Synthesis and biological evaluation of aminomethyl and alkoxy methyl derivatives as carbonic anhydrase, acetylcholinesterase and butyrylcholinesterase inhibitors

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ABSTRACT

Compounds containing nitrogen and sulfur atoms can be widely used in various fields such as industry, medicine, biotechnology and chemical technology. Therefore, the reactions of aminomethylation and alkoxy methylation of mercapto benzothiazole, mercaptobenzoxazole and 2-aminothiazole were developed. Additionally, the alkoxy methyl derivatives of mercaptobenzoxazole and 2-aminothiazole were synthesized by a reaction with hemiformals, which are prepared by the reaction of alcohols and formaldehyde. In this study, the inhibitory effects of these molecules were investigated against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) enzymes and carbonic anhydrase I, and II isoenzymes (hCA I and II). Both hCA isoenzymes were significantly inhibited by the recently synthesized molecules, with 5 Ki values in the range of 58–157 nM for hCA I, and 81–215 nM for hCA II. Additionally, the k values of these molecules for BChE and AChE were calculated in the ranges 23–88 and 18–78 nM, respectively.

Introduction

Chemists are interested in derivatives of mercaptobenzothiazole and mercaptobenzoxazole because a number of biologically and physiologically active compounds with bactericidal, fungicidal, tuberculostatic, anti-inflammatory, parasympatholytics and anesthetic properties have been synthesized based on them.1

The carbonic anhydrases (CAs, E.C.4.2.1.1) are a superfamily of metalloenzymes that catalyze a crucial and simple biochemical reaction, the reversible hydration of carbon dioxide (CO2) and water (H2O) to bicarbonate (HCO3) and protons (H+).2-5 This reaction, in the absence of CA cannot proceed with a perceptible rate under physiological conditions.

CO2 + H2O ⇌ H2CO3 ⇌ HCO3− + H+[6-8]

CAs are widely distributed in all kingdoms of life and are categorized in seven distinct classes: α, β, γ, δ, ε, η and θ-CAs. Each CA family demonstrates proper specific characteristics in the primary amino acid sequence.6,10 α-CAs are found in mammals. α-CAs, which have sixteen isoenzymes are expressed predominantly in vertebrates and are the only class observed in humans. They are catalytically active and differ in their subcellular localization, distribution in organs and tissues, kinetic properties, expression levels, and inhibitor binding affinities.11-13 Additionally, CAs play important roles in a multitude of physiological activities in eukaryotes, such as CO2 transport, respiration, photosynthesis and electrolyte secretion.14-16

The production of novel CA inhibitors (CAIs) is a growing priority for pharmaceutical research and discovery. In addition to the defined role of CAs as antiglaucoma drugs and diuretics, their potential as anti-obesity, anti-convulsant, anti-inflammatory and anti-cancer has been recently described.17,18 hCA II inhibitors has been widely studied from structural and design points-of-view and in dynamics simulations.19-21 In addition, it is the most widespread physiologically relevant CA isoenzyme.

Alzheimer’s disease (AD) is the most prevalent cause of dementia in elderly people.22-24 Recoveries in cognitive capabilities in AD patients were obtained by disrupting or blocking the acetylcholinesterase (AChE) activity with inhibitor compounds.25-27 Alkaloid compounds are some of the strongest acetylcholinesterase inhibitors (AChEIs); therefore searches for novel alkaloids with inhibitory compounds have been conducted.28-30 The AChE enzyme by prompting hydrolyses of the neurotransmitter acetylcholine (ACh), concluding an impulse transmissions at the cholinergic synapses in neurons.31,32 As can be seen in Figure 1, the active site of AChE consists of two parts: (i) the anionic part that accommodates the positively charged section of acetylcholine and (ii) the catalytic part where the ester bond is hydrolysed.33,34 AChE is the target of many drugs and neurotoxins that bind particularly to its active site.35,36 Inhibition of AChE is used for the treatment of senile dementia, AD, myasthenia gravis, ataxia and Parkinson’s disease.37-39 AChE can also serve as a probe for biosensors that are capable of binding to and potentially discovering new AChE inhibitor compounds; these compounds have applications as...
possible neurotoxins, such as nerve factors, pesticides and therapeutic drugs.\textsuperscript{46,47} X-ray structures have indicated that the although the butyrylcholinesterase (BChE) and AChE structures are similar, multiple structural discrepancies in the active-site gorges and the active sites have been observed.\textsuperscript{42,43} BChE has of toxicological and pharmacological importance because it scavenges ChEIs, including potent organophosphorus nerve factors, before they bind synapses and hydrolyzes ester-containing drugs.\textsuperscript{44} BChE is also important for drug metabolism such as cocaine.\textsuperscript{45} Both BChE and AChE, which have molecular roles beyond normal neurons and differentiated kinetics recorded in the brain, accumulate within tangles and amyloid plaques.\textsuperscript{46}

