Regular Article

ORGANIC CHEMISTRY RESEARCH
Published by the
Iranian Chemical Society
www.orgchemres.org

Org. Chem. Res., Vol. 7, No. 2, 139-156, July 2021.
DOI: 10.22036/org.chem.2022.313484.1266

Design, Synthesis and Biological Evaluation of Novel 1,3,4-Thiadiazol Derivatives as Selective Inhibitors of Butyrylcholinesterase

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(Received 4 November 2021, Accepted 7 February 2022)

Butyrylcholinesterase (BChE) is considered a promising drug target which plays an essential part in the progression of the late stage of Alzheimer's disease (AD). Selective BChE inhibitors could be capable drug candidates for a neurodegenerative disorder. So that, a set of novels 1,3,4-thiadiazole derivatives (1c-17c) were designed and synthesized in order to selective inhibitory of BChE. Physicochemical and spectroscopic approaches proved the structures of the synthesized compounds, and the purity of the compounds was confirmed by CHN analysis. The compound's interactions with amino acid residues in the active sites of acetylcholinesterase (AChE) and BChE were examined by in silico molecular docking studies. In vitro cytotoxicity effects measured by MTT method. For evaluating synthetic substances' inhibitory activity, Ullman's method was used by AChE from electric eel and BuChE from equine serum. Based on the in vitro inhibitory assay results, compounds 1c (IC50 = 18.34 µM) and 3c (IC50 = 10.45 µM) exhibited inhibitory potency on BChE. Results indicate that these compounds (1c and 3c) possibly will be subjected to further investigations. The present study may be hoped to stimulate efforts to develop of novel BChE inhibitor agents.

Keywords: Heterocyclic compounds, Thiadoazole, Bioorganic chemistry, Computational chemistry, Drug research

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder in cholinergic neurons, a kind of dementia in adults [1,2]. It is characterized by progressive and steady mental capacity deterioration and negative behavioral and psychiatric symptoms. The disease currently affects over 45 million people worldwide and continues to have a significant public health impact as the aging population increases [3]. There are three types of symptoms that accrue during AD. The first type consists of language difficulties, forgetfulness, and loss of planning and intellectual coordination skills. The Second type includes psychiatric symptoms and behavioral disorders like depression, hallucination, illusion, and distress, all non- cognitive symptoms. Furthermore, the third type of symptoms comprises difficulties with activities like shopping, driving, wearing clothes [4-6].

Up to now, the most commonly recommended treatments for minor to moderate stage AD are inhibitors which are from the family of enzymes known as cholinesterases (ChEs), like donepezil, galantamine, and rivastigmine [7]. The function of ChEs in the brain is to conclude the excitatory synaptic activity by altering the neurotransmitter acetylcholine. These drugs follow the classical cholinergic theory, suggesting that improved synaptic concentration of acetylcholine, due to acetylcholinesterase (AChE) inhibition, affords some relief of dementia AD-related cognitive decline [8,9]. Due to continuous dosing requirements of reversible inhibitors and the unique expression of AChE in peripheral tissues, such compounds tend to have severe systemic side effects related to disruption of the peripheral nervous system (PNS) gastrointestinal

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toxicity.

An essential isoform of AChE is butyrylcholinesterase (BChE). This enzyme is present in the peripheral and central nervous system and has been notably upregulated in AD brains, whereas AChE levels remain unchanged or decreased [10]. Indeed, during the development of the disease, the level of BChE increases, so the AChE rectifies by decreasing [11]. Nakayama *et al.,* in their work with organophosphorus (OP) inhibitors, emphasized the importance of BChE-selective inhibitors that do not change AChE activity [12]. In another study on transgenic mouse models, Greig *et al*. have find out that selective inhibition of BChE overexpressing amyloid precursor protein and Aβ peptide exhibited a significant reduction (47-54%) of synthesis and secretion of neurotoxic Aβ and increased cognitive performance in elderly rats. They were administering cymserine and analogs as inhibitors of BChE to human neuroblastoma cells significantly reduced intracellular and secreted APP levels and secreted Aβ40 without altering cellular viability [13].

These results confirm the importance and high clinical potential of BChE-selective inhibitors according to their ability to mediate AD's critical neuropathological markers.

Another compound studied in treating Alzheimer's disease is 3-aminopropan-1-sulfonic acid, also known as tramiprosate or homotaurine. Tramiprosate is an oral amyloid anti-aggregation agent evaluated in mild to moderate AD patients. Unfortunately, this compound failed in phase 3 clinical trials due to its side effects associated with gastrointestinal[14-16].

Heterocycles compounds, natural and synthetic, are important classes of compounds due to their varied biological properties. For example, imidazo[1,2-a]pyridine, tetrahydropyridine, 1,3,4-oxadiazole scaffolds can be significantly found in natural products and pharmacological compounds [17-20].

Also, some heterocyclic compounds are effective catalysts like TTSA@Ni nanocomposite and LDH@MPS-GMA-TZ-CuI. These catalysts are used to synthesize sulfonamides [21,22].

