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RESEARCH ARTICLE

Synthesis and inhibitory properties of some carbamates on carbonic anhydrase and acetylcholine esterase

Süleyman Yılmaz[1], Yusuf Akbaba[1,2], Bünyamin Özgeriş[1,2], Leyla Polat Köse[1], Süleyman Göksu[1], İlhami Gülçin[1,3], Saleh H. Alwasel[3], and Claudiu T. Supuran[4,5]

[1]Department of Chemistry, Faculty of Science, Atatürk University, Erzurum, Turkey, [2]Department of Basic Science, Faculty of Science, Erzurum Technical University, Erzurum, Turkey, [3]Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia, [4]Dipartimento di Chimica Ugo Schiff, Universita Degli Studi di Firenze, Sesto Fiorentino, Firenze, Italy, and [5]Neurofarba Department, Section of Pharmaceutical and Nutriceutical Sciences, Universita Degli Studi di Firenze, Sesto Fiorentino, Florence, Italy

Abstract

A series of carbamate derivatives were synthesized and their carbonic anhydrase I and II isoenzymes and acetylcholinesterase enzyme (AChE) inhibitory effects were investigated. All carbamates were synthesized from the corresponding carboxylic acids via the Curtius reactions of the acids with diphenyl phosphoryl azide followed by addition of benzyl alcohol. The carbamates were determined to be very good inhibitors against for AChE and hCA I, and II isoenzymes. AChE inhibition was determined in the range 0.209–0.291 nM. On the other hand, tacrine, which is used in the treatment of Alzheimer's disease possessed lower inhibition effect (K_i: 0.398 nM). Also, hCA I and II isoenzymes were effectively inhibited by the carbamates, with inhibition constants (K_i) in the range of 4.49–5.61 nM for hCA I, and 4.94–7.66 nM for hCA II, respectively. Acetazolamide, which was clinically used carbonic anhydrase (CA) inhibitor demonstrated K_i values of 281.33 nM for hCA I and 9.07 nM for hCA II. The results clearly showed that AChE and both CA isoenzymes were effectively inhibited by carbamates at the low nanomolar levels.

Keywords

Acetylcholinesterase, carbamates, carbonic anhydrase, enzyme inhibition, synthesis

History

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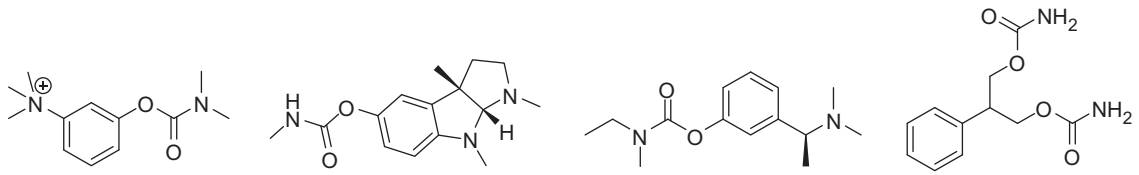
Introduction

Organic carbamates (urethanes) have unique applications in pharmaceutical chemistry. Many drugs contain carbamate functional groups in their structures[1]. Neostigmine (**1**)[2], Physostigmine (**2**)[3] and Rivastigmine (**3**)[4] are acetylcholinesterase inhibitors. A carbamate drug felbamate (**4**) commercially known as felbatol is an anticonvulsant drug and it is used in the treatment of epilepsy[5] (Figure 1). Besides these drugs, the synthesis and biological evaluation of some carbamates have also been reported, e.g. anticancer[6], HIV protease inhibition[7], antimicrobial properties[8], β-secretase inhibition[9], CA and AChE inhibitory effects of carbamates and sulfamoylcarbamates have been investigated by different research groups[10–12].

