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Synthesis and bioactivity of several new hetaryl sulfonamides

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ABSTRACT

1-(4-Methylsulfonyl)-2-thione-4-aryl-5-*Z*-6-methyl and oxyalkyl-imidazoles were synthesized from different tetrahydropyrimidinethiones and aryl sulfonyl chloride. These compunds were tested for metal chelating effects and to determine the phrase in which inhibition occured between two physiologically pertinent compunds and carbonic anhydrase (CA) isozymes I and II (hCA I and II), butyrylcholinesterase (BChE) and acetylcholinesterase (AChE). AChE was detected in high concentrations in the brain and red blood cells. BChE is another enzymes that is abundant available in the liver and released into the blood in a soluble form. Newly synthesized hetaryl sulfonamides exhibited impressive inhibition profiles with Ki values in the range of 1.42–6.58 nM against hCA I, 1.72–7.41 nM against hCA II, 0.20–1.14 nM against AChE and 1.55–5.92 nM against BChE. Moreover, acetazolamide showed Ki values of 43.69 ± 6.44 nM against hCA I and 31.67 ± 8.39 nM against hCA II. Additionally, tacrine showed Ki values of 25.75 ± 3.39 nM and 37.82 ± 2.08 against AChE and BChE, respectively.

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Acetylcholinesterase; aryl sulfonyl chlorides; butyrylcholinesterase; carbonic anhydrase; heterocyclic amines;

Introduction

Pyridine thione compounds are the building blocks of drugs that improve cerebral blood flow (nimodipinum, nifedipinum (calcium channel blockers). At present, non-glycoside and nonadrenergic cardiotonics with a large range of therapeutic actions are actively being sought. Their synthetic analogs-amrinone, peroximon and milrinone- are widely used in intensive treatment. Furthermore, sulfonamides that contain pyrimidine moieties exhibit cytotoxic effects that permit their use as antiviral and anticancer drugs. However, these compounds are also potent bactericides. Their anti-microbial activity and effect on various microorganisms depends on the nature of the heterocycle and various functional groups. Therefore, the synthesis of new sulfonamides containing bicyclic compounds is relevant, particularly the reaction of aryl sulfonyl chlorides with heterocyclic amines. The influence of the heterocycle's composition and the location of the amine are studied. Interestingly, the amino group's position in the isoxazole greatly affects its reactivity. Within the reaction of sulphonic-acid chloride with 5-amino 3,4-dimethyl isoxazole[1] and biphenyl isoxazole[2], hetaryl sulfonamides were obtained in high yields. The reaction of sulfonyl chlorides with five-membered aminoheterocycles such as oxazole[3] and pyrazole also proceed in high yield. However, the reaction of sulfonyl chlorides with benzoxazole requires a long boiling period in pyridine solution[4].

The reaction of aryl sulfonyl chlorides with piperazines, connected via oxygen, sulfur, *N*-pyridyl or pyrimidine diyl moieties proceeds very easily. The influence of the radicals and functional groups on the reaction was not observed[5]. These sulfonamides are used for treating diseases of the central nervous system and kidney function disorders. Despite the presence of bicinchoninic acid in the 4-amino benzenesulfonyl chloride moiety, the reaction of 2-amino-4,6-dimethylpyrimidine proceeds very easily to form sulfonamides that exhibit anti-inflammatory and analgesic activity[6].

It was observed that the reaction of acetamide benzenesulfonyl chloride in the presence of DMSO is easily connected with chitosan derivatives[7]. These sulfonamides have antifungal activity against Alternaria Solani and Phomopsis asparage. Pyridazinyl sulfonamide derivatives obtained by the reaction of sulfonyl chlorides with pyridazinyl also have antimicrobial activity[8]. Thus aminoheterocycle reactivity depends on the structure, location and presence of functional groups. Studies of aryl sulfonyl chloride reactions with heterocyclic and bicyclic amines are important for both obtaining new sulfonamide compounds and investigating their germicidal and other properties[8]. It was discovered that the reaction of aryl sulfonyl chlorides in heterocyclic amine solvents results in the separation of hydrogen chloride when using freshly distilled pyridine. A similar separation of hydrogen chloride was also observed when aryl sulfonyl chlorides were reacted in ethyl alcohol or triethylamine solvent. New hetaryl sulfonamides were observed by continuing focused research on synthesing various classes of organic sulfur compounds and the studying their transformations.