Due to the multiple benefits of heterocyclic compounds, they have always been considered by chemists, and they have become the subject of various investigations [23,24].

Among the wide world of heterocyclic compounds, 1,3,4-thiadiazoles are attractive groups of heterocyclic compounds. Thidiazole rings are bioisosteres of pyrimidines and oxadiazoles [25].

The sulfur atom's liposolubility in 1,3,4-thiadiazole and the mesoionic nature of these compounds allowed them to interact with biological targets. Moreover, studies have shown that these compounds have extensive uses in anti-cancer, anti-microbial, antiviral, antiepileptic, anti-inflammatory, antifungal, antidiabetic, and analgesic activities [26-31]. Furthermore, some derivatives of 4-(5-phenyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol have been evaluated as suitable inhibitors of AChE and BChE, which shows 1,3,4-thiadiazole scaffold has a good potential for designing new cholinesterase inhibitors [32].

In this work, we design and synthesize new derivatives of 1,3, and 4-thiadiazoles with 1,3-propane sulfonic acid moiety and study their molecular interactions with AChE and BChE using AutoDock Vina software version 1.1.2 [33]. The compound's cytotoxicity was assessed against HDF by MTT colorimetric assay. The modified Ullman's method was used to evaluate the inhibitory activity of synthesized compounds against AChE and BchE [34].

RESULT AND DISCUSSION

The general synthesis method that consists of a three-step reaction, as shown in Schemes 1-5, was used to synthesize novel 1,3,4-thiadiazole derivatives. The result products were obtained in good yield. The structure of products was deduced by ^{1}H NMR, ^{13}C NMR, FT-IR, and CHN analysis. For the Docking study on AChE and BuChE, the blind docking method with Autodock Vina software has been used. The Best conformer was detected, and the binding interactions are shown in Figs. 1-6. The inhibitory activity range represents biding affinity for AChE is -9.3 to -4.2 and

for BuChE is -8.9 to -5.2 Kcal mol^{-1} (Table 2). As the result of molecular docking with AchE shows the receptor, among all the compounds, compounds 8c, 12c, and 13c have the lowest binding affinity with an amount of -9.3 Kcal mol^{-1} compared to the rest of them. Compound 8c has hydrogen binding with Ser293, Arg296, and Phe295, and π-alkyl interaction with Tyr341. Moreover, it has unfavorable acceptor-acceptor interaction with TRP286. Compound 12c has hydrogen bonds with Tyr341, Ser293, Phe295, and Arg286. Compound 13c has hydrogen bonds with His287, Trp286, Ser293, Phe295, and Arg296. The result of molecular docking with BuChE as the receptor administrate shows that compounds 3c,5c, and 8c have the lowest binding affinity with -8.9 Kcal mol^{-1} compared to the rest of the compounds. Compound 3c has hydrogen bonds with Thr120, Gly116, Gly117, π-sulfur bounds with Phe329, His438, π-π interactions with Leu286, Trp231, and van der Waals interactions with Ser198, Trp231. Compound 5c shows hydrogen bonds with His438 and Ser287. Compound 5c shows unfavorable doner-doner interactions with Asn289 and Gly117 as well. Compound 8c shows hydrogen bonds with Tyr332, Trp430, Tyr440, Leu286, π-sulfur bound with His438, π-alkyl interactions with Trp231, and Phe329, and van der Waals interactions with Trp82, and Trp231. Compound 8c has unfavorable interaction with Ser198 as well.

In vitro cytotoxicity effect of all synthesized compounds on HDF was evaluated by MTT colorimetric assay, and it has been found that up to the concentration of 100 mM, none of the compounds showed cytotoxicity against HDF which indicates their non-toxicity.

Based on the in silico modeling of design compound inhibitory actives, it has been decided to do the *in vitro* studies. The modified Ulman's method has been used for in vitro inhibitory assay of synthesized compounds using AChE from electric eel (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, 1000 unit) and BuChE from equine serum (BuChE, E.C. 3.1.1.8). As shown in Table 2, the compounds (1c-17c) didn't show biological activity against AChE, and only compounds 1c and 3c have inhibitory activity against the BuChE.

