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RESEARCH ARTICLE

Synthesis, characterization and *in vitro* evaluation of substituted *N*-(2-phenylcyclopropyl)carbamates as acetyl- and butyrylcholinesterase inhibitors

Eva Horáková[1], Pavel Drabina[1], Břetislav Brož[1], Šárka Štěpánková[2], Katarína Vorčáková[2], Karel Královec[2], Radim Havelek[2], and Miloš Sedlák[1]

[1]*Institute of Organic Chemistry and Technology, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic and* [2]*Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic*

Abstract

A serie of *O*-substituted *N*-2-phenylcyclopropylcarbamates was prepared and characterized. These carbamates were tested as inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). It was found, that these compounds exhibit moderate inhibition activity with values of IC_{50} in the range of 54.8–94.4 μM (for AChE) and up to 5.8 μM (for BChE). The AChE/BChE selectivity for each carbamate was calculated. These values varied from 0.50 to 9.46, two carbamate derivatives inhibited only AChE selectively. The most promising derivative was prepared in all optically pure forms (four isomers). It was found that individual stereoisomers differed only slightly in the inhibition ability. The cytotoxicity of all carbamates was evaluated using the standard *in vitro* test with Jurkat cells. With regard to their inhibition activity and cytotoxicity as well as easy preparation, *O*-substituted *N*-2-phenylcyclopropylcarbamates can be considered as promising compounds for potential medicinal applications.

Keywords

Carbamates, cholinesterase inhibitors, cyclopropane derivatives, *in vitro* cytotoxicity, lipophilicity

History

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Introduction

Alzheimer's disease (AD) is known as progressive neurodegenerative brain disorder with characteristic clinical and pathological symptoms[1–3]. AD belongs among most often causation of formation of dementia in the elderly population and it is supposed that about 6% of the population worldwide aged over 65 is affected by AD[1–4]. AD represents the complex disease, whose exact cause of attendance is unknown at this time[5]. One of clinical manifestation of AD is connected with low acetylcholine (ACh) concentration in cholinergic synapses caused by its excessive degradation; ACh being very important neurotransmitter of the cholinergic central nervous system of human organism[6,7]. ACh is hydrolyzed by cholinesterases (ChEs) to choline and acetic acid. In vertebrates, there are two enzymes which are usually defined as ChEs: acetylcholinesterase (AChE, EC: 3.1.1.7) and butyrylcholinesterase (BChE, EC: 3.1.1.8). AChE plays a crucial role in ACh breaking down in cholinergic brain synapses and neuromuscular junctions[8]. The main function of BChE is still unclear. BChE enables the hydrolysis of ACh as well as other esters[9–12] and can also act as a scavenger for some toxins by reacting with them before they reach AChE[13–15]. The AD therapy is based on inhibition of the ChEs in order to maintain the proper level of ACh[16,17]. The ChEs are inhibited by several compounds, which can act as reversible or irreversible inhibitors. In AD therapy only reversible inhibitors are used[8,18,19].

On the other hand, BChE substitutes the function of AChE in the later stages of AD, where its activity is significantly lower[10,20–23]. According to this published results, the ratio of BChE/AChE in the normal brain was estimated 0.2, whereas the ratio in the brain of people with AD reaches the value ca. 11[24]. Moreover, selective BChE inhibitors do not exhibit the adverse cholinergic effects, which are characteristic for AChE inhibitors[25–28]. Therefore, the research concerned in the selective BChE inhibitors is currently a promising direction in medicinal chemistry research. Furthermore, the AChE and BChE inhibitors respectively can be applied in the therapy of other diseases, including myasthenia gravis, some other dementias, parasitic infections, glaucoma, obstipation or to antagonize muscle relaxation[29–31].

Many of the potential or approved commercially available pharmaceutical substances acting as AChE inhibitors contain carbamate functional group among others[16,32]; for instance, human drug Rivastigmin[33,34], which is used in the therapy of patients in the early or middle stage of AD. Besides this, many of differently substituted carbamate derivatives are described in literature[35–46], which embody significant inhibition activity against AChE and/or BChE. Unfortunately, the clinical applicability of these compounds is limited with regard to many side effects or demerits, e.g. general or specific toxicity, periphery side effect, short half-life, or gastrointestinal tract disorders[47].