Carbonic anhydrase (CA) enzymes (EC 4.2.1.1) are present in many living systems and play a role in a diversity of pathological and physiological effects including neurological disorders, fluid balance, pH regulation, bone resorption, carboxylation reactions, glaucoma, calcification, osteoporosis, cancer, tumorigenicity and the synthesis of bicarbonate (HCO_3^-)[9–13]. CAs are a player main role in the physiology of coral calcification[14]. CA has active confidants in the gastric mucosa, brain, kidney, salivary glands, eye lens, pancreas, nerve myelin sheath, prostate and uterus[15]. In

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addition, CA amounts are considerably decreased in the brain tissue of patients suffering from Alzheimer's disease, proving an essential involvement of various human CA isozymes (hCA I, II, IV, and VII) in cognitive and learning functions[16]. CA inhibitors (CAIs) are classified into two principal groups: the unsubstituted sulfonamides and the metal complexing anions. Sulfonamides are very momentous CAIs and sulfonamide varieties are effective organic compounds in medicinal chemistry[17,18]. The detection of CA inhibition with sulfanilamide by Mann and Keilin was the beginning of these applications and many additional scientific discoveries[19].

CAs are biological catalysts that convert water and carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and a proton (H^+)[20–23]. This reaction is one of the fastest determined reactions (kcat, 10^6/s) in the environment and is required by animals and plants for survival[24].

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

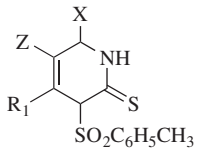
CAs have six distinct enzyme families: the α-, β-, γ-, δ-, ζ- and ɳ-CA. α-Cas (expressed in vertebrates and algae)[25] usually have monomer structures and occasionally a dimer form. β-CAs (in plants and prokaryotes)[25] have dimer, tetramers or octamer forms. γ-CAs (in archaea)[28] are trimers and the δ- and ζ-CAs (in marine diatoms)[25] are less well understood up to now[26,27]. All human CAs (hCAs) belong to the α-class. Thus far, 16 isoenymes have been identified[28]. All CA families are metalloenzymes, which have Cd^{2+}, Zn^{2+} or Fe^{2+} at their active sites[29]. Different isoforms of CA have been recognized for their therapeutic effects toward several diseases. Therefore, the purpose of developing isoform-specific inhibitors is to develop new and improved treatments[30].

Cholinesterases (ChEs) have a role in catalyzing the hydrolysis of acetylcholine (ACh) into choline and acetic acid, a fundamental process for the restoration of the cholinergic neurotransmission[31]. Acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) are among the various ChEs[31].

Acetylcholine (ACh) molecules are synthesized in pre-synaptic finals from choline and they are necessary for cholinergic neurotransmission in the peripheral nervous systems (PNS) and the central nervous system (CNS)[31]. Alzheimer's disease (AD) is characterized by dementia, memory loss and cognitive impairment[32]. Perception is affected in of derangements such as AD[32]. An unusually low concentration of ACh can be create several neuropsychological and neuropsychiatric perturbations such as AD and Parkinson's disease[33]. Generally, the treatment of AD is centralized on AChE inhibitors, such as rivastigmine, tacrine, galantamine and donepezil[34]. AD is shown as a complex syndrome where various agents are responsible for its etiology such as tau protein aggregation, β-amyloid aggregation and low levels of ACh[35]. Additionally, AChE inhibitors are used in the treatment of multiple neuromuscular diseases, and they were implemented in the treatment of AD because AChE accelerates hydrolysis and enables the regulation of ACh[36].

BChE is commonly referred to as plasma ChE and is also a non-specific ChE enzyme that hydrolyzes several choline-based esters[36]. BChE exhibits high activity levels in the intestine, liver, kidney, heart and lung; wheras high levels of AChE are present in the brain, muscle and erythrocyte membrane[36]. Because BChE and AChE have up to 84% sequences similarity, they display similar activity in a variety of therapeutic applications and, thus, play an important role in medical science[26]. In this study, we synthesized several new hetaryl sulfonamides (**1**–**12**), characterized their inhibition confidants against hCA I, hCA II, AChE and BChE and evaluated their inhibition confidants against acetazolamide (AZA), which is a clinical standard used as a CA inhibitor. Additionally, tacrine (TAC) was used as a standard for BChE and AChE inhibition.

Table 1. The synthesis route of hetaryl sulfonamides **1**–**12**.