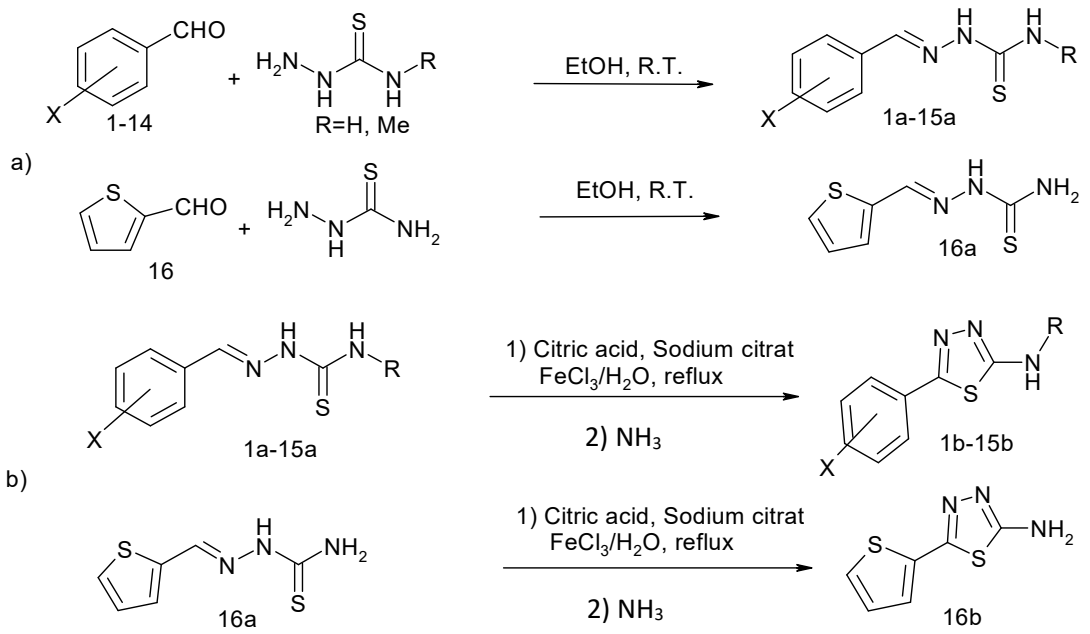
The study of the relationship between chemical structure and biological activity in synthesized 1,3,4-thiadiazole derivatives shows that the existence of an aromatic ring in the position of 5 of the thiadiazole ring is necessary. Therefore, biological activity is not seen in derivatives without an aromatic group (compounds 16c, 17c), and probably there is an aromatic ring in the enzyme's active site that can create an aromatic-aromatic attraction with the aromatic ring in the sample (compounds 1c, 3c) [35,36].

EXPERIMENTAL

General

Melting points were recorded using a ThermoElectrothermal model lA9200. ^{1}H and ^{13}C NMR spectra were recorded by 300 MHZ BRUKER model DXR-300 spectrometers. The DMSO-d6 solvent was used for ^{1}H and ^{13}C NMR spectra detection by using TMS as an internal chemical shift were accounted in ppm (δ). Relative to TMS: δ value multiplicity (s, singlet; d, doublet; t, triplet; quint, quintet; m, multiplet), number, and count of the protons. IR spectra were recorded using KBr disks on PU 9624 FTIR spectrometer. The elemental analysis (C, H, N) was applied on a LECO FP 528 CHN analyzer, and their results were found to be in good agreement (±0.3%) with the calculated values. Thin-layer chromatography (TLC) on silica gel-protected aluminum sheets (Type 60 F_{254}, Merck) was used to monitor the reaction's progress, and a UV λ 254 nm lamp detected the spots. Solvents were evaporated with a BUCHI R-114 rotary evaporator.

General procedure for synthesis compounds 1a-15a. In a round-bottom flask, Aryl aldehyde (10 mmol) was added to ethanol 95% (20 ml). Thiosemicarbazide (10 mmol) (N-methylhydrazine carbothioamide in case of 15a) was solved in hot water (20 ml), and the product solution was added to the benzaldehyde in the ethanol mixture. The resulting were filtrated with the Buchner funnel. The



Scheme 1. a) Synthesis reaction of thiosemicarbazones derivatives. X (1-14): 1 = H, 2 = 4-Me, 3 = 2-OH, 4 = 2-NO_2, 5 = 4-NO_2, 6 = 2-Cl, 7 = 4-Cl, 8 = 4-NH_2, 9 = 4-Methoxy, 10 = 4-Br, 11 = 3-OH, 12 = 4-OH, 15 = 2,4-di OH; R = H, Me. b) Ring forming reaction of 5-aryl-1,3,4-thiadiazol-2-amine derivatives (1b-16b) and 5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (16b).

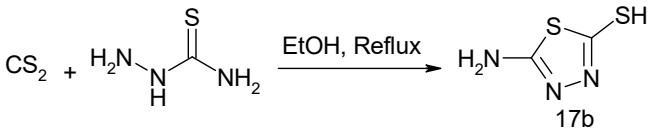
resulting thiosemicarbazones (1a-16a) were dried for the next step (Scheme 1).

General procedure for synthesis 5-aryl-1,3,4-thiadiazol-2-amine derivatives (1b-15b). In a round-bottom flask, thiosemicarbazone (1a-15a, 10 mmol), sodium citrate (10 mmol), citric acid (20 mmol), and $FeCl_3$ (50 mmol) were added to the 100 ml of distilled water and stirred under reflux condition for 2 h. The mixture was then cooled down to room temperature and neutralized by NH_3 (28%). The resulting sediments were filtered and recrystallized from ethanol [37] (Scheme 1).