Carbon dioxide (CO_2) and bicarbonate (HCO_3^-) are essential components in living organism. The required CO_2 by the cell is transported into the cell by hydration reaction depending upon the HCO_3^- and CO_2 concentration inside and outside the cells. This transportation is performed depending upon the amount of CO_2 conversion into HCO_3^- and occurs very frequently in the cells. This conversion reaction is very slow and should be speed up somehow. Carbonic anhydrase (CA, EC 4.2.1.1) catalyzes this reaction with typical catalytic rates of the different forms of this enzyme ranging between 10^4 and 10^6 reactions per second[13–18]. Carbonic anhydrases are mainly Zn^{2+} containing metalloenzymes that catalyze the reversible interconversion of CO_2 and H_2O to HCO_3^- and a proton (H^+) for the hydration reaction or consumes one equivalent of H^+ for the dehydration reaction[15–20]. This makes these isoenzymes crucial for many physiological and biochemical processes including electrolyte secretion, respiration, pH and CO_2 homeostasis, bone calcification, ureagenesis, gluconeogenesis, tumorigenicity, lipogenesis, transport of CO_2/HCO_3^- between metabolizing tissues and the lungs, and some other physiologic or pathologic processes[21–25]. This enzyme class is present either in eukaryote or prokaryote cells. There are sixth main genetic families encoding classes of these enzymes: α-, β-, γ-, δ-, ζ- and η-CAs. It was reported that α-CAs are normally monomers and rarely dimers; β-CAs are dimers, tetramers or octamers; γ-CAs are trimers, whereas the δ- and ζ-CAs are less well understood at this moment[26–30]. α-CAs are found in algae, vertebrates, bacteria and cytoplasm of green plants. β-CAs are present in bacteria, algae and chloroplasts of monocotyledons and dicotyledons. On the other hand, the δ-CAs exists in diatoms and

Address for correspondence: Prof. Dr Süleyman Göksu, Department of Chemistry, Faculty of Science, Atatürk University, Erzurum, 25240, Turkey. Tel: +90 442 2314389. Fax: +90 442 234109. E-mail: sgoksu@atauni.edu.tr; Prof. Dr İlhami Gülin, Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia. Tel: +90 442 2314375. Fax: +90 442 234109. E-mail: igulcin@atauni.edu.tr



1 Neostigmine **2** Physostigmine **3** Rivastigmine **4** Felbamate

Figure 1. Some selected carbamate drugs.

other marine eukaryotes and ζ-CAs are found in diatoms[31]. Human CAs (hCAs) all belongs to the α-family and so far 16 different CA isoforms discovered in this class[32–34]. In humans, CAs are dispersed in different tissues including the reproductive tract, the gastrointestinal tract, kidneys, the nervous system, skin, eyes, lungs, and among some others[35–38]. Carbonic anhydrase isoenzymes contain a zinc ion (Zn^{2+}) in their active site, coordinated by three His residues and a H_2O molecule/hydroxide ion (−OH) in the α- and γ-CAs or by two Cys and one His residues (in the β class), with the fourth ligand being a H_2O molecule/−OH ion acting as nucleophile in the catalyzed reactions[39–43]. Cytosolic hCA I and II isoforms are spread throughout the human body and are drug targets for clinically used antiglaucoma, anticonvulsants and diuretics drugs[44–47]. Five of them (CA I, II, III, VII and XIII) are cytosolic, four of them (CA IV, IX, XII and XIV) are membrane bound, CA VA and VB are mitochondrial, and CA VI is secreted in saliva[48–53]. It was recently reported that CA XV isoform is not expressed in humans or in living primates. However, it is plentiful in rodents and other higher vertebrates. Also, three catalytic forms are also known and called CA-related proteins (CARP)[54–58]. All isoenzymes contain a zinc ion (Zn^{2+}) located at the base of a 15 Å deep funnel-shaped active site cavity, that is, coordinated to the imidazole groups of three His residues and to the substrate H_2O/hydroxide (−OH) that reacts with CO_2[59–63].

Acetylcholinesterase (AChE, EC. 3.1.1.7) is a crucial enzyme used to control transmission between neurons when the process is either mediated or modulated by the neurotransmitter acetylcholine (ACh)[64–67]. ACh is released by the axon terminal or varicosities of the transmitter neuron into the extracellular space to interact with the receptors of the other neuron. To maintain control of neurotransmission, it is necessary for AChE, after ACh executes its function, to catalyze ACh hydrolysis, converting ACh to choline (Ch) and acetate. After ACh hydrolysis, Ch is reabsorbed by the axon terminal to produce more ACh[68,69]. ACh acts as an excitatory neurotransmitter for voluntary muscles in the somatic nervous system (NS) and as a preganglionic and a postganglionic transmitter in the parasympathetic NS of vertebrates and invertebrates[70–72]. If AChE is inhibited in the central NS, the concentration of ACh increases in the synaptic cleft, leading to cholinergic crisis, which affords several dangerous effects, such as convulsion and respiratory problems, which could lead to death[70,72].