Compounds	R1	Z	X
1	H	$COOCH_2CH_3$	C_6H_5
2	CH_3	$COCH_3$	C_6H_5
3	CH_3	$COCH_3$	C_6H_4OH
4	CH_3	$COOCH_2CH_2CH_3$	$CH_3C_6H_4$
5	CH_3	$COOC_2H_4OCOCCH_2CH_3$	C_6H_5
6	OC_2H_5	$COOC_2H_5$	CH_3
7	OCH_3	$COOC_2H_5$	CH_3
8	CH_3	$COOC_2H_5$	CH_3
9	CH_3	$COOC_2H_5$	C_6H_5
10	OC_2H_5	$COCH_3$	CH_3
11	CH_3	$COCH_3$	CH_3
12	OC_2H_5	$COOC_2H_5$	C_6H_5

Experimental

General methodology

Synthesis of 1-(4-methylsulfonyl)-2-thione-4-aryl-5-Z-6-methyl and oxyalkyl-imidazoles (1–12)

In this work, 0.01 mmol of the appropriate heterocyclic amine was dissolved in 10 mL of ethanol. Then, 0.01 mmol of aryl sulfonyl chloride and 0.011 mmol of triethylamine were slowly added to the solution. The mixture was heated at 70–80 °C for 1.5–2 h, cooled and diluted with acetone or water until crystals developed. The crystals were filtered and recrystallized from ethanol. The physical and chemical characteristics of the hetaryl sulfonamides are shown in Tables 1 and 2.

Biochemical studies

CA isoenzyme purification and inhibition studies

Affinity chromatography is an essential purification method because it offers high resolution, high selectivity and high capacity for protein purification[37–40]. Recently there has been a strong interest in two of the most important hCA isoforms that are particularly common proteins found in many tissues. Both cytosolic hCA isoforms were purified by the Sepharose-4B-L-tyrosine-sulfanilamide affinity segregation method using a single purification step[41–43]. In this study, the proteins presnt in the column eluates were spectrophotometrically determined at 280 nm[44]. We then performed the sodium dodecyl sulfate-polyacrylamide gel electrophoresis method (SDS-PAGE) after purifying of the enzymes[45,46]. Upon visualizing the proteins by SDS-PAGE techniques, a single band was identifed for each isoenzyme[47]. After spectrophotometrically purifying the samples, the protein concentrations were measured at 595 nm according to the Bradford technique[48–51]. The Sepharose-4B-L-tyrosine-sulfanilamide affinity gel was cleaned with Tris-HCl (25 mM)/Na_2SO_4 (22 mM) at pH 8.7. Both CA isozymes were washed with NaCl (1.0 M)/Na_2HPO_4 (25 mM) at pH 6.3 and $NaCH_3COO$ (0.1 M)/$NaClO_4$ (0.5 M) at pH 5.6, respectively[52,53]. The effects of the new hetaryl sulfonamides derivatives (**1**–**12**) were inwestigated by measuring the hydratase activity and determined in triplicate analysis at each concentration used[54–56]. In this study,

