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RESEARCH ARTICLE

Synthesis of diaryl ethers with acetylcholinesterase, butyrylcholinesterase and carbonic anhydrase inhibitory actions

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Abstract

A series of diaryl ethers were synthesized and their human (h) carbonic anhydrase (CA) isoenzymes hCA I and II, acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE) inhibitory actions were investigated. The new compounds were synthesized from the corresponding phenols and bromobenzenes via the Ullmann reaction, by using dipicolinic acid as a copper (I) complexing ligand. hCA I and II were inhibited with K_is in the low nanomolar range of 102.01–127.13 nM against hCA I, and of 73.71–113.40 nM against hCA II, whereas the inhibition constants against AChE were of 15.35–18.34 nM and against BChE in the range of 9.07–22.90 nM. The CA inhibition mechanism with these ethers is unknown, but may be similar to that of aryl methyl ethers investigated earlier by computational approaches.

Keywords

Acetylcholinesterase, butyrylcholinesterase, carbonic anhydrase, diaryl ethers, enzyme inhibition

History

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Introduction

Diaryl ethers are important compounds in synthetic organic chemistry and pharmaceutical chemistry. These compounds are found in the substructures of some natural products. Hormones such as triiodothyronine (**1**) and thyroxine (**2**)[1], a trace amine thyronamine (**3**) which is active at trace amine associated receptors[2], incorporate diaryl ether scaffolds in their structures. The diaryl ether **4** is a natural product and is isolated from the arid plant *Prosopis cineraria* (Fabaceae)[3]. Lactoperoxidase (LPO), cyclooxygenase-1 and 2 (COX1 and COX2) enzymes inhibition of **4** has been reported by Liu et al.[3] Another natural product, obavatol (**5**), isolated from *Magnolia obovata*, has anti-inflammatory[4] and anti-tumor activities[5] (Figure 1). In addition, γ/δ PPAR agonistic[6], *Candida albicans* isocitrate lyase inhibitory[7], antimicrobial[7], nitric oxide synthase inhibitory[8] and some other important inhibitory properties of diaryl ethers have been reported[9]. Furthermore, CA inhibitory properties of diarylthioethers[10], coumarines and thiocoumarines were also studied[11,12]. In our early studies, we explained the synthesis, carbonic anhydrase (CA)[13–16] and acetylcholinesterase (AChE)[17–19] inhibition of some aryl methyl ether derivatives.

The carbonic anhydrases (CAs, EC 4.2.1.1) are a superfamily of metalloenzymes, which catalyze the interconversion between CO_2 and HCO_3^- by using a metal hydroxide nucleophilic mechanism. They are ubiquitous metalloenzymes and present in almost all living organism[18,20–23]. In humans, CAs are present in a large variety of tissues such as the gastrointestinal tract, the nervous system, the reproductive tract, lungs, kidneys, skin and eyes[24–26]. This regulatory reaction supports many biochemical and physiological processes associated with pH control, fluid secretion and ion transport[27–29].

$$CO_2 + H_2O \overset{CA}{\Leftrightarrow} H_2CO_3 \Leftrightarrow HCO_3^- + H^+$$

Six distinct genetic CA families, the α-, β-, γ-, δ-, ζ- and η-CAs, are known to date, constituting an interesting example of convergent evolution at the molecular level[30–32]. Also, to date, 16 different CA isoenzymes are described in various organisms[33,34]. These enzymes differ in their subcellular localization, catalytic activity and susceptibility to different classes of inhibitors. Some of them are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), others are membrane bound (CA IV, CA IX, CA XII and CA XIV), two are mitochondrial (CA VA and CA VB), and one is secreted in saliva (CA VI)[35–37]. It has been recently reported that CA XV isoform is not expressed in humans or in living primates. It is abundant in rodents and other higher vertebrates. Three catalytic forms, namely CARP VIII, X and XI are the only known CA-related proteins (CARP)[38–40].