The goal of this paper is to design and synthesize some novel aminomethyl and alkoxymethyl derivatives (1–17) and to generate more potent BChE and AChE enzymes, CA II and I isoforms.

**Experimental**

**Chemistry**

**Synthesis of aminomethyl derivatives of benzothiazole and benzoxazolthiones (1–10)**

Aminomethylation was carried out at the temperature of 10°C by adding the corresponding aminal to a solution of mercaptobenzothiazole (or mercaptobenzoxazole) in ethanol. The resulting product was recrystallized from methanol. The aminomethyl derivatives of benzothiazole and benzoxazolthiones 2–8 were reported in the literature.\textsuperscript{47–53} However, there is no information about the synthesis of compounds 9 and 10 in the literature.

Initial aminals were obtained by condensing of secondary amines with formaldehyde. The physico-chemical characteristics of the obtained products are shown in Table 1.

Formaldehyde was used as a form of paraformaldehyde. The reaction was carried out in an absolute ethanol solution. Hemiformals reacted immediately after its preparation without isolation. The resulting reaction water was separated by azeotropic distillation with benzene. The crystals were obtained after distilling the solvents, including ethanol and benzene, and recrystallizing. The melting points and yields are given in Table 2.

**Synthesis of the alkoxymethyl derivatives of benzoxazolthione and 2-aminothiazole (11–17)**

To do this, hemiformal was obtained from 0.05 mol of a formaldehyde (used as paraformaldehyde) and 40 mL of the corresponding alkanol (taken in excess as a solvent). Hemiformal reacted immediately after its preparation without isolation. Then, 0.05 mol of mercaptobenzoxazole (or 2-aminothiazole) dissolved in ethanol was added to hemiformal at the temperature of 10°C. The resulting reaction water was separated by azeotropic distillation with benzene. The crystalline substances were obtained after distilling off the solvent (ethanol, benzene) and recrystallization.

**Biological studies**

**Purification of carbonic anhydrase I and II isoforms and inhibition studies**

To observe of inhibition effects of novel aminomethyl and alkoxymethyl derivatives (1–17) on CA I, and II isoforms, which purified from fresh human erythrocyte using an affinity chromatography procedure.\textsuperscript{54,55} CA activity was determined using the previously described spectrophotometric procedure of Verpoorte et al.\textsuperscript{56} as explained previously.\textsuperscript{21,57,58} In this procedure, changes in activity were obtained during 3 min at 22°C. \(p\)-Nitrophenylacetate (PNA) compound was used as a substrate, and it was converted by both isoforms to \(p\)-nitrophenolate ions.\textsuperscript{59,60} The quantity of protein was measured according to the previously described by Bradford method\textsuperscript{61–64} and bovine serum albumin was used as the standard.\textsuperscript{55,66} After the purification method of the CA isoforms, samples were subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE).\textsuperscript{57–69} The change in activity was spectrophotometrically obtained at 348 nm.\textsuperscript{70,71} The I_{50} values were calculated from activity (%) against compounds inhibition.\textsuperscript{72–74} Three various concentrations were used to calculate \(K_i\) values.\textsuperscript{75–77}