Some inhibitors of AChE have medical applications and are particularly important for the treatment of Alzheimer's disease (AD). When people develop AD, their neurons degenerate, leading to the low production of neurotransmitters, a process that induces serious memory problems. In this case, the inhibition of AChE increases the concentration of ACh in synaptic clefts, improving the neurotransmission process and brain function. For this reason, AChE inhibitors are very important agents for the treatment of AD, but some of these inhibitors are toxic, such as tacrine, requiring the development of new agents. Interestingly, some AChE reactivators also display competitive inhibition of the enzyme[73], and the reversible inhibitor and AD drug Galantamine protects animals from soman, sarin and paraoxon intoxication, suggesting that novel compounds may have dual application, for AD and organophosphorus intoxication[74].

As described above, carbamates show beneficial biological activities. Therefore, here we focused on the synthesis of some novel carbamates (**10–12**). Then, we investigated AChE and hCA I, and II isoenzymes inhibitory properties of carbamates **(10–16)** for the first time.

Experimental

General information

All chemicals and solvents are commercially available. All solvents were distilled and dried according to standard procedures. Silica gel (SiO_2, 60 mesh; Merck, Darmstadt, Germany) was used for column chromatography (CC). 1 mm of SiO_2 60 PF (Merck) on glass plates was used for preparative thick layer chromatography. Melting point of all compounds was determined with capillary melting-point apparatus (BUCHI 530; Meierseggstrasse 40, 9230 Flawil, Switzerland) and uncorrected. IR spectra were recorded as solutions in 0.1 mm cells with a Mattson 1000 FT-IR spectrophotometer (Unicam. Ltd., York Street, Cambridge, U.K.) ^{1}H- and ^{13}C-NMR spectra were recorded on 400 (100)-MHz Varian spectrometer (Danbury, Connecticut, USA) in deuterated solvents ($CDCl_3$ and D_2O) with tetramethylsilane (TMS, $SiMe_4$) as an internal standard for protons and solvent signals, as internal standard for carbon spectra. Chemical shift values were mentioned δ in ppm. Elemental analyses were recorded on Leco CHNS-932 apparatus (Saint Joseph, MI). Carbonic anhydrase and acetylcholinesterase inhibitory properties of samples were determined on a spectrophotometer (UV-1208, Shimadzu Co., Kyoto, Japan).

The synthesis of methyl 4,6-dimethoxy-2,3-dihydro-1H-indene-2-carboxylate (6)

Methyl 5,7-dimethoxy-1-oxo-2,3-dihydro-1H-indene-2-carboxylate (**5**) was synthesized according to the literature procedure[13]. Compound **5** (1.0 g, 4.0 mmol) was dissolved in TFA (6.12 mL, 79.92 mmol). Et_3SiH (2.55 mL, 15.98 mmol) was added to this mixture under N_2 gas and refluxed for 4 h. At the end of this time, TFA was evaporated. Then, saturated Na_2CO_3 (20 mL) solution was added to this mixture up to pH: 8.0 and extracted with EtOAc (3 × 10 mL). Diluted HCl (20 mL) was added to aqueous phase up to pH: 5.0 and it was extracted with EtOAc (3 × 10 mL). Combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. Column chromatography on silica gel (30 g) with 10% EtOAc-hexane was applied to the residue to give carboxylate **6** (0.83 g, 88%). White solid; m.p. 118–120 °C. IR (CH_2Cl_2, cm^{-1}): 3674, 2997, 2952, 2839, 1733, 1601, 1494, 1455, 1438, 1340, 1320, 1263, 1216, 1197, 1166, 1145, 1093, 1047, 934; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.37 (bs, 1H, H-5), 6.27 (bs, 1H, H-7), 3,78 (s, 6H, 2xOCH_3), 3,71 (s, 3H, OCH_3), 3.40–3.00 (m, 5H, 2XH-1, H-2, 2XH-3). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 176.0 (CO), 160.9 (C6)[a], 156.6 (C4)[a], 144.2 (C8),

121.6 (C9), 100.8 (C7), 77.2 (C5), 55.7 (OMe), 55.4 (OMe), 52.0 (OMe), 43.5 (C2), 36.9 (C1), 32.8 (C3). Anal. Calcd for ($C_{13}H_{16}O_4$): C, 66.09; H, 6.83. Found: C, 65.46; H, 7.03.