The cyclopropane cycle occurs in many of natural products and biologically active substances[48], e.g. terpenes, pheromones, fatty acids and unusual amino acids. The natural, as well as

Address for correspondence: Pavel Drabina, Institute of Organic Chemistry and Technology, University of Pardubice, Studentská 573, 532 10 Pardubice, Czech Republic. Tel.: +420 466 037 010. Fax: +420 466 037 068. E-mail: pavel.drabina@upce.cz

Table 1. The list of prepared carbamates **3a–o** and their inhibition activity against AChE and BChE expressed as IC_{50}; lipophilicity $\log P_{ow}$ and AChE/BChE selectivity.

Compound		IC_{50} (μM)			
Code	R	AChE	BChE	AChE/BChE selectivity	$\log P_{ow}$
***trans*(±)3a**	*i*Pr	72.70 ± 7.36	33.64 ± 0.32**	2.16	4.61 ± 0.73
***trans*(±)3b**	Bu	66.32 ± 2.10	17.40 ± 0.33*	3.81	5.17 ± 0.26
***trans*(±)3c**	isoBu	71.45 ± 4.19	17.00 ± 1.09*	4.21	6.63 ± 0.45
***trans*(±)3d**	*t*Bu	62.87 ± 0.29	21.06 ± 0.25*	2.99	2.90 ± 0.31
***trans*(±)3e**	*s*Bu	77.63 ± 4.23	40.77 ± 1.52*	1.90	4.37 ± 0.62
***trans*(±)3f**	*c*Hex	62.78 ± 0.92	29.05 ± 2.57*	2.16	4.87 ± 0.88
***trans*(±)3g**	(–)-menthyl	72.25 ± 2.03	9.05 ± 0.01**	7.98	4.93 ± 0.19
***trans*(±)3h**	3-Ph$(CH_2)_3$–	60.51 ± 1.07	27.15 ± 0.39*	2.22	5.18 ± 0.52
***trans*(±)3i**	2-Ph$(CH_2)_2$–	60.45 ± 2.72	33.33 ± 2.75**	1.81	4.31 ± 0.32
***trans*(±)3j**	PG-galactosyl	57.85 ± 0.08	9.79 ± 0.43*	5.91	5.00 ± 0.46
***trans*(+)3j**	PG-galactosyl	54.84 ± 4.80	5.80 ± 0.95*	9.45	4.80 ± 0.33
***trans*(–)3j**	PG-galactosyl	85.87 ± 4.99	32.74 ± 1.92*	2.62	4.85 ± 0.78
(1*S*,2*S*)-*cis*(–)3j	PG-galactosyl	94.39 ± 0.18	37.59 ± 1.81*	2.51	5.87 ± 0.93
(1*R*,2*R*)-*cis*(–)3j	PG-galactosyl	82.38 ± 2.20	20.24 ± 0.04**	4.07	5.63 ± 0.98
***trans*(±)3k**	PG-glucosyl	63.30 ± 2.99	105.57 ± 2.76*	0.60	4.10 ± 0.93
***trans*(±)3l**	PG-pinitolyl	83.41 ± 1.10	109.13 ± 0.53*	0.76	4.84 ± 0.22
***trans*(±)3m**	Galactosyl	89.63 ± 1.37	–	–	1.78 ± 0.11
***trans*(±)3n**	Glucosyl	75.59 ± 4.28	152.46 ± 2.83*	0.50	1.70 ± 0.13
***trans*(±)3o**	Pinitolyl	80.01 ± 0.08	–	–	1.44 ± 0.15

Data are presented as mean values ± SD from two independent experiments. *Significantly different to control ($p \leq 0.01$); **significantly different to control ($p < 0.05$), *t*-test.

synthetic compounds containing cyclopropane moiety, possess a wide spectrum of biological effects, including enzyme inhibition, fungicidal, herbicidal, antimicrobial, antibiotic, antiviral and cytostatic activity[49]. Therefore, they can be considered as an interesting class of the organic chemicals. Well known (±)-*trans*-2-phenylcyclopropyl-1-amine can be mentioned as such cyclopropane derivative, used in human medicine as the monoamine oxidase inhibitor[50].