Table 2. Physical and chemical characteristics of hetaryl sulfonamides **1–12.**

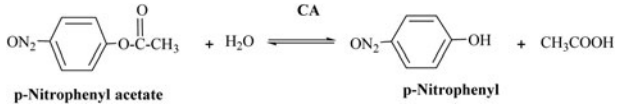
No	Z	R^1	X	Yield (%)	Mp (°C)	Brutto formula	Element analysis, Found/Calculated (%)		Spectrum analysis
							N	S	
1	2	3	4	6	7	8	9	10	11
1	$COOCH_2CH_3$	H	C_6H_5	70.1	254–256	$C_{13}H_{13}N_2O_4S_2$	8.58/8.62	19.61/19.69	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.30 (t, 3H, CH_3); 3.98 (q, 2H, CH_2); 7.20–7.44 (m, 4H, CH–Ar); 9.73 (bs, 1H, NH).**13C NMR** (75 MHz, DMSO-d_6): 24.3; 55.1; 61.7; 111.6; 128.6; 142.9; 184.7. **IR** ν, sm^{-1}: 3313; 1672; 1574; 3175 (NH); 1588–1696 (C = O); 1094 (C = S); 1190–1458 (SO_2N)
2	$COCH_3$	CH_3	C_6H_5	45.5	190–192	$C_{13}H_{14}N_2O_3S_2$	8.95/9.03	20.58/20.64	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.27 (s, 3H, CH_3); 2.43 (s, 3H, CH_3); 5.06–5.17 (dd, 2H, CH_2); 7.25 (d, 2H, 2CH–Ar); 7.30 (t, 2H, 2CH–Ar); 2.33 (s, 3H, Ar–CH_3); 7.77 (s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6) 24.3; 27; 52.8; 104.5; 126.8; 150.0; 196. **IR** ν, sm^{-1}: 3714; 3672; 3565; 3236; 3109; 2510; 1699 (C = O); 1599 (–OCO); 1462 (SO_2N); 1090 (C = S)
3	$COCH_3$	CH_3	C_6H_4OH	68.6	208–210	$C_{13}H_{15}N_2O_4S_2$	8.49/8.56	19.49/19.57	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.11 (t, 3H, CH_3); 6.61–7.04 (m, 4H, CH–Ar); 2.33 (s, 3H, Ar–CH_3); 8.90 (d, 1H, OH); 9.20 (s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6): 12; 24.3; 27.0; 46.6; 104.5; 115.7; 122.8; 128.3; 136.7; 141.6; 150.0; 196.5.**IR** ν, sm^{-1}: 1570; 3185; 1650–1680 (C = O); 1076–1493 (SO_2N); 3300 (NH)
4	$COOCH_2CH_2CH_3$	CH_3	$CH_3C_6H_4$	35.8	205	$C_{16}H_{17}N_2O_4S_2$	7.59/7.67	17.46/17.53	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.18 (m, 3H, CH_3); 2.06 (s, 3H, CH_3); 2.35 (s, 3H, CH_3); 4.10 (m, 2H, CH_2); 7.35 (m, 2H, CH_2); 7.81 (s, 1H, CH–Ar); 6.34 (s, 1H, CH–Ar); 5.76 (s, 1H, CH–Ar); 5.70 (s, 1H, CH–Ar); 2.33 (s, 3H, Ar–CH_3); 5.43 (s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6): 11.9; 17.9; 24.3; 54.6; 104.2; 128.6; 125.2; 141.6; 143.2; 152.3.**IR** ν, sm^{-1}: 3237, 3116, 2973, 1687 (C = O), 1076, 1493 (SO_2N)
5	$COOC_2H_4OCOCCH_2CH_3$	CH_3	C_6H_5	44.3	171–172	$C_{18}H_{20}N_2O_6S_2$	6.54/6.60	15.01/15.09	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.17 (m, 3H, CH_3); 2. 34 (s, 3H, CH_3); 4.09 (m, 2H, CH_2); 7.30 (m, 2H, CH_2); 7.64 (s, 1H, CH–Ar); 7.12 (m, 1H, CH–Ar); 6.32 (s, 1H, CH–Ar); 5.74 (s, 1H, CH–Ar); 2.33 (s, 3H, Ar–CH_3); 5.78 (m, 2H, =CH_2); 5.40 (s, 1H, NH).**13C NMR** (75 MHz, DMSO-d_6): 17.9; 24.3; 64.0; 111.6; 125.2; 129.3; 136.7; 141.6; 185.**IR** ν, sm^{-1}: 3418 (NH); 1702 (C = O); 1093; 1403 (SO_2N)
6	$COOC_2H_5$	OC_2H_5	CH_3	9.20	145–146	$C_{10}H_{16}N_2O_5S_2$	9.03/9.09	20.69/20.78	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.12 (3H, CH_3); 2.02 (s, 3H, CH_3); 4.02 (2H, CH_2); 8.13 (s, 1H, NH); 7.42–7.05 (m, 4H, Ar–CH).**13C NMR** (75 MHz, DMSO-d_6): 166.5; 152.7; 149.8; 144.4; 137.9; 135.6; 130.0; 129.9; 129.1; 128.0; 126.6; 103.9; 53.4; 51.6; 21.1; 18.5.**IR** ν, sm^{-1}: 3472; 1694; 1211; 1076; 1712 (C = O); 1090; 1412 (SO_2N)
7	$COOC_2H_5$	OCH_3	CH_3	17.5	158–159	$C_9H_{14}N_2O_5S_2$	9.45/9.52	21.69/21.77	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.08 (t, 3H, CH_3), 2.23 (s, 3H, CH_3), 3.80 (q, 2H, CH_2), 7.31 (m, 1H, CH–Ar), 9.19 (s, 1H, NH); 3.58 (s, 3H, OCH_3). **^{13}C NMR** (75 MHz, DMSO-d_6): 183.6; 167.2; 152.3; 144.4; 137.9; 135.6; 130.0; 129.9; 129.1; 128.0; 126.6; 103.9; 53.4; 51.6; 21.1; 18.5. **IR** ν, sm^{-1}: 3175 (NH); 1709 (C = O); 1092 (C = S); 756; 852; 974; 1230
8	$COOC_2H_5$	CH_3	CH_3	89.80	212	$C_9H_{14}N_2O_4S_2$	10.01/10.07	22.95/23.02	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.13 t (3H, CH_3); 2.47 t (3H, CH_3); 2.64–2.89 m (2H, CH_2); 7.31 m (1H, NH); 3.03 m (1H, CH). **^{13}C NMR** (75 MHz, DMSO-d_6): 18.6; 53.3; 57.6; 36.2; 184.7. **IR** ν, sm^{-1}: 3223; 3126; 2931; 1719 (C = O); 1070–1450 (SO_2N)
9	$COOC_2H_5$	CH_3	C_6H_5	90.67	220	$C_{14}H_{16}N_2O_4S_2$	8.19/8.23	18.78/18.82	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.10 (t, 3H, CH_3); 2.31 (s, 3H, CH_3); 4.00 (q, 2H, OCH_2); 6.92–7.50 (m, 4H, CH–Ar); 9.50 (s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6): 24; 65.0; 113; 126.2; 128.3; 137; 146; 195. **IR** ν, sm^{-1}: 3325; 3177; 1674; 1726 (C = O); 1087–1459 (SO_2N)
10	$COCH_3$	OC_2H_5	CH_3	26.3	198–199	$C_9H_{14}N_2O_4S_2$	10.01/10.07	22.95/23.02	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.14 (t, 3H, CH_3); 2.35 (s, 3H, CH_3); 4.03 (q, 2H, CH_2); 6.84–7.20 (m, 4H, CH–Ar); 8.60 (s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6): 19; 23; 68.0; 116; 122; 136.7; 140.6; 180. **IR** ν, sm^{-1}: 3220;