The synthesis of 4,6-dimethoxy-2,3-dihydro-1H-indene-2-carboxylic acid (7)

Ester **6** (0.90 g, 3.81 mmol) was dissolved in MeOH (60 mL). Saturated NaOH solution (20 mL) was added to this mixture and stirred for 24 h at room temperature. Then, MeOH was evaporated and extracted with CH_2Cl_2 (3×10 mL). HCl was added to aqueous phase up to pH: 1.0 and extracted with CH_2Cl_2 (3×20 mL). Combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. Carboxylic acid **7** was synthesized with a yield of 87% (0.74 g). Brown solid; m.p. 159–161 °C. IR (CH_2Cl_2, cm^{-1}): 3681, 3014, 2947, 2880, 2838, 1698, 1599, 1496, 1463, 1425, 1340, 1315, 1270, 1221, 1202, 1183, 1180, 1148, 1102, 1063, 1046, 929; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.79 (bs, 1H, OH), 6.38 (s, 1H, H-7), 6.29 (s, 1H, H-5), 3.79 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.39–3.08 (m, 5H, 2xH-1, H-2, 2xH-3). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 182.0 (CO), 160.9 (C4 or C6), 156.6 (C4 or C6), 143.9 (C8), 121.5 (C9), 100.7 (C7), 97.1 (C5), 55.8 (OMe), 55.4 (OMe), 52.0 (OMe), 43.4 (C2), 36.8 (C1 veya C3), 32.6 (C1 or C3). Anal. Calcd for ($C_{12}H_{14}O_4$): C, 64.85; H, 6.35. Found: C, 63.99; H, 6.31.

The synthesis of carboxylic acids 8 and 9

Carboxylic acids **8**[75] and **9**[76] were synthesized according to the procedures as described earlier.

General procedure for the synthesis of carbamates: benzyl(4,6-dimethoxy-2,3-dihydro-1H-inden-2-yl) carbamate (10)

Carboxylic acid **7** (0.45 g, 2.02 mmol) was dissolved in benzene (30 mL). NEt_3 (0.34 mL, 2.43 mmol) and DPPA (diphenylphosphoryl azide) (0.52 mL, 2.43 mmol) were added to this solution, respectively, and refluxed for 4 h. Then, benzyl alcohol (0.63 mL, 6.07 mmol) was added to this mixture and refluxed for 30 h. At the end of the reaction time, the benzene was evaporated. Column chromatography on silica gel (30 g) with 20% EtOAc-hexane was applied to the residue to give carbamate **10** (0.46 g, 70%). White solid; m.p. 79–81 °C. IR (CH_2Cl_2, cm^{-1}): 3676, 3317, 2950, 2891, 2837, 2140, 1692, 1597, 1524, 1493, 1455, 1340, 1320, 1250, 1208, 1145, 1097, 1046, 931. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.36–7.26 (m, H-5, ph), 6.38 (s, 1H, H-5 or H-7), 6.30 (s, 1H, H-5 or H-7), 5.28 (bs, 1H, NH), 5,10 (s, 2H, OCH_2), 4.52 (m, 1H, H-2) 3.26 (dd, 1H, H-1 or H-3, $J_{1,2trans}$ = 7.0 Hz, ^2J = 16,1 Hz), 3.16 (dd, 1H, H-1 or H-3, $J_{1,2trans}$ = 7.0 Hz, ^2J = 16,1 Hz), 2.77 (dd, 1H, H-1 L or H-3 L, $J_{1,2cis}$ = 4.1 Hz, ^2J = 16,1 Hz), 2.67 (dd, 1H, H-1 L or H-3 L, $J_{1,2cis}$ = 4.1 Hz, ^2J = 16.1 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 160.9 (CO), 156.9 (C4 or C6), 156.2 (C4 or C6), 143.4 (C8), 136.8 (CH (Ph)), 128.7 (2CH (Ph)), 128.3 (2CH (Ph)), 127.2 (CH (Ph)), 120.9 (C9), 101.3 (C7), 97.1 (C5), 66.8 (OMe), 55.8 (OMe), 55.4 (OMe), 52.5 (C2), 41.3 (C1), 36.5 (C3). Anal. Calcd for ($C_{19}H_{21}NO_2$): C 69.71; H 6.47; N 4.28. Found: C 68.58; H 6,43; N 4.66.