**AChE/BChE activity determination and inhibition studies**

The inhibitory effects of novel aminomethyl and alkoxymethyl derivatives (1–17) on AChE and BChE activities were measured according to Ellman \textit{et al.}\textsuperscript{78} Acetylthiocholine iodide (AChI) and butyrylthiocholine iodide (BChI) were used as substrates for the reaction. 5,5’-Dithio-bis-(2-nitro-benzoic)acid (DTNB) was used for the measurement of the AChE/BChE activities. Briefly, 1.0 mL of Tris/HCl buffer (1.0 M, pH 8.0), and 10 μL of sample solution were dissolved in deionized water at different concentrations and 50 μL AChE/BChE solution were mixed and incubated for 10 min at 25°C. Next 50 μL of DTNB (0.5 mM) was added. The reaction was then initiated by the addition of 50 μL of AChI or BChI. The hydrolysis of these substrates was monitored spectrophotometrically by the formation of the yellow 5-thio-2-nitrobenzoate anion, as a result of the reaction of DTNB with thiococholine, which released by enzymatic hydrolysis of AChI or BChI, with absorption maximum at 412 nm.
Results and discussion

Synthesis

Many physiologically active natural compounds contain > N-CH$_2$-O- > N-CH$_2$-N < structural fragments. This study sought to build a structure that combines physiologically active benzothiazole or benzoxazole groups with alkoxymethyl or aminomethyl fragments. Therefore, the aminomethylation and alkoxymethylation reactions of mercaptobenzothiazole, mercaptobenzoxazole and 2-aminothiazole are developed.

The structure of the products was established by NMR spectroscopy and the composition was confirmed by elemental analysis. Spectra were measured on a Bruker device in acetone. A singlet at 4.6 ppm corresponding to N-CH$_2$-N was observed in the $^1$H NMR spectra of all aminomethyl derivatives. A singlet at 5.2–5.8 ppm characterized the presence of the fragment N-CH$_2$-O in the $^1$H NMR spectra of alkoxymethyl derivatives. Methylene-bis-amines, which have good alkylation (amino-methylation) properties, were used as amino-methylation reagents.