The aim of this work was the preparation and characterization of a series of *N*-(2-phenylcyclopropyl)carbamate derivatives as compounds, which would contain both above mentioned types of pharmacophores. Subsequently, the inhibition activity of these compounds against AChE and BChE respectively as well as their cytotoxicity should be studied and evaluated. With respect to the fact, that *N*-(2-phenylcyclopropyl)carbamate derivatives are chiral compounds, the most promising derivative with the highest inhibition activity should be prepared in all of the configuration forms (four stereoisomers), which would enable verification of the influence of absolute configuration at stereogenic centers on resulting biological activity.

Materials and methods

Lipophilicity

The traditional shake-flask method was used for determination of $\log P_{ow}$ values[51,52]. First, the two solvents were mutually saturated at the temperature of the experiment in a way described in literature[52]. Then, the determination $\log P_{ow}$ was performed subsequently: *n*-octanol (1.5 ml) and *n*-octanol solution of tested compound (10 µl, 0.01 M) were placed into the test tube. This mixture was intensively shaken for 15 min. Then, 1 ml of this mixture was placed into the quartz cuvette and its absorbance at the absorption maximum wavelength was measured. Thereby the value of absorbance corresponding to 100% of the tested compound in *n*-octanol was obtained. The reference solution was *n*-octanol. Into the other test tube *n*-octanol (1.5 ml), water (1.5 ml) and *n*-octanol solution of tested compound (10 µl, 0.01 M) were placed. This mixture was intensively shaken for 15 min and then centrifuged (3000 rpm, 10 min). Then 1 ml of this mixture was placed into the quartz cuvette and its absorbance at the absorption maximum wavelength was measured. The reference solution was *n*-octanol again. Thereby the percentage content of tested compound in the *n*-octanol layer (%) was obtained. Subsequently, the $\log P_{ow}$ ($\log P_{ow} = \log(c_1/c_2)$, where c_1 and c_2 are molar concentrations of tested compound in *n*-octanol and water) was calculated. For each compound, at least two determinations were performed. Obtained results are shown in Table 1.

Biological assay

AChE, BChE, acetylthiocholine (ATCh), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and *n*-octanol were purchased from Sigma-Aldrich (St. Louis, MO). KH_2PO_4, $Na_2HPO_4 \cdot 12H_2O$, KCl, NaCl, dimethyl sulfoxide (DMSO) were purchased from Penta (Prague, Czech Republic).

All tested compounds were dissolved in DMSO (concentration 0.01 M) and diluted in demineralized water (concentration 0.001 M). The ability of tested compounds to inhibit AChE (from electric eel) and BChE (from equine serum) was determined using modified Ellman's method at 25 °C in the presence of phosphate-buffered saline (PBS, 0.1 M, pH 7.4) in glass cuvette with 1 cm optical path. The enzyme activity in the total reaction mixture (2 ml) was 0.2 U/ml, the concentration of ATCh 40 µM and concentration of DTNB 0.1 mM for all reactions. The IC_{50} value was obtained from the dependence v_0/v_i versus concentration of tested compound (inhibitor), where v_0 is the reaction rate of uninhibited reaction and v_i is the reaction rate of inhibited reaction (for given concentration of inhibitor).

First, v_0 was determined. Into the cuvette, PBS (0.1 M, pH 7.4), DTNB and ATCh were placed. The enzymatic reaction was started by adding the enzyme. The dependence of absorbance ($\lambda = 412$ nm) versus time was observed for 70 s (reference solution contained PBS, DTNB and ATCh) and then reaction rate (v_0) was calculated ($v = \Delta A/\Delta t$). The measurement was performed in triplicate at least and average v_0 was determined.

Then, v_i (for given concentration of inhibitor) was determined. Into the cuvette DTNB, ATCh, chosen volume of suitably diluted inhibitor (to achieve required concentration of

inhibitor in the total reaction mixture) and certain volume of PBS (to achieve a total volume of reaction mixture 2 ml after adding of enzyme) were placed. The enzymatic reaction was started by adding of the enzyme. The dependence of absorbance ($\lambda = 412$ nm) versus time was observed for 70 s (reference solution was the same as for uninhibited reaction) and then reaction rate (v_i) was calculated. Five different concentration of inhibitor were used and each measurement was performed in duplicate at least.