Synthesis approach of (E)-2-(thiophen-2-ylmethylene) hydrazine-1-carbothioamide (16a). In a round-bottom flask, thiophene-2-carbaldehyde (10 mmol) was added to ethanol 95% (20 ml). Thiosemicarbazide (10mmol) was solved in hot water (20 ml), and this solution was added to the mixture of thiophene-2-carbaldehyde in the ethanol. The resulting reaction was stirred at room temperature, and sedimentations were filtrated with the Buchner funnel.

Synthesis approach of 5-(thiophen-2-yl)-1, 3,4-thiadiazol-2-amine (16b). In a round-bottom flask, (E)-2-(thiophen-2-ylmethylene) hydrazine-1-carbothioamide (10 mmol), sodium citrate, citric acid, and $FeCl_3$ were added to the water and stirred under reflux condition for 2 h. The mixture was then cooled to room temperature and neutralized by NH_3 (28%). The resulting sediments were filtered and recrystallized from ethanol.

Synthesis of 5-amino-1,3,4-thiadiazoles-2-thiol (17b). Thiosemicarbazide (0.25 mol) was suspended in ethanol (98%). Then anhydrous sodium carbonate (24 g) and carbon disulfide (0.25 mol) was added slowly. The mixture was stirred under reflux for 1 h and then heated at 80 °C for 4 h. After removing the solvent, the residue was dissolved in water (200 ml). Then the resulting mixture was acidified with concentrate HCl to give the product as the hydrochloride salt [38] (Scheme 2).



Scheme 7. Synthesis reaction of 5-amino-1,3,4-thiadiazoles-2-thiol (17b)

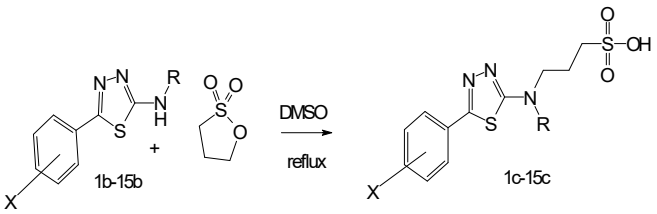
Synthesis approach of 3-((5-aryl-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid derivatives (1c-15c). In a round-bottom flask, 10 mmol of 5-aryl-1,3,4-thiadiazol-2-amine (1b-15b) was added in 2 ml of DMSO then, under the reflux condition and stirring, 10 mmol of 1,3-propane sultone were added. Also, the reaction was monitored with TLC. After finishing, the resulting sediment was filtered with the Buchner funnel and then recrystallized from methanol (Scheme 4).

Synthesis approach of 3-((5-(thiophen-2-yl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (16c). In a round bottom flask, 1 mmol of 5-(thiophen-2-yl)-1,3,4-thiadiazol-2-amine (16b) was added in 2 ml of DMSO, then under the reflux condition and stirring, 1 mmol of 1,3-propane sultone was added, the reaction was monitored with TLC. After finishing, the resulting sediment was filtered with a Buchner funnel and then recrystallized with hot methanol (Scheme 5).

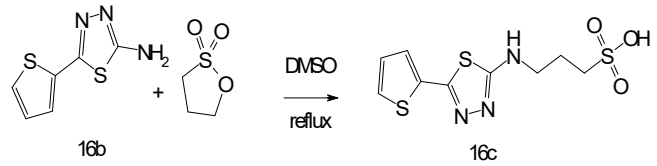
Synthesis approach of 3-((5-mercapto-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (17c). In a round-bottom flask, 1 mmol of 5-amino-1,3,4-thiadiazole-2-thiol was added in 2 ml of DMSO then, under the reflux condition and stirring, 1 mmol of 1,3-propane sultone was added, and the reaction was monitored with TLC. After finishing, the resulting sediment was filtered with the Buchner funnel and then recrystallized from methanol (Scheme 6).

Experimental Data

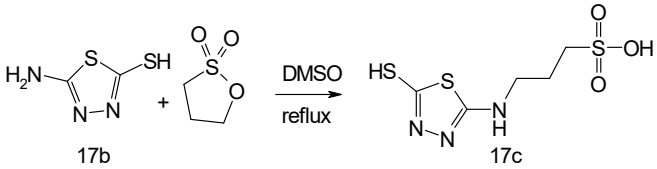
3-((5-Phenyl-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (1c). White powder, Yield: 70.5%. MW: 299.36 g mol^{-1}. melting point: 340 °C. IR (KBr, cm^{-1}): 3089, 1566, 3266, 1289, 1151, 653. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ [ppm]: 2.15 (quient, J = 6.0 Hz, 2H, CH_2), 2.58 (t, J = 6.0 Hz, 2H, CH_2,), 4.39 (t, 2H, CH_2, J = 6.1 Hz), 7.46 (m, J = 7.2 Hz, 1H, phenyl), 7.59 (m, 2H, phenyl), 7.86 (m, 2H, phenyl), 10.13 (2H, OH and NH); ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 168.64, 156.70, 131.33, 129.28, 128.92, 126.48, 60.44, 47.38, 43.23; Anal. found for $C_{11}H_{13}N_3O_3S_2$: C 44.11%, H 4.40%, N 14.08%. Calculated elemental analysis: C 44.13%, H 4.38%, N 14.04%, O 16.03%, S 21.42%.