| No | Compound | The melting point (°C) | Yield (%) | C | H | N | S | Brutto formula | NMR spectra $\delta$ (ppm) |
|----|----------|------------------------|-----------|---|---|---|---|----------------|--------------------------|
| 1  |          | 120–121                | 45        | 54.61 | 5.76 | 10.60 | 24.20 | C$_{12}$H$_{14}$N$_2$O$_2$S | 1.79 (kv. 2H, CH$_2$(CH$_3$)$_2$, 3.29(t, 2H, NCH$_2$), 3.9 (t, 2H, OCH$_3$), 4.357(t, 2H, NCH$_2$), 5.3 (s, 2H, NCH$_2$O), 6.8–7.6 (m, 4H, C$_6$H$_4$). |
| 2  |          | 124–126                | 40        | 56.00 | 5.90 | 10.30 | 26.60 | C$_{11}$H$_{12}$N$_2$O$_2$S | 1.819 (kv. 2H, CH$_2$(CH$_3$)$_2$, 3.119(t, 2H, NCH$_2$), 3.9 (t, 2H, OCH$_3$), 4.357(t, 2H, NCH$_2$), 5.3 (s, 2H, NCH$_2$O), 7.1–7.3 m. 4H (C$_6$H$_4$). |
| 3  |          | 134                    | 40        | 54.9  | 5.55 | 11.01 | 24.56 | C$_{12}$H$_{14}$N$_2$O$_2$S | 1.7 (m, 2H, CH$_2$(CH$_3$)$_2$, 1.97 (m, 4H, CH$_2$(CH$_3$)$_2$), 3.01 (t, 4H, NCH$_2$), 3.9 (t, 4H, NCH$_2$), 5.26 (s, 2H, NCH$_2$O), 7.1–7.6 (m, 4H, C$_6$H$_4$). |
| 4  |          | 152–152.5              | 42        | 57.6  | 7.5  | 11.3  | 28.8  | C$_{13}$H$_{16}$N$_2$S$_2$ | 1.5 (m, 2H, CH$_2$(CH$_3$)$_2$, 1.7 (m, 4H, CH$_2$(CH$_3$)$_2$), 3.01 (t, 4H, NCH$_2$N), 3.01 (t, 4H, NCH$_2$N), 7.1–7.6 (m, 4H, C$_6$H$_4$). |
| 5  |          | 128–130                | 35        | 54.4  | 5.98 | 8.2   | 27.0  | C$_{12}$H$_{14}$N$_2$S$_2$ | 2.19 (kv. 2H, CH$_2$(CH$_3$)$_2$, 3.12 (t, 2H, NCH$_2$), 3.6 (t, 2H, OCH$_3$), 4.67 (t, 2H, NCH$_2$N), 5.20 s. 2H (NCH$_2$O), 7.1–7.5 m. 4H (C$_6$H$_4$). |
| 6  |          | 105                    | 65        | 59.87 | 5.98 | 10.85 | 13.03 | C$_{13}$H$_{16}$N$_2$S$_2$ | 1.28 (m, 2H, CH$_2$(CH$_3$)$_2$, 3.04 (t, 2H, NCH$_2$), 3.69 t, (2H, CH$_2$), 4.59 (s, 2H, NCH$_2$N), 5.36 (s, 2H, NCH$_2$O), 6.9–7.9 (m, 4H, C$_6$H$_4$). |
| 7  |          | 125                    | 45        | 63.52 | 6.04 | 9.39  | 11.53 | C$_{13}$H$_{16}$N$_2$S$_2$ | 1.88 (m, 2H, CH$_2$(CH$_3$)$_2$, 3.18 (t, 2H, NCH$_2$), 3.9 (t, 2H, CH$_2$), 4.39 (s, 2H, NCH$_2$N), 5.56 (s, 2H, NCH$_2$O), 7.4–7.7 (m, 4H, C$_6$H$_4$). |
| 8  |          | 145–147                | 48        | 57.58 | 5.7  | 11.34 | 12.54 | C$_{12}$H$_{14}$N$_2$O$_2$S | 1.21 (d, 2H, CH$_2$(CH$_3$)$_2$, 3.23 (t, 2H, NCH$_2$), 3.7 (t, 2H, CH$_2$), 4.39 (s, 2H, NCH$_2$N), 5.86 (s, 2H, NCH$_2$O), 7.1–7.97 (m, 4H, C$_6$H$_4$). |
| 9  |          | 115–118                | 60        | 55.84 | 5.25 | 11.65 | 13.87 | C$_{12}$H$_{14}$N$_2$O$_2$S | 1.38 (m, 2H, CH$_2$(CH$_3$)$_2$, 2.08 (t, 2H, NCH$_2$), 3.39 (t, 2H, CH$_2$), 3.59 (s, 2H, NCH$_2$N), 5.16 (s, 2H, NCH$_2$O), 7.1–8.7 (m, 4H, C$_6$H$_4$). |
| 10 |          | 145–147                | 67.6      | 56.0  | 5.51 | 11.0  | 13.3  | C$_{12}$H$_{14}$N$_2$O$_2$S | 1.8 (m, 2H, CH$_2$(CH$_3$)$_2$, 3.08 (t, 2H, NCH$_2$), 3.9 (t, 2H, CH$_2$), 4.59 (s, 2H, NCH$_2$N), 5.56 (s, 2H, NCH$_2$O), 7.4–7.7 (m, 4H, C$_6$H$_4$). |

The alkoxymethyl derivatives of mercaptobenzoxazole and 2-aminothiazole were synthesized by reacting them with hemiformal, which were prepared by the reaction of alcohols.
with formaldehyde.

\[
\text{ROH} + \text{CH}_2\text{O} \rightarrow \text{ROCH}_2\text{OH}
\]