Finally, the dependence v_0/v_i versus concentration of inhibitor was constructed and IC_{50} was calculated from obtained equation of regression curve for $y = 2$ (coming out from the definition of IC_{50}). The obtained IC_{50} values of all tested compounds are shown in Table 1. The comparison of both IC_{50} values (for AChE and BChE) of each compounds **3a–o** was statistically analyzed with GraphPad Prism (version 5.00; La Jolla, CA) statistical software using unpaired *t*-test: $p \leq 0.01$ or $p < 0.05$.

In vitro cytotoxicity assay

Cell lines

The human T-cell acute lymphoblastic leukemia cell line Jurkat was purchased from European Collection of Cell Cultures (UK). These cells were cultured in Roswell Park Memorial Institute 1640 medium (Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum, 1% non-essential amino acid, 1% penicillin/streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate and 10 mM 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid buffer (all supplements from Life Technologies) in a humidified atmosphere containing 5% CO_2 at 37 °C.

Cytotoxicity measurement

All compounds were tested using a standard colorimetric method measuring a tetrazolium salt reduction via mitochondrial dehydrogenase activity in Jurkat cells. The cells were seeded in density 133 000 cells per well in a 96-well plate. The cells were treated with each of the tested substances dissolved in DMSO. All compounds were prepared in six incubation concentrations (1–500 μM) in triplicates. Also, the vehiculum controls (0.2% DMSO) were prepared in triplicates. After 48 h incubation, cell survival was determined using Cell Proliferation Kit II (XTT, Roche, Germany) according to manufacturer's instructions. In brief, XTT reagent (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) was added to each well and incubated for 3 h at 37 °C; absorbance was then measured at 470 nm using a 96-multiwell microplate reader Tecan Infinite M200 (Tecan Group Ltd., Männedorf, Switzerland). Viability was calculated as described in the paper by Havelek et al. using the following formula: (%) viability = (A470sample − A470blank)/(A470control − A470blank) × 100, where A470 is the absorbance of utilized XTT formazan measured at 470 nm[53]. Data were statistically analyzed with GraphPad Prism (version 5.00; La Jolla, CA) statistical software using unpaired *t*-test: $p \leq 0.01$.

Results

Chemistry

The serie of *N*-(2-phenylcyclopropyl)carbamate derivatives **3a–o** was prepared by three-step synthesis with good yields. All of the newly prepared carbamates **3a–o** were characterized by means of melting point, ^{1}H and ^{13}C NMR spectroscopy and high-resolution mass spectroscopy. The optical purity of non-racemic derivatives was determined by means of chiral HPLC and their optical rotatory power was measured. The purity of all compounds was verified by means of elemental analysis.

Lipophilicity

The values of partition coefficient were determined using traditional shake-flask method[51,52,54]. The obtained results are shown in Table 1. All tested compounds could be divided into two groups: *Group 1* includes compounds **3a–i** ($R = 9$ alkyls) and *Group 2* includes compounds **3j–o** ($R = 6$ glycosyls). All compounds in the *Group 1* fulfill in fact the condition of Lipinski's rule of five (except ***trans*(±)3c** with $\log P_{ow} = 6.63$). The lowest value of $\log P_{ow}$ in the *Group 1* was obtained for compound ***trans*(±)3d**. But there is no significant trend in increasing/decreasing of $\log P_{ow}$ values depending on chain length. The compounds in *Group 2* containing protecting isopropylidene groups show high lipophilicity, the $\log P_{ow}$ values are in range from 4.10 for ***trans*(±)3k** to 5.87 for **(1*S*,2*S*)-*cis*(–)3j**.

Inhibition studies

The ability of all prepared carbamates to inhibit AChE from electric eel (*Electrophorus electricus*) and BChE from equine serum was determined *in vitro* using modified Ellman's method. The effectiveness of the inhibitors was expressed as IC_{50} value representing the concentration of an inhibitor which is necessary for reduction of enzyme activity (or reaction rate) to 50%. The obtained results are shown in Table 1.