Inhibitors of carbonic anhydrase enzymes (CAIs) have a large number of applications in therapy, including anticancer, antiglaucoma, and anti-osteoporosis agents. They used as diuretics, anti-obesity, and anti-infective drugs. Also, these CAIs have been used for the management of Alzheimer's disease and a variety of neurological disorders, among others. Many types of CAIs such new derivatives have been reported recently, together with their potential applications[34,41]. These chemical groups are used for the clinical treatment of some conditions for decades[42,43].

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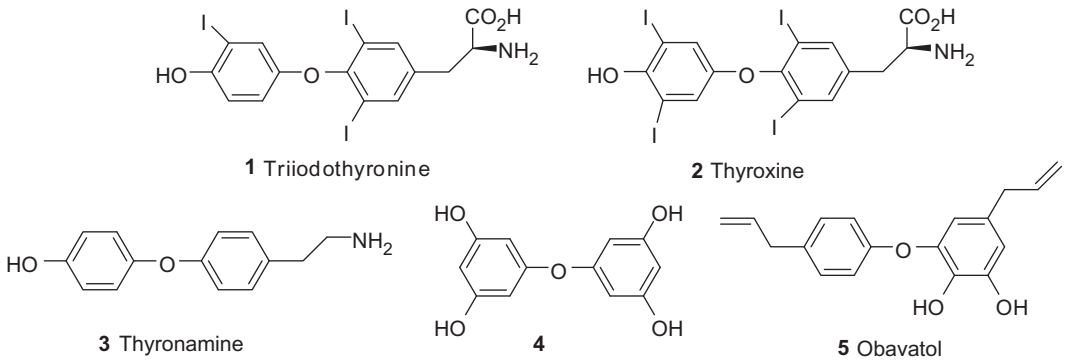


Figure 1. Some selected natural diaryl ethers **1–5**.

Acetylcholinesterase (AChE) is an important component of central and peripheral cholinergic vertebrate synapses, where it hydrolyzes acetylcholine (ACh) to choline (Ch) and acetate (CH_3COO^-)[44–46]. ACh is one of the most important neurotransmitters found in the human central and peripheral nervous systems. An abnormally low concentration of ACh can cause various neuropsychological and neuropsychiatric disorders such as Alzheimer's diseases (AD) and Parkinson's diseases (PD)[47,48]. AD is a progressive neurodegenerative disorder of the brain, which most commonly leads to dementia among the elderly. The main reason behind the AD is the decreased levels of ACh and the enzymes responsible for its synthesis and degradation in the brain[49,50]. Also, butyrylcholinesterase (BChE, E.C. 3.1.1.8) is involved in hydrolysis and regulation of butyrylcholine. AChE and BChE sequences are similar up to 84% and hence, their responses to a definite therapy almost yield in similar results[51,52]. However, during the progression of AD, brain AChE levels decline while BChE activity increases, suggesting that ACH hydrolysis may occur to a greater extent via BChE catalysis[53].

To date, most of the drugs and chemicals approved for treating AD are AChE inhibitors (AChEIs), such as tacrine, rivastigmine, galantamine and donepezil. However, some undesirable side effects including vomiting, nausea and weight loss were observed with these AChEIs while tacrine was found to be hepatotoxic[54]. So, the design of novel agents such as AChEIs is still urgently needed for AD treatment.

To the best of our knowledge, hCA, AChE and BChE inhibition of diaryl ethers have not been investigated properly. As the title compounds show a wide biological activity spectrum, synthesis of these compounds and their derivatives attract scientific attentions for synthetic and biological properties. In this context, in the present study, we aimed to extend our studies on the synthesis and biological investigation of some novel diaryl ethers. For this purpose, the synthesis of novel diaryl ethers was achieved via Ullmann nucleophilic coupling. All synthesized compounds were investigated for their inhibition against AChE, BChE and hCA I, II isoenzymes.