| No | Compounds | Melting point (°C) | Yield (%) | C       | H       | N       | S       | Brutto Formula | NMR spectra δ (ppm) |
|----|-----------|--------------------|-----------|---------|---------|---------|---------|----------------|---------------------|
| 11 | ![Structure](image1) | 130 | 71.72 | 55.40 | 4.58 | 7.13 | 16.35 | C4H8NO2S | 2.06 (s., 3H, OCH3); 5.781 (s., 2H, NCH2O); 7.373–7.55 (m., 4H, C6H4). |
| 12 | ![Structure](image2) | 132–133 | 77 | 57.37 | 5.15 | 6.64 | 15.23 | C8H11NO2S | 2.06–2.096 (t., 3H, CH3); 3.07 (t., 2CH2CH3); 5.766 (s., 2H, NCH2O); 7.33–7.52 (m., 4H, C6H4). |
| 13 | ![Structure](image3) | 126–127 | 24 | 57.32 | 5.63 | 6.13 | 14.18 | C12H15NO2S | 2.01–2.16 (d., 6H, CH3); 2.999 (m., 1H, OCH3); 7.27 (s., 2H, NCH2O); 7.34–7.53 (m., 4H, C6H4). |
| 14 | ![Structure](image4) | 120–121 | 19 | 55.17 | 5.35 | 5.75 | 13.28 | C12H15NO2S | 1.06–2.01 (t., 3H, CH3); 3.072 (m., 4H, C6H4). |
| 15 | ![Structure](image5) | 120–121 | 30 | 43.52 | 6.28 | 14.78 | 17.15 | C12H15NO2S | 2.86 (s., 1H, NH); 5.11 (t., 4H, -OCH2CH2O-); 5.29 (s., 3H, -OCH3); 5.82 (s., 2H, NCH2O); 6.86 (d., 1H, SCH); 7.61 (d., 1H, SCH). |
| 16 | ![Structure](image6) | 118–122 | 118–120 | 39.78 | 6.35 | 15.97 | 17.80 | C16H19NOS | 1.11–2.10 (t., 3H, CH3); 3.10 (t., 2CH2CH3); 5.20 (s., 2H, NCH2O); 6.83–7.7 (m., 4H, C6H4). |
| 17 | ![Structure](image7) | 126–127 | 27 | 40.52 | 5.03 | 18.37 | 23.12 | C10H13NO3S | 2.86–2.47 (s., 1H, NH); 5.19 (t., 4H, -OCH2CH2O-); 5.19 (s., 3H, -OCH3); 5.12 (s., 2H, NCH2O); 6.68 (d., 1H, SCH); 7.71 (d., 1H, SCH). |

Table 2. Physico-chemical characteristics of the alkoxymethyl derivatives of benzoxazolthione and 2-aminothiazoles.

Biological results

Sulfamate and sulfonamide CAIs demonstrated fundamental anti-glaucoma and anti-tumour activities in vivo and in vitro; therefore new therapeutic approaches targeting either hCA IX/XII (for antitumor activity) or hCA II (for antiglaucoma action) have been developed. Heterocyclic molecules with primitive sulfonamide compounds are the most extensively evaluated class of CAIs, which has led to the advancement of diverse classes of clinical drugs like methazolamide (MZA), acetazolamide (AZA) and others. In this work, both the Ki and IC50 of the aminomethyl and alkoxymethyl derivatives (1–17) were calculated and they are given in Table 3.

1. Cytosolic hCA I, and II isoenzymes are widely distributed throughout the human body and interference with these enzymes may cause side effects. For the cytosolic hCA I enzyme, aminomethyl and alkoxymethyl derivatives (1–17) had Ki values in the range of 58 ± 15 to 157 ± 38 nM (Table 3). Especially, compound 8 (Ki: 58 ± 15 nM); N-morfolinomethylbenzoxazoline-2-thion and compound 5 (Ki: 70 ± 15 nM); N-diethylaminomethylbenzothiazoline-2-thione) inhibited the hCA I isofrom more potently than the standard compound AZA (Ki: 333 ± 28 nM), which is used to treat glaucoma, cystinuria, periodic paralysis, epileptic seizure, dural estasia and central sleep apnea. hCA I is involved in retinal edema and cerebral and the inhibition of hCA I can be a significant factor for eliminating of these conditions.

2. The role of hCA II in diseases such as glaucoma has been well characterized. Indeed, HCO3− production serves as a mechanism to transport sodium ions (Na+) into the eye along with the influx of water, which leads to an increase in intraocular pressure. Inhibition of CA II decreases HCO3− production and
3. BChE and AChE were very significantly inhibited by novel AZA (ratio 0.9774) and AChE (ratio 0.9774) inhibitors. The most significant inhibition result was recorded by N-oxazinomethylbenzothiazoline-2-thione (ratio 1.285) and 2-(methoxy)methylaminothiazole (17) compounds were weaker inhibitors compared to other compounds for this isoform. The molecule 8 was shown to had the excellent inhibitory efficacy on hCA I isoenzyme activity while the molecule 1 was shown to had the excellent inhibitory efficacy on hCA II isoenzyme activity. For hCA II isoform, the best inhibitors of them were N-oxazinomethylbenzothiazoline-2-thione (1) and N-isopropoxymethylbenzoxazo- line-2-thione (13). The 2-(methoxyethoxy) methylaminothiazole (15) and 2-methoxymethylaminothiazole (17) molecules are weaker inhibitors compare with other molecules for this isoform. As seen in Table 3 and Figure 2(b), IC50 values are in the range of 89–187 nM towards hCA I, while for hCA II is in the range of 79–156 nM. The IC50 values for standard molecule TAC towards hCA II and I are 520 and 373 nM, respectively. All molecules have lower IC50 value compare with AZA towards hCA II and hCA I isoenzymes.