In vitro cytotoxicity assay

The cytotoxicity of all prepared compounds was screened using a standard tetrazolium salt XTT cytotoxicity assay after 48 h of treatment. As shown in Table 2, most of the evaluated compounds of *Group 1* exhibited cytotoxic activity against Jurkat cells at concentrations ≥100 μM. Contrary thereto, ***trans*(±)3g** at all tested concentrations and ***trans*(±)3d** at concentrations ≤ 250 μM do not significantly affect cell viability of Jurkat cells. Similarly, also the majority of the compounds in *Group 2* exhibited cytotoxic activity against Jurkat cells at concentrations ≥100 μM (Table 2). Exceptions are ***trans*(±)3m** with statistically insignificant effect on Jurkat cell viability at concentrations ≤ 250 μM and ***trans*(±)3n** together with ***trans*(−)3j** with statistically insignificant effect on Jurkat cell survival at concentrations ≤ 100 μM.

Discussion

The key intermediate for the synthesis of carbamate derivatives **3a–l** was 2-phenylcyclopropanecarboxylic acid **2**. It was prepared by standard two-step synthesis, including at first cyclopropane cycle formation by the action of ethyl diazoacetate onto styrene afforded cyclopropanecarboxylate **1** and subsequent hydrolysis of ester group under basic condition (Scheme 1).

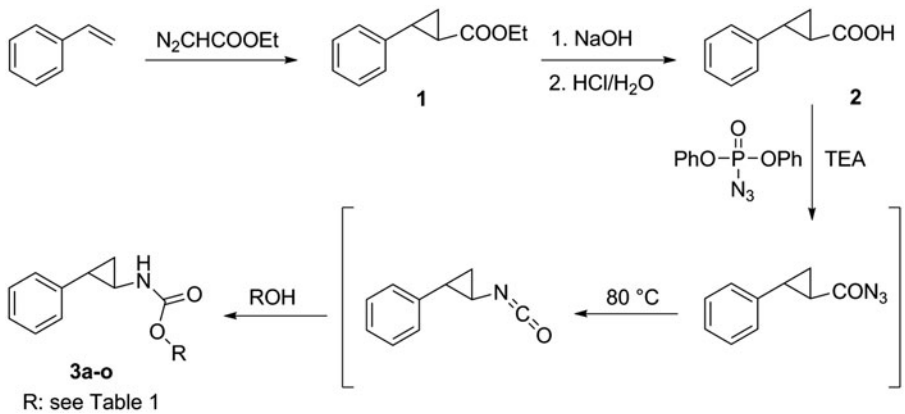
The cyclopropanation leading to racemic ***trans*/*cis* (±)-1** was conducted at high temperature (120–130 °C) without the presence of any catalyst with the yield of 62%[55]. The individual isomers formed ***trans*(±)-1** and ***cis*(±)-1** were not separated, the minor *cis* isomer was removed from the mixture by recrystallization after its hydrolysis to the corresponding acid ***trans*/*cis* (±)-2**. The optically pure forms of ester **1** were prepared according to the protocol described by Evans et al.[56] employing the enantioselective catalyst based on copper(I) complex of commercially available chiral bisoxazoline derivative. This method can be considered as the most convenient for the preparation of all of the isomers of **1**, because, according to the original paper, it enables the formation of the esters **1** with excellent optical purity (95–98% ee) and with diastereomeric ratio 77/23 (*trans*/*cis*), i.e. relatively favorable ratio for less thermodynamically stable *cis* form. In our case, we observed optical purity 96% ee, the ratio of *trans*/*cis*, we found 75/25 and isolated yields were 79–85%. The asymmetric

Table 2. Cell viability of Jurkat cells measured by using XTT cytotoxicity assay after treatment with different concentrations of the tested compounds. Treatment for 48 h. Viability is referred to cells treated with DMSO 0.2% (control). Negative control (NC) was treated with the vehicle (DMSO 0.2%) used for diluting the tested substance.