(*continued*)

Table 2. Continued

No	Z	R^1	X	Yield (%)	Mp (°C)	Brutto formula	Element analysis, Found/Calculated (%)		Spectrum analysis
							N	S	
1	2	3	4	6	7	8	9	10	11
11	$COCH_3$	CH_3	CH_3	23.30	168	$C_8H_{12}N_2O_3S_2$	11.21 / 11.29	25.77 / 25.81	3100–2900; 1680 (C = O); 1093; 1403 (SO_2N) **^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.05 (m, 3H, CH_3); 2.29 (s, 3H, CH_3), 3.33 (s, 3H, CH_3), 7.09 (s, 1H, NH), 7.81 (s, 1H, CH–Ar); 2.33 (s, 3H, Ar–CH_3). **^{13}C NMR** (75 MHz, DMSO-d_6): 186, 162, 153, 144, 139, 135.6, 130.0, 129, 105, 54, 51, 21. **IR** ν, sm^{-1}: 3196, 2980, 1750, 1690 (C = O), 1093, 1403 (SO_2N)
12	$COOC_2H_5$	OC_2H_5	C_6H_5	19.2	226	$C_{15}H_{18}N_2O_5S_2$	7.49 / 7.57	17.23 / 17.30	**^{1}H NMR** (300 MHz, (DMSO-d_6, δ): 1.13 (m, 3H, CH_3); 2.42 (s, 3H, CH_3), 2.98 (q, 2H, CH_2); 4.02 (s, 2H, CH_2); 7.20 (m, 1H, CH–Ar); 9.73 (s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6): 23.4; 51.5; 67.1; 74.5; 116.1; 128.6; 140.9; 171.7; 184.7. **IR** ν, sm^{-1}: 3214; 1682; 1564; 3185 (NH); 1699 (C = O); 1599 (OCO); 1144–1462 (SO_2N)

the variations in activity were determined at 348 nm by measuring the conversion of the p-nitrophenylacetate substrate (NPA) to p-nitrophenolate (NP) and recording measurements in 3 min intervals at the room temperature (25 °C) by using a spectrophotometer (Shimadzu, UV-VIS Spectrophotometer, UVmini-1240, Kyoto, Japan)[57,58].



AChE/BChE activity determination

The inhibitory effects of several new hetaryl sulfonamides (**1**–**12**) on AChE/BChE activity were recorded according to the spectrophotometric technique of Ellman et al.[59]. Butyrylthiocholine iodide and acetylthiocholine iodide (BChI/AChI) were applicated as substrates for both repercussions. 5,5′-Dithio-bis(2-nitro-benzoic)acid (DTNB, D8130-1G, Sigma-Aldrich, Steinheim, Germany) was used for the measurement of the AChE/BChE reactions. In this study, 100 mL of Tris/HCl buffer (1 M, pH 8.0), and 10 mL of sample solution were dissolved in distilled water at varying concentrations and then 50 mL AChE/BChE (5.32×10^{-3} EU) solution was added and the reaction was incubated for 10 min at 25 °C. Then, 50 mL of DTNB (0.5 mM) was added. The activity was then initiated by the addition of 50 mL of AChI/BChI[31].