Benzyl 6-methoxy-1,2,3,4-tetrahydronaphthalen-2-ylcarbamate (11)

Carbamate **11** was synthesized from acid **8** with a yield of 93% (2.70 g). White solid; m.p. 104–106 °C. IR (CH_2Cl_2, cm^{-1}): 3329, 3061, 2938, 1710, 1578, 1432, 1344, 1254, 1093, 903. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.38–7.32 (m, 5H, Ar-H), 6.97 (d, J = 8.4 Hz, 1H, Ar-H), 6.71 (dd, J = 2.7 Hz, 8.4 Hz, 1H, Ar-H), 6.63 (d, J = 2.3 Hz, 1H, Ar-H), 5.11 (s, 2H, CH_2), 4.91 (d, J = 6.9 Hz, 1H, NH), 4.16–4.03 (m, 1H, CH-N), 3.78 (s, 3H, OCH_3), 3.06 (dd, 1H, CH_2, A part of AB system, J = 4.1 Hz, 15.9 Hz), 2.92–2.80 (m, 2H, CH_2), 2.59 (dd, 1H, CH_2, B part of AB system, J = 8.1 Hz, 15.9 Hz), 2.08–2.04 (m, 1H, CH_2), 1.81–1.72 (m, 1H, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 158.2 (CO), 156.0 (C), 136.8 (C), 130.6 (2CH), 130.0 (C), 128.8 (2CH), 128.4 (2CH), 126.2 (C), 113.6 (CH), 112.6 (CH), 66.9 (OCH_2), 55.5 (OCH_3), 47.2 (CH-N), 35.4 (CH_2), 29.1 (CH_2), 27.6 (CH_2). Anal. Calcd for ($C_{20}H_{24}NO_3$): C 73.59; H 7.41; N 4.29. Found: C 73,03; H 7.48; N 4.20.

Benzyl 7-methoxy-1,2,3,4-tetrahydronaphthalen-2-ylcarbamate (12)

Carbamate **12** was synthesized from acid **9** with a yield of 90% (2.61 g). White solid; m.p. 105–107 °C. IR (CH_2Cl_2, cm^{-1}): 3334, 3058, 2942, 1721, 1598, 1437, 1345, 1259, 1103, 918. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.37–7.32 (m, 5H, Ar-H), 7.00 (d, J = 8.4 Hz, 1H, Ar-H), 6.71 (dd, J = 2.7 Hz, 8.4 Hz, 1H, Ar-H), 6.59 (d, J = 2.3 Hz, 1H, Ar-H), 5.10 (s, 2H, CH_2), 4.83 (d, J = 7.1 Hz, 1H, NH), 4.13–4.00 (m, 1H, CH-N), 3.76 (s, 3H, OCH_3), 3.10 (dd, 1H, CH_2, A part of AB system, J = 4.3 Hz, 16.4 Hz), 2.83–2.79 (m, 2H, CH_2), 2.64 (dd, 1H, CH_2, B part of AB system, J = 7.8 Hz, 16.4 Hz), 2.07–2.02 (m, 1H, CH_2), 1.82–1.73 (m, 1H, CH_2).^{13}C-NMR (100 MHz, $CDCl_3$): δ = 158 (CO), 136.7 (C), 135.3 (C), 129.9 (2CH), 128.8 (3CH), 128.4 (CH), 127.7 (C), 114.1 (CH), 112.8 (2CH), 66.9 (OCH_2), 55.5 (OCH_3), 46.9 (CH-N), 36.4 (CH_2), 29.2 (CH_2), 26.3 (CH_2). Anal. Calcd for $C_{20}H_{24}NO_3$: C 73.59; H 7.41; N 4.29. Found: C 73.16; H 6.64; N 4.58.

The synthesis of carbamates **13**[77] and **14**[78], sulfamoyl carbamates **15**[33] and **16**[33] were achieved according to our previous procedure.