	Cell viability (% of control)						
Compound	0.2% DMSO	5 μM	10 μM	50 μM	100 μM	250 μM	500 μM
***trans*(±)3a**	100.00 ± 3.32	96.30 ± 1.22	85.77 ± 4.30	82.27 ± 5.81	69.59 ± 2.13*	64.70 ± 2.82*	27.26 ± 2.17*
***trans*(±)3b**	100.00 ± 3.64	89.76 ± 7.00	79.73 ± 2.73	64.99 ± 1.75*	33.82 ± 0.66*	3.70 ± 0.31*	0.25 ± 0.08*
***trans*(±)3c**	100.00 ± 1.41	85.16 ± 10.99	83.10 ± 9.97	65.54 ± 5.90*	64.76 ± 2.37*	43.35 ± 1.50*	3.16 ± 0.75*
***trans*(±)3d**	100.00 ± 8.35	94.50 ± 2.15	93.80 ± 5.50	82.93 ± 7.08	78.03 ± 8.97	68.47 ± 5.70	25.29 ± 1.44*
***trans*(±)3e**	100.00 ± 3.12	100.39 ± 9.28	99.39 ± 1.78	91.87 ± 5.34	77.60 ± 3.06*	25.79 ± 1.93*	0.68 ± 0.24*
***trans*(±)3f**	100.00 ± 2.42	94.82 ± 3.39	83.04 ± 0.80*	62.78 ± 0.39*	49.06 ± 1.39*	35.83 ± 2.34*	33.30 ± 1.02*
***trans*(±)3g**	100.00 ± 7.96	86.90 ± 2.05	82.93 ± 3.42	69.74 ± 2.01	64.58 ± 2.31	62.64 ± 5.03	62.91 ± 3.56
***trans*(±)3h**	100.00 ± 3.01	80.55 ± 1.41*	72.66 ± 3.01*	3.42 ± 1.66*	0.29 ± 0.19*	0.78 ± 0.22*	0.11 ± 0.05*
***trans*(±)3i**	100.00 ± 0.13	97.71 ± 0.67	93.52 ± 1.90	81.01 ± 1.93*	40.89 ± 1.72*	1.98 ± 0.30*	1.20 ± 0.35*
***trans*(±)3j**	100.00 ± 4.02	98.03 ± 0.55	89.45 ± 0.22	82.37 ± 1.63	79.18 ± 1.62*	61.62 ± 0.37*	59.70 ± 1.05*
***trans*(+)3j**	100.00 ± 2.52	92.89 ± 1.52	91.30 ± 2.17	89.47 ± 3.22	80.27 ± 2.47*	56.88 ± 0.69*	41.41 ± 0.95*
***trans*(–)3j**	100.00 ± 2.52	93.47 ± 1.52	93.17 ± 3.46	98.98 ± 16.46	88.75 ± 3.44	73.38 ± 3.14*	44.32 ± 0.55*
(1*S*,2*S*)-*cis*(–)3j	100.00 ± 4.87	75.12 ± 6.72	71.67 ± 2.78*	72.73 ± 2.60*	74.85 ± 0.72*	76.91 ± 1.19*	76.81 ± 0.70*
(1*R*,2*R*)-*cis*(–)3j	100.00 ± 4.87	70.26 ± 1.13*	68.41 ± 1.24*	68.49 ± 4.72*	63.52 ± 2.06*	56.42 ± 0.02*	51.34 ± 0.67*
***trans*(±)3k**	100.00 ± 3.63	91.09 ± 1.89	84.96 ± 0.81	80.96 ± 0.14*	73.13 ± 1.46*	56.86 ± 0.37*	19.26 ± 0.58*
***trans*(±)3l**	100.00 ± 2.26	91.16 ± 4.37	88.92 ± 6.46	83.27 ± 5.19	74.95 ± 1.47*	62.45 ± 1.11*	28.75 ± 0.70*
***trans*(±)3m**	100.00 ± 6.02	83.97 ± 3.26	82.78 ± 1.81	79.40 ± 1.06	77.49 ± 3.39	73.61 ± 1.19	71.36 ± 0.70*
***trans*(±)3n**	100.00 ± 2.32	91.50 ± 6.53	89.32 ± 2.73	89.84 ± 1.18	93.65 ± 4.38	88.30 ± 0.82*	66.93 ± 0.47*
***trans*(±)3o**	100.00 ± 3.17	97.14 ± 7.85	90.13 ± 2.09	88.86 ± 3.76	78.99 ± 2.72*	77.31 ± 2.27*	67.86 ± 0.64*

Data are presented as mean values ± SD from three independent experiments. *Significantly different to control ($p \leq 0.01$), *t*-test.