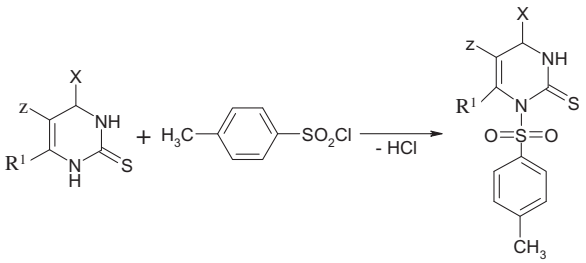
Metal chelating activity

Metal chelating capacity of hetaryl sulfonamides **1**–**12** was determined according to Dinis et al.[60]. The Fe^{2+}-binding capacity of the hetaryl sulfonamides was spectrophotometrically recorded at 562 nm. Simultaneously, to a mixture of $FeCl_2$ (0.1 mL, 0.6 mM), three concentrations (10–30 μg/mL) of hetaryl sulfonamides **1**–**12** in ethanol (0.4 mL) were added. The activities were initiated by the addition of ferrozine molecules (0.1 mL, 5 mM). Additionally, the reaction was mixed and maintained in a dark room for 10 min. Finally, the activities of the hetaryl sulfonamide mixture was recorded spectrophotometrically at 562 nm[41].

Results and discussion

Synthesis

The synthesis of 1-(4-methylsulfonyl)-2-thione-4-aryl-5-*Z*-6-methyl and oxyalkyl-imidazoles is reported in Scheme 1. The reaction of tetrahydropyrimidinethiones substituted with different heterocyclic amines and aryl sulfonyl chloride in the presence of triethylamine led to the desired new compounds (**1**–**12**). The reactions completed within 2.5–3.0 h at 70–80 °C. The newly synthesized compounds were crystalline and their structures were confirmed



Scheme 1. The synthesis routes of the 1-(4-methylsulfonyl)-2-thione-4-aryl-5-*Z*-6-methyl and oxyalkyl-imidazoles.

Table 3. The inhibition profiles of a dozen 1-(4-methylsulfonyl)-2-thione-4-aryl-5-*Z*-6-methyl and oxyalkyl-imidazoles **1–12** against human carbonic anhydrase isoenzymes I and II (hCA I and II), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes.

	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
1	4.33	0.9899	3.37	0.8518	3.17	0.9746	6.24	0.9674	1.43 ± 0.09	2.85 ± 0.58	1.14 ± 0.26	2.02 ± 0.45
2	4.31	0.9540	3.14	0.9338	2.62	0.9426	5.50	0.9827	5.74 ± 2.89	2.44 ± 0.62	0.44 ± 0.01	2.24 ± 0.19
3	4.51	0.9325	3.19	0.9216	2.77	0.9737	5.97	0.9661	4.31 ± 2.43	1.72 ± 0.62	0.94 ± 0.17	3.51 ± 1.04
4	4.78	0.9925	6.60	0.9745	0.76	0.9817	5.87	0.9827	2.31 ± 0.88	5.86 ± 0.99	0.21 ± 0.04	2.45 ± 0.32
5	4.61	0.9755	5.49	0.9771	0.95	0.9546	5.25	0.9868	1.92 ± 0.26	3.62 ± 0.87	0.20 ± 0.05	2.59 ± 0.34
6	4.99	0.9911	7.69	0.9821	1.59	0.9625	7.88	0.9637	1.42 ± 0.24	6.97 ± 2.25	0.49 ± 0.17	5.92 ± 1.63
7	6.03	0.9930	7.22	0.9834	1.95	0.9666	8.06	0.9941	4.27 ± 0.53	4.59 ± 1.74	0.47 ± 0.21	5.53 ± 0.91
8	4.08	0.8833	2.86	0.9349	2.21	0.9939	4.71	0.9709	5.62 ± 1.33	3.35 ± 1.16	0.46 ± 0.04	2.75 ± 0.45
9	3.61	0.9704	6.29	0.9679	1.54	0.9943	7.29	0.9656	3.46 ± 0.49	6.34 ± 3.12	0.72 ± 0.21	2.75 ± 0.38
10	5.06	0.9729	5.47	0.9836	1.12	0.9539	7.53	0.9782	4.39 ± 1.50	5.31 ± 2.07	0.38 ± 0.11	1.55 ± 0.44
11	4.50	0.9970	6.42	0.9355	1.60	0.9595	6.79	0.9613	6.58 ± 2.01	7.41 ± 2.32	0.39 ± 0.13	2.17 ± 0.66
12	5.37	0.9838	6.03	0.9611	2.06	0.9810	6.03	0.9703	5.21 ± 1.07	6.47 ± 2.21	0.62 ± 0.23	1.87 ± 0.25
AZA	46.19	0.9925	34.65	0.9947	–	–	–	–	43.69 ± 6.44	31.67 ± 8.39	–	–
TAC	–	–	–	–	62.43	0.9984	86.63	0.9721	–	–	25.75 ± 3.39	37.82 ± 2.08

by spectral and physico-chemical methods, including IQ, ^1H and ^{13}C NMR spectroscopies.