Scheme 4. Synthesis reaction of 3-((5-aryl-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid derivatives



Scheme 5. Synthesis reaction of 3-((5-(thiophen-2-yl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid



Scheme 6. Synthesis reaction of 3-((5-mercapto-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid

3-((5-(p-tolyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (2c). White powder, Yield: 72%. MW: 313.39 g mol^{-1}. melting point: 329 °C. IR (KBr, cm^{-1}): 3010, 3301, 1315, 1201, 651. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]:

3.32 (t, J = 6.3 Hz, 2H, S-CH_2), 4.34 (t, J = 6.8 Hz, 2H, N-CH_2), 7.12 (dd, 2H, J = 8.2 Hz, phenyl), 7.78 (dd, J = 8.0 Hz, 2H, phenyl), 10.13 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 165.32, 153.61, 130.33, 128.20, 127.12, 125.40, 59.72, 46.20, 43.19; Anal. found for $C_{12}H_{15}N_3O_3S_2$: C 45.97%, H 4.80%, N 13.37%; Calculated elemental analysis: C 45.99%, H 4.82%, N 13.41%, O 15.32%, S 20.46%.

3-((5-(2-Hydroxyphenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (3c). White powder, Yield: 91%. MW: 315.36 g mol^{-1}. melting point: 312 °C. IR (KBr, cm^{-1}): 1376, 3092, 2989, 630. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.16 (quint, J = 6.0 Hz, 2H, CH_2), 2.58 (t, J = 6.1 Hz, 2H, S-CH_2), 4.39 (t, J = 6.0 Hz, 2H, N-CH_2), 7.14, 7.2, 7.214, 7.26 (4H, phenyl), 10.12 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 168.60, 157.12, 148.62, 131.30, 128.25, 126.60, 122.71, 120.42, 60.32, 47.20, 43.21; Anal. found for $C_{11}H_{13}N_3O_4S_2$: C 41.89%, H 4.12%, N 13.28%; Calculated elemental analysis: C 41.89%, H 4.15%, N 13.32%, O 20.29%, S 20.33%.

3-((5-(2-Nitrophenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (4c). Yellow powder, Yield: 67%. MW: 344.36 g mol^{-1}. melting point: 270 °C. IR (KBr, cm^{-1}): 3052, 3012, 1214, 1151, 1569, 1564. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.11 (quint, J = 6.0 Hz, 2H, CH_2), 2.86 (t, J = 6.1 Hz, 2H, S-CH_2), 4.31 (t, J = 6.0 Hz, 2H, N-CH_2), 7.9 (2H, phenyl), 8.25 (2H, phenyl), 10.11 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 166.23, 155.72, 142.36, 134.23, 130.43, 128.41, 127.59, 124.48, 59.74, 46.25, 43.52; Anal. found for $C_{11}H_{12}N_4O_5S_2$: C 38.39%, H 3.56%, N 16.22%; Calculated elemental analysis: C 38.37%, H 3.51%, N 16.27%, O 23.23%, S 18.62%.

3-((5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (5c). Yellow powder, Yield: 85%. MW: 344.36 g mol^{-1}. melting point: 320 °C. IR (KBr, cm^{-1}): 3009, 3056, 1211, 1149, 1567, 1562. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.13 (quint, J = 6.0 Hz, 2H, CH_2), 2.56 (t, J = 6.7 HZ, 2H, S-CH2), 4.38 (t, J = 6.0 Hz, 2H, N-CH_2), 7.36 (dd, J = 7.5 Hz, 2H, phenyl), 8.07 (dd, J = 7.3 Hz, 2H, phenyl), 10.03 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 165.82, 155.62, 142.16, 133.50, 128.92, 126.28, 59.81, 46.27, 43.58; Anal. found for $C_{11}H_{12}N_4O_5S_2$: C 38.35%, H 3.57%, N 16.32%; Calculated elemental analysis: C 38.37%, H 3.51%, N 16.27%, O 23.23%, S 18.62%.

3-((5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (6c). White powder, Yield:90%. MW: 333.81 g mol^{-1}. melting point: 225 °C. IR (KBr, cm^{-1}): 2993, 3023, 1213, 1145. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.16 (quint, J = 6.1 Hz, 2H, CH_2), 2.59 (t, J = 6.0 Hz, 2H, S-CH_2), 4.4 (t, J = 6.0 Hz, 2H, N-CH_2), 7.56, 7.64, 7.69, 7.98 (4H, phenyl), 10.03 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 164.51, 162.98, 132.17, 130.28, 129.66, 128.20, 128.94, 126.14, 78.63, 43.64, 41.37; Anal. found for $C_{11}H_{12}ClN_3O_3S_2$: C 39.56%, H 3.68%, N 12.53%; Calculated Elemental analysis: C 39.58%, H 3.62%, Cl 10.62%, N 12.59%, O 14.38%, S 19.21%.