As seen in Table 3 and Figure 2(c), IC50 amounts were in the range of 36–89 nM towards AChE, while they were in the range of 48–145 nM towards BChE (Figure 2(d)). The IC50 amounts of the entire compounds are shown in Table 3 and Figure 2(b). All inhibitors except for 17 and 13 have lower IC50 amount than TAC towards AChE and BChE. CHEs have shown excellent efficacy than placebo in clinical tests and are extensively prescribed as symptomatic therapy to ameliorate behavior and recognition in AD patients with moderate dementia. TAC (9-Amino-1,2,3,4-tetrahydroacridine) compound is a reversible inhibitor of BChE and AChE and the first drug to be agreed by the Drugs and Foods Administration of America for the placative therapy of AD.

For AChE and BChE enzymes were good inhibited by entire of AzA and TAC. The most promising compound 14 obtained 2.2-fold of inhibitory activity against AChE/BChE than that of TAC. It can be as a potential factor for the therapy of AD. Also, as shown in Table 3, the compound 14 (N-(methoxyethoxy)methylbenzoxazole-2-thione) showed the highest selectivity for AChE over BChE (ratio: 0.388) and weakest compound was 6 (N-diethylaminomethylbenzoxazole-2-thione) (ratio 1:500).

### Discussion

The synthesized molecules are shown to inhibit hCA II and I isoenzymes by the interplay of aminomethyl and alkoxymethyl derivatives (1–17) with cofactor Zn\(^{2+}\) ions in the structure of the isozymes. For hCA I isoform (generally defined an important isoform when CAIs for anticancer activity or antiglaucoma are encountered) was good inhibited by entire of the evaluated molecules, the best inhibitors of them were N-diethylaminomethylbenzothiazoline-2-thione (5), N-morfolinomethylbenzoxazole-2-thion (8) and N-oxazolinomethylbenzoxazole-2-thione (9) (Figure 2(a)). The 2-isopropoxymethylaminothiazole (16) and 2-(methoxy)methylaminothiazole (17) compounds are weaker inhibitors compared to other compounds for this isoform. The molecule 8 was shown to had the excellent inhibitory efficacy on hCA I isoenzyme activity while the molecule 1 was shown to had the excellent inhibitory efficacy on hCA II isoenzyme activity. For hCA II isoform, the best inhibitors of them were N-oxazinomethylbenzothiazoline-2-thione (1) and N-isopropoxymethylbenzoxazoline-2-thione (13). The 2-(methoxyethoxy) methylaminothiazole (15) and 2-methoxymethylaminothiazole (17) molecules are weaker inhibitors compare with other molecules for this isoform. As seen in Table 3 and Figure 2(b), IC50 values are in the range of 89–187 nM towards hCA II, while for hCA I is in the range of 79–156 nM. The IC50 values for standard molecule TAC towards hCA II and I are 520 and 373 nM, respectively. All molecules have lower IC50 value compare with AZA towards hCA II and hCA I isoenzymes.

As seen in Table 3 and Figure 2(c), IC50 amounts were in the range of 36–89 nM towards AChE, while they were in the range of 48–145 nM towards BChE (Figure 2(d)). The IC50 amounts of the entire compounds are shown in Table 3 and Figure 2(b). All inhibitors except for 17 and 13 have lower IC50 amount than TAC towards AChE and BChE. CHEs have shown excellent efficacy than placebo in clinical tests and are extensively prescribed as symptomatic therapy to ameliorate behavior and recognition in AD patients with moderate dementia. TAC (9-Amino-1,2,3,4-tetrahydroacridine) compound is a reversible inhibitor of BChE and AChE and the first drug to be agreed by the Drugs and Foods Administration of America for the placative therapy of AD.