Biochemistry

A variety of special isoforms from the CA family of zinc containig metalloenzymes been applied in the treatment of diseases[61]. Classical CAIs, generally sulfonamide-based compounds and their features, are known for their antiepileptic, antiobesity, antiglaucoma, anticancer and antineuropathic pain applications[25]. Anticonvulsant drugs such as topiramate acetazolamide and zonisamide are recocnized CA inhibitors[62]. Classic CA enzymes, which are typically inhibited by compounds that contain a sulfonamide-based (SO_2NH_2) zinc-binding group (ZBG) or their bioisosteres such as sulfamides and sulfamates. Because CA II enzymes are the most physiologically abundant isoforms, they are often regarded as the predominant all-purpose isozymes of which inhibitions are to be eschewed. Recently, multiple types of compounds have been categorized as non-classical CAIs, including polyamines, phenols, coumarins, carboxylic acids and their derivatives, and also fullerenes[25]. The hCA I and II isoenzyme inhibitory activity of the new hetaryl sulfonamides (**1**–**12**) is shown in Table 3. Cytosolic hCA I enzyme is found in the body and also could be observed in high concentrations in the erythrocytes and gastrointestinal tissues[63].

i. The low cytosolic isoform hCA I was measured for each new hetaryl sulfonamides (**1**–**12**), and the resulting Ki values ranged between 1.42 ± 0.24–6.58 ± 2.01 nM. (Table 3). In addition, acetazolamide (AZA), which is kbown to exhibit mediocre activity as an hCA I inhibitör, showed a Ki of 43.69 ± 6.44 nM. Likewise, the strongest hCA I inhibition level was obseved for hetaryl sulfonamide **6,** with a Ki value of 1.42 ± 0.24 nM. The inhibitory activity of hetaryl sulfonamides (**1**–**12**) was also assessed against the low cytosolic hCA I isoform, the higher activity cytosolic hCA II isoenzyme and the AChE enzyme. Interestingly, these hetaryl sulfonamide compounds were determined to be effective due to their ferrous ions (Fe^{2+})-chelating effects[63–70].

ii. The importance of an isoenzyme and its effects on the physiologically dominant cytosolic hCA II enzyme varies by the disease target. For hCA II, new hetaryl sulfonamides **1**–**12** had Ki values in the range of 1.72 ± 0.62–7.41 ± 2.32 nM. Hetaryl sulfonamide **3** demonstrated the best inhibition profile, with a Ki value of 1.72 ± 0.62 nM. In addition, acetazolamide (AZA, 5-aceta-mido-1,3,4-thiadiazole-2-sulfonamide), a clinical standard used in this study as a medium strength CA II inhibition for this isoenzyme, exhibited a stable level of inhibition at 31.67 ± 8.39 nM.

iii. A significant cause contributing to the onset of AD is the reduced amount of ACh and other enzymes responsible for its synthesis and reduction in the brain tissue[71]. Generally, a person who has suffered from AD will exhibit lower amounts of ACh (i.e., less than 0.20 μM ACh)[71]. Recently, Wei and coworkers have determined the levels of ACh using carbon dots[70]. Schistosome AChE plays a significant role in limiting this action and other reactions by inhibiting the AChE emulator ligand causibg receptor desensitization[71]. AChE can increase the deposition of aging β-amyloid plaques in aging brain tissue[71]. It has been recorded that the use of AChE inhibitors can strongly reduce some of the cognitive symptoms of AD and other behavioral traits[71]. Recently, Chen and his coworkers recorded a sensor for determining the activity of AChE using a changed BSA preserved cluster[72]. During the blood lodging steps of schistosomes, AChE is available on the parasite stratum membrane[73]. Effective BChE and AChE inhibitors can also be used for AD therapy. Thus far, the typical drugs for treating AD on the market -including rivastigmine, tacrine, donepezil and galantamine- are BChE or AChE inhibitors[72,64]. AChE orginated in the brain and in erythrocyte cells with higher prudaction levels and it is an important enzyme for neural devices[73]. The activity (%)-[Hetaryl sulfonamides] graphs were plotted and the IC_{50} values of each hetaryl sulfonamide against AChE were computed after appropriate each dilution[73]. Accordingly, the manufacturing of new inhibitors is important for thedeveloping improved therapies to treat AD. The inhibitory effects of hetaryl sulfonamides **1**–**12** on AChE and BChE are shown in Table 3. In this study, the recently synthesized compounds exhibited strong inhibitory activity against AChE, with Ki values ranging from 0.20 ± 0.05 nM to 1.14 ± 0.26 nM. In addition, tacrine as a standard inhibitor for AChE exhibited a Ki value of 25.75 ± 3.39 nM. Based on these results, the inhibition of AChE by hetaryl sulfonamides **1**–**12** is significantly stronger than that of tacrine, a standard AD medication.