Biochemical studies

Carbonic anhydrase isoenzymes (hCA I and II) were purified by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography in a single purification step[62]. Sepharose-4B-L-tyrosine-sulfanilamide was prepared according to a reported method[61]. Thus, pH of the solution was adjusted to 8.7, using solid Tris. Then, supernatant was transferred to the previously prepared Sepharose-4B-L-tyrosine-sulphanilamide affinity column[50]. Subsequently, the proteins from the column were spectrophotometrically determined at 280 nm[79–85]. For determination of the purity of the hCA isoenzymes, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), having 10 and 3% acrylamide as an eluent and packing gel, respectively, with 0.1% SDS[86–90], was performed, through which a single band was observed for each isoenzyme.

Carbonic anhydrase isoenzymes activities were determined following the methods described by Verpoorte et al.[91] and the methods reported previously[60]. Absorbance change at 348 nm from *p*-nitrophenylacetate (NPA) to *p*-nitrophenolate (NP) was recorded by 3 min intervals at the room temperature (25 °C) using a spectrophotometer (Shimadzu, UV-VIS Spectrophotometer, UVmini-1240, Kyoto, Japan). Quantity of the protein was measured spectrophotometrically at 595 nm during the purification steps according to the Bradford method[92]. As reported previously, bovine serum albumin was used as a standard protein. An activity (%)-[Carbamates] graph was depicted to determine the inhibition effect of each carbamate derivative. For K_i values, three different carbamate derivatives were tested. NPA was used as a substrate at five different concentrations, and Lineweaver–Burk curves[93] were drawn as described previously[90].

In the third part of this study, the inhibitory effects of carbamates **(10–16)** on AChE activities were determined according to the Ellman test[94]. Acetylthiocholine iodide (AChI) was used as substrate for this reaction. 5,5′-Dithio-bis(2-nitro-benzoic)acid (DTNB) was used for the measurement of the AChE activity. Briefly, 100 mL of Tris/HCl buffer (1.0 M and pH 8.0), 10 mL of carbamates solution dissolved in deionized water at different concentrations and 50 mL AChE (5.32×10^{-3} EU) solution were mixed and incubated for 10 min at 25 °C. Then a portion of DTNB (50 mL, 0.5 mM) was added. Subsequently, the reaction was initiated by the addition of 50 mL of AChI (10 mM). The hydrolysis of these AChI was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of AChI, at a wavelength of 412 nm[67,95]. For determination of the effect of carbamates **(10–16)** on AChE, different carbamates **(10–16)** concentrations were added into the reaction solution. AChE activity was measured, and an experiment in the absence of drug was used as control. The IC_{50} values were obtained from activity (%) versus carbamates **(10–16)** concentration plots. For determination of *K*i constants in the media with carbamates **(10–16)** as inhibitor, the different ACh concentrations were used as substrate.

Results and discussion

Synthesis

β-Keto ester **5** was synthesized according to the literature procedure[96]. Reduction of keto esters with Et_3SiH in the presence of trifluoroacetic acid (TFA) has been reported[97]. Therefore, applying this method to compound **5** for conversation of C=O functional group to CH_2 with Et_3SiH in the presence of TFA gave ester **6**. Hydrolysis of ester group of compound **6** with NaOH in MeOH–H_2O followed by acidification with dilute HCl gave acid **7** in good yield. Carboxylic acids **8**[75] and **9**[76] were also synthesized according to the procedures described previously. Curtius reaction is one of the most efficient methods for the conversion carboxylic acids to the corresponding alkyl isocyanates[78]. It is also very well known that the reactions of alkyl isocyanates with alcohols yield related carbamates[98]. In this context, the reactions of acids **7–9** with diphenyl phosphoryl azide (DPPA) in the presence Et_3N at 80 °C for 4 h then addition of benzyl alcohol (BnOH) and heating the reaction mixture at the same temperature for 30 h furnished carbamates **10–12** in good yields (Scheme 1). The structures of the synthesized novel compounds were elucidated by ^1H, ^{13}C-NMR spectroscopy. Functional groups were determined by IR spectroscopy techniques. In addition, some synthetically known carbamate **13**[77] and **14**[78], sulfamoyl carbamates **15**[33] and **16**[33] were also synthesized for biological investigation. These compounds were synthesized according to the procedure described previously by us (Figure 2).