For AChE and BChE enzymes were good inhibited by entire of the evaluated compounds, the best inhibitors of AChE were N-Piperidinomethylbenzothiazoline-2-thione (4), N-(methoxyethoxy)methylbenzoxazole-2-thione (14) and also for BChE were N-diethylaminomethylbenzoxazole-2-thione (6) and N-morfolinomethylbenzoxazole-2-thione (8), respectively.

### Conclusions

In this paper, nanomolar levels of IC50 amounts were obtained for entire novel aminomethyl and alkoxymethyl derivatives (1–17) and subsequently aqueous humor secretion, which leads to decreased pressure in the eye. For the ubiquitous cytosolic isoform hCA II, novel aminomethyl and alkoxymethyl derivatives (1–17) had Ki values ranging from 81 ± 19–215 ± 40 nM. In addition, AZA compound applied as a standard CA inhibitor, which obtained Ki value of 353 ± 60 nM. As can be observed in hCA II, the most considerable inhibition result was recorded by N-oxazinomethylbenzothiazoline-2-thione (1) (81 ± 19) (Table 3).

| Compounds | hCA I | IC50 (nM) | hCA II | IC50 (nM) | AChE | IC50 (nM) | BChE | IC50 (nM) |
|-----------|-------|-----------|-------|-----------|-------|-----------|-------|-----------|
| 1         | 79    | 0.9639    | 0.9527 | 51        | 0.9762 | 99        | 0.9694 |
| 2         | 79    | 0.9852    | 0.9839 | 39        | 0.9885 | 89        | 0.9773 |
| 3         | 83    | 0.9597    | 0.9555 | 54        | 0.9670 | 75        | 0.9619 |
| 4         | 86    | 0.9588    | 0.9774 | 36        | 0.9852 | 82        | 0.9630 |
| 5         | 82    | 0.9755    | 0.9670 | 52        | 0.9699 | 83        | 0.9539 |
| 6         | 102   | 0.9359    | 0.9649 | 44        | 0.9857 | 80        | 0.9750 |
| 7         | 79    | 0.9597    | 0.9457 | 65        | 0.9874 | 79        | 0.9520 |
| 8         | 94    | 0.9533    | 0.9619 | 38        | 0.9484 | 49        | 0.9911 |
| 9         | 103   | 0.9652    | 0.9440 | 62        | 0.9769 | 84        | 0.9904 |
| 10        | 98    | 0.9350    | 0.9452 | 50        | 0.9819 | 94        | 0.9710 |
| 11        | 112   | 0.9607    | 0.9711 | 89        | 0.9865 | 133       | 0.9621 |
| 12        | 119   | 0.9752    | 0.9695 | 63        | 0.9908 | 93        | 0.9947 |
| 13        | 105   | 0.9664    | 0.9483 | 63        | 0.9859 | 83        | 0.9807 |
| 14        | 112   | 0.9426    | 0.9556 | 38        | 0.9860 | 68        | 0.9704 |
| 15        | 128   | 0.9783    | 0.9644 | 43        | 0.9888 | 109       | 0.9752 |
| 16        | 156   | 0.9757    | 0.9659 | 76        | 0.9949 | 127       | 0.9590 |
| 17        | 142   | 0.9774    | 0.9562 | 80        | 0.9912 | 144       | 0.9749 |
| Aza       | 373   | 0.9774    | 0.9616 | 520       | 0.9016 | 174       | 0.9513 |
| TACb      | —     | —         | —     | —         | —     | —         | —     |

TAC (TAC) was used as a standard inhibitor for BChE and AChE enzymes.

\(^{a}\)Acetazolamide (AZA) was used as a standard inhibitor for both carbonic anhydrase I, and II isoenzymes (hCA I and II).

\(^{b}\)Calculated that TAC (ratio 1:500).
these molecules can be considerable inhibitor of AChE, BChE enzymes and both hCA isoforms. The molecules 5 and 8 towards hCA I and molecules 1 and 13 towards hCA II and molecules 4 and 14 towards AChE and molecules 6 and 8 towards BChE enzymes recorded which can to be the leader molecules of the parts for subsequent evaluations.

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