In this context many efforts have been made for the development of specific CAIs, and some remarkable results have been achieved in the past 15 years[55–59]. In this study, we synthesized some novel diaryl ethers **11–14**. We determined their inhibition properties against hCA I, hCA II, AChE and BChE and compared their inhibition properties to acetazolamide (AZA) as a clinical used carbonic anhydrase inhibitor. On the other hand, tacrine (TAC) was used as standard AChE and BChE inhibitor.

Experimental section

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 recorded uncorrected. IR spectra were recorded as solutions in 0.1 mm cells with a Mattson 1000 FT-IR spectrophotometer (Unicam. Ltd., York Street, Cambridge, UK). ^{1}H- and ^{13}C-NMR spectra were recorded on 400 (100)-MHz Varian spectrometer (Danbury, CT) 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 in ppm. Elemental analyses were recorded on Leco CHNS-932 apparatus (Saint Joseph, MI). CA, AChE and BChE inhibitory properties of samples were determined as spectrophotometericaly (UV-1208, Shimadzu Co., Kyoto, Japan).

General procedure for the synthesis of diaryl ethers via Ullmann Coupling

Dipicolinic acid (DPA, 0.4 mmol) and Cs_2CO_3 (2.8 mmol) was dissolved in dry DMSO (10 mL) and it was stirred for neutralization for 10 minutes under N_2 atmosphere in a pressure tube. To the solution CuI (0.2 mmol), phenol (1.2 mmol) and aryl halide (1 mmol) was added under N_2 atmosphere and nitrogen gas was passed through the tube until it was closed. After the sealed tube was stirred for 18 h at 120 °C, the reaction mixture was cooled to RT and diluted with CH_2Cl_2 (40 mL). Organic layer was washed with 1 N NaOH (2×20 ml) and dried over $MgSO_4$. Column chromatography of the residue with (Silica gel, 20 g; EtOAc:Hexane 1:20) gave light yellow oily diaryl ethers **11–14**.

3,3′-Oxybis(methoxybenzene) (11)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (1H, dd, $J = 15.2$ Hz, $J = 6.2$ Hz, Ar-H), 6.68–6.59 (2H, dm, $J = 6.2$ Hz, Ar-H), 6.58–6.57(1H, m, Ar-H), 3.77 (9H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (2OC), 158.45 (2OC), 130.3 (2CH), 111.3 (2CH), 109.2 (2CH), 105.2 (2CH), 55.6 ($2OCH_3$). IR (CH_2Cl_2, cm^{-1}): 3052, 2924, 1587, 1455, 1264, 1149, 862, 686. Anal. Calcd for ($C_{14}H_{14}O_3$): C, 73.03; H, 6.13. Found: C, 73.30; H, 6.25

1,2-Dimethoxy-4-(3-methoxyphenoxy)benzene (12)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (1H, t, $J = 8.4$ Hz, Ar-H), 6.81 (1H, d, $J = 8.4$ Hz, Ar-H), 6.65–6.52 (4H, m, Ar-H), 3.86 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.75 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (OC), 159.8 (OC), 150.3 (OC), 150.1 (OC), 145.8 (OC), 130.3 (CH), 128.6 (CH), 111.9 (CH), 111.3 (CH), 108.3 (CH), 104.8 (CH), 103.9 (CH), 56.5 (OCH_3), 56.1

(OCH_3), 55.7 (OCH_3). IR (CH_2Cl_2, cm^{-1}): 3065, 2929, 1663, 1487, 1260, 1196, 841, 729. Anal. Calcd for ($C_{15}H_{16}O_4$): C, 69.22; H, 6.20. Found: C, 69.40; H, 6.32.