3-((5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (7c). White powder, Yield:85%; MW: 333.81 g mol^{-1}. melting point: 225 °C. IR (KBr, cm^{-1}): 2998, 3025, 1218, 1147. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.15 (quint, J = 6.1 Hz, 2H, CH_2), 2.58 (t, J = 6.4 Hz, 2H, S-CH_2), 4.38 (t, J = 6.1 Hz, 2H, N-CH_2), 7.64 (dd, J = 8.1 Hz, 2H, phenyl), 7.87 (dd, J = 6.6 Hz, 2H, phenyl), 10.13 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 164.28, 163.18, 131.12, 129.57, 128.42, 126.90, 78.70, 43.9, 40.5; Anal. Found for $C_{11}H_{12}ClN_3O_3S_2$: C 39.53%, H 3.61%, 12.52%; Calculated elemental analysis: C 39.58%, H 3.62%, Cl 10.62%, N 12.59%, O 14.38%, S 19.21%.

3-((5-(4-Aminophenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (8c). Dark yellow powder, Yield: 52%. MW:314.38 g mol^{-1}. melting point: 328 °C. IR (KBr, cm^{-1}): 3016, 1343, 1212, 680. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.15 (quint, J = 6.3 Hz, 2H, CH_2), 2.57 (t, J = 6.1 Hz, 2H, S-CH_2), 4.27 (t, J = 6.3 Hz, 2H, N-CH_2), 4.91 (s, 2H, NH_2), 6.59 (dd, J = 7.5, 0.3 Hz, 2H, phenyl), 6.94(d, J = 7.0 Hz, 2H, phenyl), 10 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 168.51, 156.49, 138.41, 132.28, 129,81, 126.45, 59.83, 47.12, 42.63; Anal. found for $C_{11}H_{14}N_4O_3S_2$: C 42.05%, H 4.53%, N 17.77%.; Calculated elemental analysis: C 42.02%, H 4.49%, N 17.82%, O 15.27%, S 20.40%.

3-((5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (9c). White powder, Yield: 91%; MW: 329.39 g mol^{-1}; melting point: 312 °C. IR (KBr, cm^{-1}): 3058, 1351, 1201, 1211, 1041, 670. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.13 (m, 2H, CH_2), 2.58 (t, J = 6.0 Hz, 2H, S-CH_2), 3.84 (t, J = 6.5 Hz, 2H, N-CH_2), 4.32 (s, 3H, O-CH_3), 7.12(d, J = 7.5 Hz, 2H, phenyl), 7.79 (d, J = 7.8 Hz, 2H, phenyl), 9.95 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 167.94, 156.52, 152.31, 126.28, 125.91, 120.52, 63.44, 53.42, 47.31, 43.51; Anal. found for $C_{12}H_{15}N_3O_4S_2$: C 43.72%, H 4.58%, N 12.47%; Calculated elemental analysis: C 43.76%, H 4.59%, N 12.76%, O 19.43%, S 19.47%.

3-((5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (10c). White powder, Yield: 69%; MW: 327.26 g mol^{-1}. melting point: 218 °C. IR (KBr, cm^{-1}): 3002, 1205, 1151, 651, 1035. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.14 (quint, J = 6.0 Hz, 2H, CH_2), 2.57 (t, J = 6.1 Hz, 2H, S-CH_2), 4.39(t, J = 6.0 Hz, 2H, N-CH_2), 6.96 (m, 2H, phenyl), 7.29 (m, 2H, phenyl), 10.12 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 163.92, 162.78, 130.52, 129.37, 127.42, 126.92, 48.70, 43.82, 42.77; Anal. found for $C_{11}H_{12}BrN_3O_3S_2$: C 34.95%, H 3.26%, N 11.21%. Calculated elemental analysis: C 34.93%, H 3.20%, Br 21.12%, N 11.11%, O 12.69%, S 16.95%.

3-((5-(3-Hydroxyphenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (11c). Yield: 89%; MW: 315.36 g mol^{-1}. melting point: 367-370 °C. IR (KBr, cm^{-1}): 3081, 2892, 1385, 632. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.14 (quint, J = 6.0 HZ, 2H, CH_2), 2.57 (t, J = 6.0 HZ, 2H, S-CH_2), 4.39 (t, J = 6.1 HZ, 2H, N- CH_2), 7.50 (m, 4H, phenyl), 10.01 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 167.94, 157.82, 148.02, 131.36, 128.75, 125.61, 120.17, 119.12, 59.82, 47.54, 43.51; Anal. found for $C_5H_9N_3O_3S_3$: C 42.19%, H 4.02%, N 13.01%; Calculated elemental analysis: C 41.89%, H 4.15%, N 13.32%, O 20.29%, S 20.33%.

