int. J. Neuropsychoph. 9 (2006) 101–124.
[27] F. Marcelo, F.V.M. Silva, M. Goulart, J. Justino, P. Sinay, Bioorg. Med. Chem. 17 (2009) 5105–5116.
[28] M.A. Kamal, P. Klein, W. Luo, Y. Li, H.W. Holloway, D. Tweedie, et al., Nerochem. Res. 33 (2008) 745–753.
[29] G. Campiani, C. Fattorusso, S. Butini, A. Gaeta, M. Agnusdei, S. Gemma, et al., J. Med. Chem. 48 (2005) 1919–1929.
[30] G.L. Ellman, K.D. Courtney, V. Andres Jr., R.M. Feather-Stone, Biochem. Pharmacol. 7 (1961) 88–95.
[31] P. Camp, X. Formosa, C. Galdeano, D. Muñoz-Torrero, L. Ramirez, E. Gómez, et al., J. Med. Chem. 52 (2009) 5365–5379.
[32] L. Yu, R. Cao, W. Yi, Q. Yan, Z. Chen, L. Ma, et al., Bioorg. Med. Chem. Lett. 20 (2010) 3254–3258.
[33] J. Sterling, Y. Herzig, T. Goren, N. Finkelstein, D. Lerner, W. Goldenberg, et al., J. Med. Chem. 45 (2002) 5260–5279.
[34] M.L. Bolognesi, M. Bartolini, A. Cavalli, V. Andrisano, M. Rosini, A. Minarini, et al., J. Med. Chem. 47 (2004) 5945–5952.
[35] R. Sheng, X. Lin, J. Li, Y. Jiang, Z. Shang, Y. Hu, Bioorg. Med. Chem. Lett. 15 (2005) 3834–3837.
[36] W. Luo, Q.S. Yu, M. Zhan, D. Parrish, J.R. Deschamps, S.S. Kulkarni, et al., J. Med. Chem. 48 (2005) 986–994.