1,3-Dimethoxy-5-(4-methoxyphenoxy)benzene (13)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (2H, d, $J = 9.2$ Hz, Ar-H), 6.87 (2H, d, $J = 9.2$ Hz, Ar-H), 6.17–6.15 (2H, m, Ar-H), 6.12–6.09 (1H, m, Ar-H), 3.80 (3H, s, OCH_3), 3.73 (6H, s, OCH_3). ^{13}C NMR (100 MHz, CDCl3) δ 161.7(2OC), 160.8 (OC), 156.3 (OC), 149.8 (OC), 121.3 (2CH), 115.0 (2CH), 96.3 (2CH), 94.8 (CH), 55.8 (OCH_3), 55.7 (OCH_3), 55.5 (OCH_3). IR (CH_2Cl_2, cm^{-1}): 3024, 2925, 1595, 1463, 1212, 1131, 825, 720. Anal. Calcd for ($C_{15}H_{16}O_4$): C, 69.22; H, 6.20. Found: C, 69.40; H, 6.53.

5,5′-Oxybis(1,3-dimethoxybenzene) (14)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.16 (2H, bs, Ar-H), 6.13 (4H, bs, Ar-H), 3.68 (6H, s, OCH_3), 3.67 (6H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7 (2OC), 160.8 (2OC), 158.8 (2OC), 97.7 (CH), 95.8 (CH), 55.5 (4 OCH_3). IR (CH_2Cl_2, cm^{-1}): 3094, 2924, 1600, 1428, 1205, 1129, 823, 737. Anal. Calcd for ($C_{16}H_{18}O_5$): C, 66.20; H, 6.25. Found: C, 66.45; H, 6.35.

Biochemical studies

Carbonic anhydrase I and II isoenzyme purification and inhibition studies

Recently, a specific interest has been oriented toward the slow cytosolic isoform hCA I, and dominant physiologic isoform hCA II[60]. Two physiologically relevant CA isoforms, hCA I, and II, were included[61,62]. In this study, both hCA I, and II isoenzymes were purified by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography[63–65]. The affinity chromatography consists Sepharose-4B-L-tyrosine-sulfanilamide that acts as affinity matrix for selective retention of CA isoenzymes[66–68]. The column material was prepared according to a previous method[69]. Thus, homogenate solution acidity was adjusted and supernatant was transferred to the previously prepared column[70]. The proteins flow in the column eluates was spectrophotometrically determined at 280 nm. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was applied for detection for of both isoenzymes purity[71]. After the visualizing by SDS–PAGE process, a single band was observed for each isoenzyme. This protein-imaging method was previously described[72]. In this application, the imaging method was performed out in 10 and 3% acrylamide for the running and the stacking gel, respectively, with 0.1% SDS[61].

Both CA isoenzymes activities were determined according to the method of Verpoorte et al.[73] and described previously[74]. The protein quantity was spectrophotometrically measured at 595 nm during the purification steps according to the Bradford method[75]. Bovine serum albumin was used as the standard protein[61]. For determining the inhibition effect of each isoenzyme, some novel diaryl ether (**11–14**) derivatives and an Activity (%) – [Diaryl ethers] graph was drawn. To determine K_i values, three different novel diaryl ether **11–14** concentrations were tested. In these experiments, different substrat concentration was used and Lineweaver–Burk curves were drawn[76], as previously described[70].

AChE/BChE activity determination

The inhibitory effect of some novel diaryl ethers **11–14** on AChE/BChE activities were measured according to spectrophotometric method of Ellman et al.[77] Acetylthiocholine iodide or butyrylthiocholine iodide (AChI/BChI) were used as substrates for both reactions. 5,5′-Dithio-bis(2-nitro-benzoic)acid (DTNB, D8130-1G, Sigma-Aldrich, Steinheim, Germany) was used for the measurement of the AChE/BChE activities. Briefly, 100 mL of Tris/HCl buffer (1 M, pH 8.0), 10 mL of sample solution dissolved in deionized water at different concentrations and 50 mL AChE/BChE (5.32×10^{-3} U) solution were mixed and incubated for 10 min at 25 °C. Then 50 mL of DTNB (0.5 mM) was added. The reaction was then initiated by the addition of 50 mL of AChI/BChI. The hydrolysis of these substrates of AChI/BChI was monitored spectrophotometrically by the formation of the yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by enzymatic hydrolysis of AChI/BChI, with absorption maximum at a wavelength of 412 nm.