iv. The BChE enzyme play a major role as an ACh hydrolyzing enzyme in environmental mammalian systems[37]. The BChE enzyme has a particular role in cholinergic neurotransmission and it has been a key factor in AD[37]. An improved Ellman method[59] was defined to quantify the activity of BChE.

Table 4. Determination of the half maximal concentrations (IC_{50}, μg/mL) of Fe^{2+} chelating of several new hetaryl sulfonamides **1–12** and standard compounds including BHA, BHT, α-Tocopherol, Trolox and EDTA.

Antioxidant compounds	Fe^{2+} chelating (IC_{50})	r^2
BHA	46.20	0.9984
BHT	76.96	0.9664
α-Tocopherol	40.76	0.9421
Trolox	31.13	0.9442
EDTA	9.36	0.9773
1	173.25	0.9727
2	196.11	0.9866
3	72.32	0.9694
4	169.02	0.9341
5	43.31	0.9170
6	135.88	0.9766
7	97.65	0.9782
8	86.63	0.9092
9	115.40	0.9912
10	76.95	0.9636
11	131.50	0.9626
12	95.86	0.9689

Currently, the most commonly prescribed cholinesterase inhibitors are galantamine, donepezil and rivastigmine[61]. Donepezil and galantamine are short-lived reversible competitive inhibitors, whereas rivastigmine actively reacts with ChE. However, rivastigmine has an equal affinity for both the BChE and AChE enzymes, which makes it a very momentous drug currently prescribed for the therapeutic treatment of AD[41]. It has also been shown that phenothiazines inhibit ChEs, especially BChE. In this study, hetaryl sulfonamides **1–12** inhibited BChE, with Ki values in the range of 1.55 ± 0.44–5.92 ± 1.63 nM. Additionally, hetaryl sulfonamide **10** was potent BChE inhibitor (Ki: 1.55 ± 0.44 nM). Morever, all of the hetaryl sulfonamides displaed higher BChE inhibition activity than tacrine (Ki: 37.82 ± 2.08 nM).

v. The metal chelating procedure used in this study is also an antioxidant technique that is based on the absorbance measurement of the ferrous ion (Fe^{2+})-ferrozine molecule after subsequent treatment of a Fe^{2+} solution with the experimental sample[74–79]. The ferrozine–Fe^{2+} molecule created a red chromophore with an absorbance that was measured at 562 nm[80–82]. A weak antioxidant method for iron chelation would be strongly underestimated in low concentrations. The metal-chelating process was considerable since it decreased the concentration of the catalyzing transition metal in lipid peroxidation[83–85]. EDTA is a potent metal chelator; therefore, this compound was recorded as a standard metal chelator operative in this test[86,87]. In addition, it was determined that the IC_{50} values for hetaryl sulfonamide compounds **1–12** were calculated in the range of 43.31–196.11 μg/mL (Table 4). Moreover, the IC_{50} values corresponding to the Fe^{2+} ion-chelating method of the positive control samples – like trolox, α-tocopherol, BHA, BHT and EDTA – were determined to be in the range of 9.36–76.96 mg/mL. A lower IC_{50} value corresponds to a higher Fe^{2+} ion-binding capacity[88–90].

Conclusion

In this work, we focus on new hetaryl sulfonamides **1–12**, which exhibited effective inhibition profiles against BChE and AChE enzymes and hCA isoforms. We also defined the AZA data, as this standard sulfonamide inhibitor exhibits anticonvulsant properties. In addition, the IC_{50} values of the compounds were studied; the best inhibitor was compound **8** toward hCA II. Both the BuChE and AChE enzymes hydrolyze ACh and demonstrate retained AChE levels in AD patients. In this work, the AChEIs have been a primary drug target for regulating AD at the symptomatic level. Human AChE and BuChE also have 65% amino acid sequence similarity. Furthermore, these compounds showed effective metal chelating activity in the presence of Fe^{2+} ions and ferrozine.

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Disclosure statement

The authors declare that there is no conflict of interest.

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