Biological activity

The CA I and II examined in this study, have different activities. In mammals, CA II, which generally exists in red blood cells in lower concentrations, has approximately 10 times higher activity compare with CA I[99–102]. Cytosolic hCA I isoenzyme is ubiquitously expressed in body, and available in high concentrations in blood and gastrointestinal tract. As it was demonstrated that this isoenzyme is involved in retinal and cerebral edema, its inhibition could be a valuable tool for fighting the condition[103,104]. It is generally accepted that if K_i value of a tested compound is less than 50 μM ($K_i > 50$ μM), that compound is considered to be inactive against hCA I[18]. The results presented in Table 1 indicate that the new carbamates **(10–16)** had effective inhibition profile against slow cytosolic hCA I isoform, and cytosolic dominant rapid hCA II isoenzyme. The cytosolic hCA I isoenzyme was inhibited by all the synthesized carbamates **(10–16)** in low nanomolar levels, the K_i of which varied between 4.49 ± 1.32 and 5.61 ± 1.52 nM. On the other hand, acetazolamide (AZA), considered being a broad-specificity CA inhibitor owing to its widespread inhibition of CAs, showed K_i value of 281.33 ± 55.33 nM against hCA I. Among the inhibitors, carbamates **15** was found to be the best hCA I inhibitor with K_i of 4.49 ± 1.32 nM. The inhibition effects of the carbamates **(10–16)** were found to be greater than that of acetazolamide.

The hCA II is not only a very effective catalyst for interconversion between CO_2 and HCO_3^-, but also shows some catalytic versatility, participating in several other hydrolytic processes, which presumably involve non-physiological

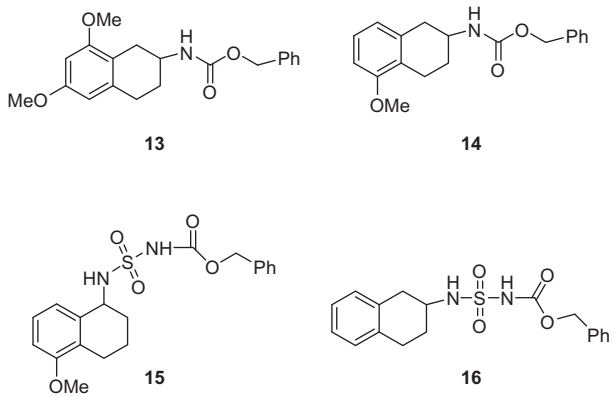
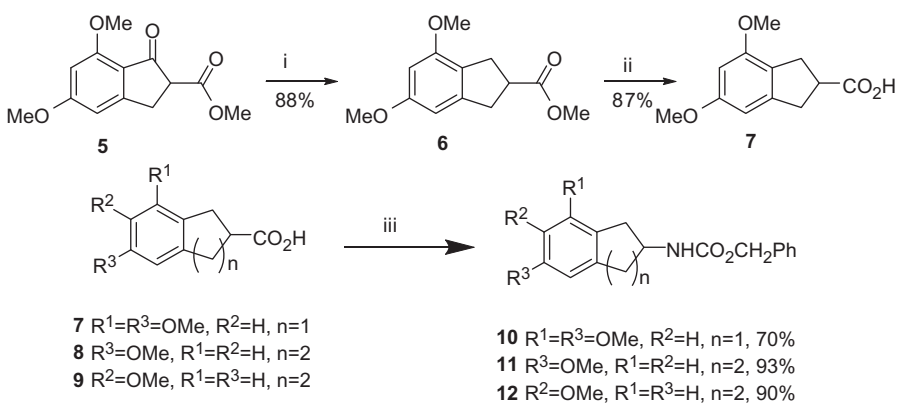


Figure 2. Carbamates **13** and **14**, sulfamoyl carbamates **15** and **16**.

Scheme 1. The synthesis of acid **7** and carbamates **10–12** (i) TFA, Et_3SiH, 75 °C, 4 h (ii) NaOH, MeOH-H_2O, 25 °C, 4 h (iii) NEt_3, DPPA, 80 °C, 4 h, then BnOH, 80 °C, 30 h.