Results and discussion

Synthesis

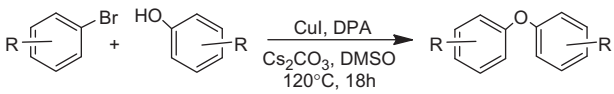
C(aryl)-O bond formation is one of the most important synthetic methods for the synthesis of diaryl ethers. Copper (I)-mediated Ullmann coupling reactions of aryl halides with phenols in the presence of bases, ligands and in different solvents have widely been used for the synthesis of diaryl ethers[78]. The main problem on the Ullmann reaction is the harsh reaction conditions used for the preparation of the ethers and the relatively moderate yields. Different catalysts such as phenanthroline, 2,2′-bipyridine, tiophen carboxylic acid, picolinic acid, N-methylmorpholine and some other compounds (i.e., acting as ligands for the copper ions) have been investigated to improve the yields[79]. In addition, different solvents (dioxane, DMF, DMSO and acetonitrile) and different bases (pyridine, Cs_2CO_3 and K_2CO_3) have also been used in the Cu (I)-catalyzed Ullmann reactions. In the present study, Copper (I)-catalyzed Ullmann reactions of methoxy-substituted bromobenzenes with methoxy-substituted phenols were carried out in the presence of several ligands (nicotinic acid, picolinic acid, dipicolinic acid, tiophene carboxylic acid), bases (Na_2CO_3, K_2CO_3, Cs_2CO_3) and solvents (acetonitrile, DMF, dioxane, DMSO) at different temperatures (25–120 °C) to penetrate the most efficient reaction conditions. From these experimental results, we found out that the most convenient method for the synthesis of diaryl ethers was CuI catalyzed the reactions of aryl bromides with phenols in the presence of dipicolinic acid (DPA), Cs_2CO_3, DMSO in pressure tube at 120 °C. Therefore, the Ullmann reaction of aryl bromides **6** and **7** with phenols **8–10** gave novel diaryl ethers **11–14** in high yields (Scheme 1 and Table 1).

Biological activity

Both physiologically relevant hCA I, and II isoforms and AChE and BChE were studied in the enzyme inhibition part of this study. A detailed in below, some novel diaryl ethers **11–14** were evaluated for their inhibition properties against hCA I, and II isoenzymes, showing generally an efficient inhibition. The chemical structures of some novel diaryl ethers **11–14** are given in Table 1. Also, CA I, and II inhibiting effects of some novel diaryl ethers **11–14** are shown in Table 2. It was well known that developing isoenzyme-specific CAIs should be highly beneficial in obtaining novel classes of drugs devoid of various undesired side-effects[80]. We declare here the first study on the inhibitory effects of novel diaryl ethers **11–14** against hCA I, and II using esterase activity.

Low cytosolic isoenzyme hCA I is found in many tissues, however, it was demonstrated that this isoenzyme is involved in retinal and cerebral edema, and its inhibition may be a valuable tool for fighting these conditions. The hCA I is ubiquitously expressed in the body and can be found in high concentrations

in the blood and gastrointestinal tract. The results obtained from this study clearly indicate that these some novel diaryl ethers **11–14** had effective inhibition profile against slow cytosolic isoform hCA I, and cytosolic dominant rapid isozymes hCA II with low nanomolar range. K_i values are in the range of 102.01 ± 2.81–108.35 ± 5.02 nM for hCA I isoenzyme. On the other hand, acetazolamide (**AZA**) was considered for broad-specificity CA inhibitor owing to its widespread inhibition of CAs, dwhich showed K_i value of 190.56 ± 1.31 nM against hCA I. However, diaryl ethers (**12**), possessing methoxy (–OCH_3) group at the *ortho*-position at the phenyl ring was the best hCA I inhibitor (K_i: 102.01 ± 2.81 nM). The inhibition effects of all novel diaryl ethers (**11–14**) are higher than that of acetazolamide (AZA; K_i: 190.56 ± 1.31 nM) (Table 2). AZA is considered the good CA inhibitor and is approved for the treatment of a range of conditions including glaucoma, epilepsy, and altitude sickness[81].