Scheme 1. Three-step synthesis of carbamates **3a–o**.

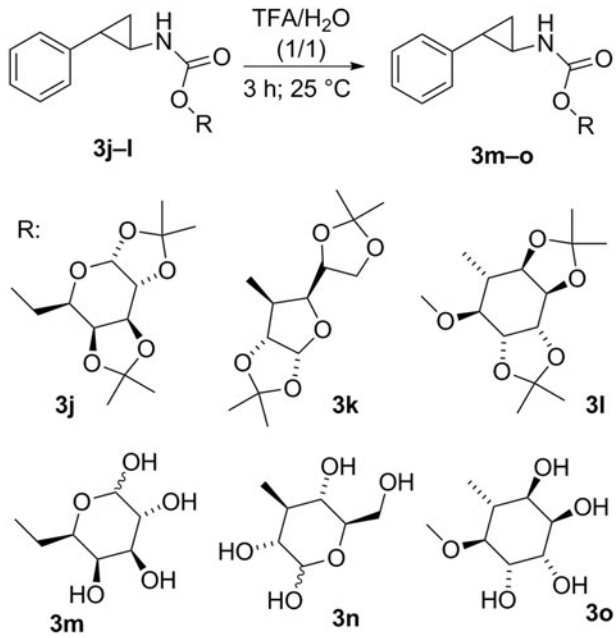


cyclopropanation with a recoverable and recyclable form of a catalyst based on copper(I) complex of chiral bisoxazoline ligand supported by Amberlite® IR-120 was also studied. Unfortunately, the chemical yields and first of all optical purity of the cyclopropane derivatives **1** obtained in this way were unsatisfactory. The individual *cis* and *trans* diastereomers of non-racemic forms of ester **1** were separated by means of column chromatography.

The carboxylic acid **2** was transformed to individual carbamates **3a–l** by the action of diphenylphosphoryl azide in the presence of triethylamine and appropriate alcohol (Scheme 1)[57]. This one-pot reaction involved at first acylazide formation, which underwent Curtius rearrangement under heating at 80 °C. The corresponding isocyanate formed was attacked by the present hydroxy derivative afforded desired carbamate **3a–l**. The presence of isocyanate derivative as the intermediate of this synthetic process needed to perform the reaction under strictly non-aqueous condition. The reacting alcohol was used as the solvent in the case of preparation of carbamates **3a–e** (Method A). The carbamates **3f–l** formed from higher alcohols were prepared in toluene (1,4-dioxane for **3j–l**) containing slight excess (1.2 eq.) of respective hydroxy derivative (Method B)[58]. The yields of this transformation (40–80%) depend on the individual alcohol used.

The serie of prepared carbamates also includes three members (**3j–l**) derived from protected monosaccharides (glucose, galactose and inositol) and their corresponding deprotected forms **3m–o**. The preparation of such designed carbamates was inspired by the hypothesis that they can possess a high level of transport ability[59–61] over blood-brain barrier via GLUT-family sugar/polyol transport facilitators[62]. Better transport of the active molecules can then lead to their enhanced concentration in intracellular space of CNS. The carbamates **3j–l** were deprotected (deacetalization of monosaccharide moiety) by the treatment of aqueous trifluoroacetic acid (TFA) (1/1)[63]. It was found out, that both isopropylidene groups were removed completely within 3 h at room temperature, without observation of any significant decomposition of carbamate group as well as ring-opening of cyclopropane cycle (Scheme 2). The mild condition (1% TFA in methanol, 10 h of reflux) led to only partial deprotection, with prevailing 1,2-monoprotected derivative.