7 R^1=R^3=OMe, R^2=H, n=1
8 R^3=OMe, R^1=R^2=H, n=2
9 R^2=OMe, R^1=R^3=H, n=2

10 R^1=R^3=OMe, R^2=H, n=1, 70%
11 R^3=OMe, R^1=R^2=H, n=2, 93%
12 R^2=OMe, R^1=R^3=H, n=2, 90%

Table 1. Human carbonic anhydrase isoenzymes (hCA I and II) and acetylcholine esterase (AChE) inhibition profile of carbamate derivatives **(10–16)**.

	IC_{50} (nM)						K_i (nM)		
Compounds	hCA I	r^2	hCA II	r^2	AChE	r^2	hCA I	hCA II	AChE
10	53.32	0.9844	7.24	0.9787	72.05	0.9725	5.61 ± 1.52	7.39 ± 2.66	0.261 ± 0.099
11	54.54	0.9802	89.79	0.9641	7.91	0.9622	5.41 ± 1.59	7.66 ± 2.73	0.286 ± 0.085
12	49.65	0.9808	157.00	0.9617	7.92	0.9679	5.51 ± 1.30	7.48 ± 2.23	0.291 ± 0.105
13	30.58	0.9845	44.69	0.9728	20.39	0.9737	4.91 ± 1.68	6.93 ± 2.49	0.265 ± 0.083
14	69.73	0.9861	6.98	0.9530	7.70	0.9693	5.89 ± 1.65	7.39 ± 2.74	0.264 ± 0.095
15	19.41	0.9865	13.43	0.9630	24.53	0.9709	4.49 ± 1.32	4.94 ± 1.76	0.209 ± 0.069
16	23.50	0.9874	21.89	0.9745	9.66	0.9676	4.59 ± 1.35	5.78 ± 1.78	0.240 ± 0.097
AZA*	8.26	0.9654	8.93	0.9676	-	-	281.33 ± 55.33	9.07 ± 2.68	-
TAC**	-	-	-	-	57.52	0.9721	-	-	0.398 ± 0.103

*Acetazolamide (AZA) was used as a standard inhibitor for CA I and II.
**Tacrine (TAC) was used as a standard inhibitor for AChE.

substrates[105,106]. Against the physiologically dominant isoform hCA II, carbamates **(10–16)** showed K_is varying from 4.94 ± 1.76 to 7.66 ± 2.73 nM (Table 1), among which the carbamates **15** was the best hCA II inhibitor (K_i: 4.94 ± 1.76 nM). Thus, these carbamates **(10–16)** had high inhibition affinity toward hCA II. On the other hand, AZA, which may interact with the distinct hydrophilic and hydrophobic halves of the CA II active site, and showed K_i of 9.07 ± 2.68 nM. Carbonic anhydrase isoenzymes are physiologically very important enzymes. Recently, very intense studies were performed on this subject[107–112].

In our study, carbamates **(10–16)** were investigated for their ability to inhibit AChE. According to our data, inhibitory effects of these carbamates **(10–16)** revealed a significant elevation in the case of AChE. Generally, these compounds showed higher inhibition and higher lipophilicity. Considering the results, all carbamates **(10–16)** expressed significantly higher inhibition activity. All the carbamates **(10–16)** derivatives had significantly higher AChE inhibitory activity than that of standard AChE inhibitors, such as Tacrine. Furthermore, the K_i values of carbamates **(10–16)** and standard compound (Tacrine) are summarized in Table 1. As can be seen in the results obtained from Table 1, AChE was effectively inhibited by carbamates derivatives **(10–16)**, with K_i values in the range of 0.209 ± 0.069 to 0.291 ± 0.105 nM. However, all of carbamates derivatives **(10–16)** had almost similar K_i values. The most active carbamate derivative is compound **15** and showed a K_i value of 0.209 ± 0.069 nM. These results clearly indicate that carbamates derivatives **(10–16)** as well as future similar derivatives may function as drugs for the treatment of Alzheimer's disease.

Conclusion

In conclusion, we synthesized a series of carbamates starting from indan or tetralin carboxylic acids. As carbamates show a broad biological activity spectrum, in the present study, AChE and CA inhibition properties of the synthesized compounds were investigated. The carbamates derivatives **(10–16)** demonstrated effective inhibition profiles against hCA I, II and, AChE. The similar inhibition profiles of these compounds for the two CA isoforms can be due to the high homology between hCA I and II isoenzymes.

Declaration of interest

There is no declaration of interest for this work.

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