3-((5-(4-Hydroxyphenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (12c). Yield: 90%; MW: 315.36 g mol^{-1}. melting point: 208-211 °C. IR (KBr, cm^{-1}): 3075, 2960, 1346, 647. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.26 (quint, J = 6.0 Hz, 2H, CH_2), 2.68 (t, J = 6.0 Hz, 2H, S-CH_2), 4.49 (t, J = 6.0 Hz, 2H, N-CH_2), 7.03 (ddd, 2H, phenyl), 7.40 (ddd, 2H, phenyl), 10.12 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 168.01, 157.31, 152.07, 128.15, 126.49, 120.72, 60.31, 47.35, 44.18; Anal. found for $C_5H_9N_3O_3S_3$: C 42.19%, H 3.92%, N 13.14%; Calculated elemental analysis: C 41.89%, H 4.15%, N 13.32%, O 20.29%, S 20.33%.

3-((5-(2,4-Dihydroxyphenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (13c). Yield: 92%; MW:331.36 g mol^{-1}. melting point: 244-247 °C. IR (KBr, cm^{-1}): 3085, 2962, 1389, 638. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]; 2.26 (quint, J = 6.0 Hz, 2H, CH_2), 2.78 (t, J = 6.0 Hz, 2H, S-CH_2), 4.59 (t, J = 6.0 Hz, 2H, N-CH_2), 7.23 (d, J = 6.5, 2.7 Hz, 1H, phenyl), 7.51 (d, J = 2.0 Hz, 1H, phenyl), 7.61 (s, 1H, phenyl), 10.01 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 169.15, 157.38, 155.18, 154.62, 131.08, 125.25, 119.60, 112.42, 60.46, 47.19, 43.91; Anal found for $C_5H_9N_3O_3S_3$: C 40.18%, H 3.75%, N 12.39%; Calculated elemental analysis: C 39.87%, H 3.95%, N 12.68%, O 24.14%, S 19.35%.

3-((5-(2-Methoxyphenyl)-1,3,4_thiadiazole-2-yl) amino) propane-1-sulfonic acid (14c). Yield: 87%; MW:329.39 g mol^{-1}. IR (KBr, cm^{-1}): 3052, 1242, 1210, 1043, 665. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.16 (quint, J = 6.0 Hz, 2H, CH_2), 2.58 (t, J = 6.0 Hz, 2H, S-CH_2), 3.89 (s, 3H, O-CH_3), 4.39 (t, J = 6.0 Hz, 2H, N-CH_2), 7.20 (d, J = 8.2 Hz, 4H, phenyl), 10.01 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 167.51, 160.98, 150.92, 129.66, 126.17, 123.62, 120.14, 119.32, 53.13, 44.17, 40.57; Anal. found for $C_5H_9N_3O_3S_3$: C 43.91%, H 4.31%, N 12.47%; Calculated elemental analysis: C 43.76%, H 4.59%, N 12.76%, O 19.43%, S 19.47%.

3-((5-(2-Hydroxyphenyl)-1,3,4-thiadiazol-2-yl) (methyl)amino) propane-1-sulfonic acid (15c). Yield: 78%; MW: 329.39 g mol^{-1}. IR (KBr, cm^{-1}): 3079, 2976, 1373, 1210, 643. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]: 2.26 (quint, J = 6.0 Hz, 2H, CH_2), 2.68 (t, J = 6.0 Hz, 2H,

S-CH_2), 2.89 (s, 3H, N-CH_3), 4.49 (t, J = 6.0 Hz, 2H, N-CH_2), 7.21 (m, 4H, phenyl), 10.12 (1H, OH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 170.62, 159.38, 150.27, 131.18, 128.25, 126.66, 122.71, 119.55, 60.81, 48.16, 43.71, 41.22; Anal. found for $C_5H_9N_3O_3S_3$: C 43.98%, H 4.38%, N 12.59%; Calculated elemental analysis: C 43.76%, H 4.59%, N 12.76%, O 19.43%, S 19.47%.

3-((5-(Thiophen-2-yl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (16c). White powder, Yield: 72%; MW: 305.39 g/mol; melting point: 332 °C. IR (KBr, cm^{-1}): 1559, 3149, 2998, 1226. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]; 2.3 (quint, J = 6.3 Hz, 2H, CH_2), 2.58 (t, J = 6.0 Hz, 2H, S-CH_2), 3.33 (t, J = 6.0 Hz, 2H, N-CH_2), 7.25 (t,1H, thiophen) 7.93 (q, 2H, J = 2.7, 3.0 Hz, thiophen); 10.11 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 171.09, 162.38, 129.41, 128.59, 128.13, 127.92, 50.27, 33.95, 26.71; Anal. found for $C_9H_{11}N_3O_3S_3$: C 35.42%, H 3.68%, N 13.70%.; Calculated Elemental analysis: C, 35.40; H, 3.63; N, 13.76; O, 15.72; S, 31.50.