Many drugs cross biological membranes through passive transport, which strongly depends on their lipophilicity. The necessary condition for exploitation of chosen compound *in vivo* as the brain AChE inhibitor is its ability to cross the blood-brain barrier. For assessment of this ability, the partition coefficient in



Scheme 2. Deprotection of *O*-glycosyl carbamate derivatives **3j–l**; the survey of individual carbamates **3j–o**.

system *n*-octanol:water (usually expressed as P_{ow} or $\log P_{ow}$) can be used[64,65]. Experimentally, it is done by partitioning the molecule between two immiscible phases (water and *n*-octanol) and determining the P_{ow} value as the ratio of the tested compound concentration in *n*-octanol and in water. The partition coefficient serves as a quantitative descriptor of lipophilicity and is one of the key determinants of pharmacokinetic properties. According to Lipinski's rule of five, $\log P_{ow}$ should not exceed 5[66]. Expectably, the corresponding deprotected compounds **3m–o** exhibit relatively low lipophilicity. Generally, it is possible to conclude, that all tested compound show adequate lipophilicity and could cross the blood-brain barrier.

Ellman's method[67] is widely used for measuring of ChE activity and effectiveness of ChE inhibitors. It is a simple, rapid and direct method to determine the SH and −S−S− groups[68]. ChE activity is measured indirectly by quantifying the concentration of 2-nitro-5-sulfanylbenzoic acid ion formed in the reaction between disulfide reagent DTNB and thiocholine, a product of substrate (i.e. ATCh) hydrolysis catalyzed by ChE.

Based on the obtained results, it is obvious that all tested compounds in *Group 1* show moderate inhibition of AChE from electric ell and BChE from equine serum. Generally, it is possible to conclude, that compound **3a–i** show higher selectivity to BChE. The IC_{50} values for AChE inhibition are in the range of 60.45–77.63 μM, whereas the most potent inhibitor is ***trans*(±)3i** and the least effective is ***trans*(±)3e**. The IC_{50} values for BChE inhibition are for all compounds lower than for AChE (9.05–40.77 μM), i.e. that compounds **3a–i** show higher selectivity to BChE. The most effective BChE inhibitor is ***trans*(±)3g** with $IC_{50} = 9.05$ μM.

All tested compounds in *Group 2* show moderate inhibition of AChE and BChE, with the exception of quite potent BChE inhibitors ***trans*(±)3j** and ***trans*(+)3j**. Compounds ***trans*(±)3j**, ***trans*(+)3j**, ***trans*(–)3j**, **(1*S*,2*S*)-*cis*(–)3j** and **(1*R*,2*R*)-*cis*(–)3j** show higher selectivity to BChE. The interesting result is, that ***trans*(±)3j** does not show significantly lower inhibitory effectiveness than ***trans*(+)3j**, ***trans*(–)3j**. On the contrary, its anticholinesterase activity is comparable to the activity of ***trans*(+)3j**. Compounds **3k–o** show (if it is possible to determine) higher selectivity to AChE. The IC_{50} values for AChE inhibition are in the range of 63.3–89.63 μM, and for BChE inhibition in the range of 105.57–152.46 μM, whereas the anti-BChE activity for ***trans*(±)3m** and ***trans*(±)3o** was undetectable under given conditions.

Among these derivatives, ***trans*(±)3d** and ***trans*(+)3j** can be considered as the most promising compounds for further study due to their moderate inhibitory activity against AChE, high inhibitory effects on BChE and concurrently low cytotoxicity against blood originated Jurkat cell line.

Conclusion

In this study, the serie of *N*-(2-phenylcyclopropyl)carbamate derivatives **3a–o** was prepared by three-step synthesis. These compounds were tested for their inhibition of AChE from electric eel and BChE from equine serum. Generally, it is possible to conclude, that all tested compounds show moderate inhibition activity, where the most effective inhibitor was found the derivative ***trans*(+)3j**. Evaluating cytotoxic activity of all derivatives, the derivative ***trans*(±)3g** can be considered as the most promising AChE and BChE inhibitor due to its potent activity and concurrently low cytotoxicity against Jurkat cells. In this regard, the further research efforts on these compounds would appear to hold great potential, especially in the light of the recent findings of the important role of BChE in the central cholinergic transmission and during the AD therapy. Overall, the obtained results indicated that substituted *N*-(2-phenylcyclopropyl)carbamates have the potential to be good candidates for AChE and BChE inhibitors and therefore they deserve further attention with the aim of their possible medicinal applications.

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Declaration of interest

The authors report no conflicts of interest.

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Supplementary material available online