Scheme 1. The synthesis of diaryl ethers **11–14**.

Cytosolic hCA II isoenzyme is involved in different diseases including edema, epilepsy, altitude sickness and glaucoma. For the physiologically dominant isoform hCA II, all novel diaryl ethers **11–14** showed K_is of 73.71 ± 1.50–113.40 ± 2.55 nM (Table 2). However, novel diaryl ether **14,** possessing two methoxy groups ($-OCH_3$) at the *meta*-position at the each phenyl ring, was the best hCA II inhibitor (K_i: 73.71 ± 1.50 nM). However, all novel diaryl ethers **11–14** have greater inhibition profiles against hCA II and these inhibition values were lower than that of AZA (K_i: 40.81 ± 1.35 nM).

The chemical possessing AChE inhibitory effects are used for the treatment of AD. However, these drugs have many undesired side effects including vomiting, nausea and weight loss. For example, tacrine as AChEIs was found to be hepatotoxic[54]. So, the design of novel agents as AChEIs is still urgently needed for AD treatment. Thus, the development and utilization of new effective AChEIs is highly desired. Currently, the most prescribed cholinesterase inhibitors (ChEIs) are donepezil, galantamine, and rivastigmine. These drugs are used to treat patients with mild-to-moderate AD. BChE has a specific role in cholinergic

Table 1. Reagents, products and yields.

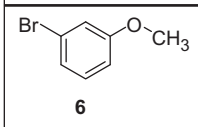
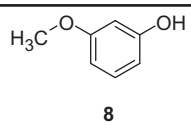
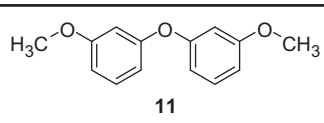
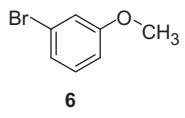
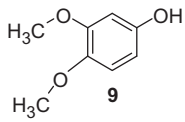
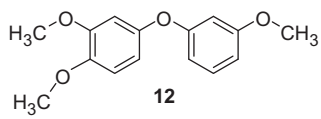
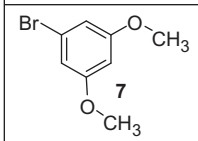
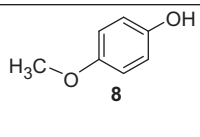
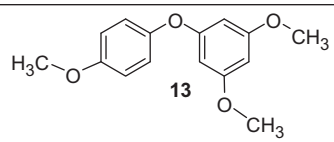
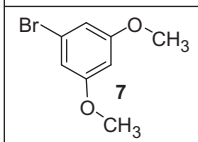
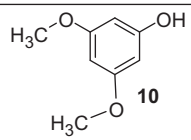
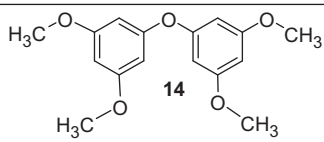
Aryl halide	Phenol	Diaryl ether	Yield (%)
 **6**	 **8**	 **11**	85
 **6**	 **9**	 **12**	82
 **7**	 **8**	 **13**	86
 **7**	 **10**	 **14**	83

Table 2. Human carbonic anhydrase I and II isoenzymes (hCA I and II), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes inhibition effects of some novel diaryl ethers **11–14**.

	IC_{50} (nM)								K_i (nM)			
Compounds	hCA I	r^2	hCA II	r^2	AChE	r^2	BChE	r^2	hCA I	hCA II	AChE	BChE
11	161.16	0.9661	111.77	0.9780	18.93	0.9671	45.89	0.9850	104.01 ± 5.05	103.84 ± 3.11	17.64 ± 0.34	14.39 ± 0.24
12	111.77	0.9768	103.43	0.9436	11.91	0.9823	37.87	0.9868	102.01 ± 2.81	113.40 ± 2.55	15.35 ± 0.22	22.90 ± 0.42
13	144.37	0.9530	97.605	0.9731	16.78	0.9867	40.06	0.9765	127.13 ± 4.92	93.85 ± 2.85	16.86 ± 0.23	12.58 ± 0.13
14	133.27	0.9577	88.846	0.9907	15.29	0.9837	19.63	0.9906	108.35 ± 5.02	73.71 ± 1.50	18.34 ± 0.43	9.07 ± 0.22
AZA*	130.74	0.9923	64.166	0.9949	–	–	–	–	190.56 ± 1.31	40.81 ± 1.35	–	–
TAC**	–	–	–	–	39.15	0.9951	15.07	0.9895	–	–	23.49 ± 0.74	46.80 ± 0.22

*Acetazolamide (AZA) was used as a standard inhibitor for both hCA I and II isoenzymes.
**Tacrine (TAC) was used as a standard inhibitor for AChE and BChE enzymes.

neurotransmission and it has been associated with AD. Individual ChEIs differ from each other with respect to their pharmacologic properties. Galantamine and donepezil are short-acting reversible competitive inhibitors, whereas rivastigmine is actively metabolized by ChE. Also, primary target of donepezil and galantamine is AChE; however, rivastigmine shows equal affinity for both AChE and BChE enzymes. BChE levels in the body exceed those of AChE in all tissues except muscle and brain. The human body contains 10 times more BChE than AChE. It was reported that in AD, AChE is lost up to 85% in specific brain regions, whereas BChE levels rise with disease progression. It was also shown that the main AChE inhibitory effect was primarily associated with aromatic compounds and, to a lesser degree, with aliphatic compounds[50,75]. Novel diaryl ethers **11**–**14**, effectively inhibited AChE and BChE with K_i values ranging 15.35 ± 0.22–18.34 ± 0.43 and 9.07 ± 0.22–22.90 ± 0.42 nM, respectively (Table 2), which were calculated from Lineweaver–Burk plots[77]. On the other hand, donepezil, which is used for the treatment of mild-to-moderate AD and various other memory impairments, had been shown to lower AChE inhibition activity (IC_{50}: 55 nM)[82]. In our study, we found that tacrine, which clinically acts as AChE/BChE inhibitors showed K_i values of 23.49 ± 0.74 nM for AChE and 46.80 ± 0.22 nM for BChE. These results clearly showed that novel diaryl ethers **11**–**14** had more inhibition profile against BChE compared to both standard AChE/BChE inhibitors (Tacrine and donepezil).

Conclusion

In conclusion, the Cu(I)-catalyzed Ullmann reaction of aryl bromides **6** and **7** with phenols **8**–**10** in DMSO in the presence of DPA as a ligand and Cs_2CO_3 base gave novel diaryl ethers **11**–**14** in good yields. Compound **14** is a methoxylated derivative of the natural product **4**. Therefore, the synthetic methodology described here is also an alternative method for the synthesis of **4**. All novel diaryl ethers **11**–**14** were evaluated against cytosolic hCA I, and II isoenzymes, AChE and BChE. These novel diaryl ethers **11**–**14** have shown effective nanomolar inhibition against hCA I, and II isoenzymes, AChE and BChE. Novel diaryl ethers **11**–**14** potently inhibited these metabolic enzymes. The CA inhibition mechanism with these ethers is unknown, and may be similar to that of the reported aryl methyl ether derivatives, investigated earlier by computational methods[13].

Declaration of interest

There is no declaration of interest for this work. The authors are indebted to the Scientific and Technological Research Council of Turkey (TÜBİTAK, Grant No. 115Z422) for financial support of this work.

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