3-((5-Mercapto-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid (17c). Yield: 69%; MW: 255.33 g mol^{-1}; melting point: 250 °C. IR (KBr, cm^{-1}): 3303, 2993, 2550, 1201, 1151, 763. ^{1}H NMR (300 MHz, DMSO-d6) δ [ppm]; 1.96 (quint, J = 6.0 Hz, 2H, CH_2), 2.57 (t, J = 6.0 Hz, 2H, S-CH_2), 3.2 (t, J = 6.0 HZ, 2H, N-CH_2), 8.5 (2H, OH and NH). ^{13}C NMR (75 MHz, DMSO-d6) δ [ppm]: 176.16, 169.85, 49.68, 33.05, 25.31; Anal. found for $C_5H_9N_3O_3S_3$: C 23.42%, H 3.61%, N 16.72%; Calculated elemental analysis: C 23.52%, H 3.55%, N 16.46%, O 18.80%, S 37.67%.

BIOLOGICAL AND PHARMACOLOGY METHOD

Physicochemical Study

Physicochemical properties including MW, number of hydrogen donors, number of hydrogen acceptors and lipophilicity (logP) of the synthesized compounds, are gathered in Table 1. According to Lipinski's rule of five (RO5, also known as Pfizer's rule of five), in general, a chemical compound for being orally active drug should have a molecular mass less than 500 Daltons, no more than 10 hydrogen bond acceptors, no more than 5 hydrogen bond donors, and logP (octanol-water partition coefficient) does not exceed 5 [39,40]. For calculating logP, we use ChemDraw software version 18.2.0.48 as its results are close to experimental amounts [41,42].

Molecular Docking Method

Molecular docking was prepared by the AutoDock Vina version 1.1.2 software using the blind docking method. AutoDock Vina uses a sophisticated gradient optimization method in its local optimization procedure [43]. AChE and BuChE crystal structures were prepared from the RCSB protein data bank (PDB) with a code of 1b41 and 1p0m. The structure of designed compounds as ligands was drawn and optimized by ChemOffice3D software version 16.0.0.82. For energy minimization, the MM2 tools were used. Python molecular viewer software version 1.5.6 (PMV) [44] was used to prepare the target proteins. The resulting file was saved in PDBQT format for using in molecular docking. Grid box spacing was 1 Å. The resulting complex in PDB format has been visualized by Discovery Studio visualizer software version v20.1.0.19295 (Figs. 1-6, Table 2).

Pharmacology

Cell line and cell cultures. The human dermal fibroblasts (HDF) were purchased from the National Cell Bank of Iran (NCBI). The HDF were cultivated in RPMI 1640 (GibcoeBRI, UK) medium supplemented with 10% heat-inactivated fetal calf serum. 100 mg ml^{-1} streptomycin and 100 U/ml penicillin incubated at 73 °C in a humidified atmosphere with 5% CO_2 in the air.

In vitro cytotoxicity effect. To assess the cytotoxicity, we use MTT colorimetric assay which is a cell-based assay. In this method, by mitochondrial dehydrogenases of the living cell, tetrazolium dye in MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) with yellow color became cleavage and turned to purple formazan crystals [45].

Table 1. Physicochemical Properties of Compounds 1c-17c

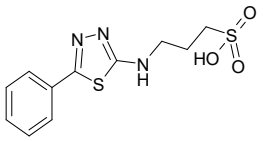
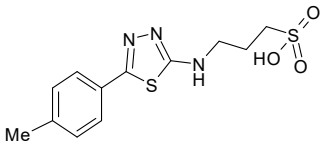
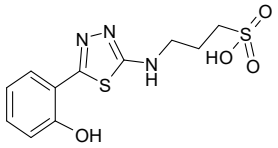
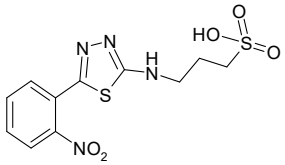
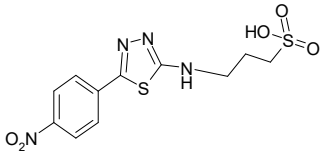
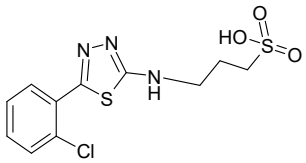
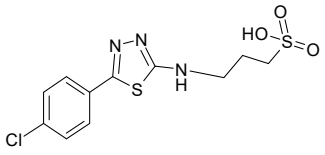
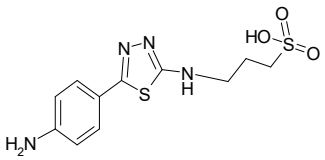
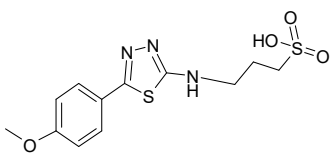
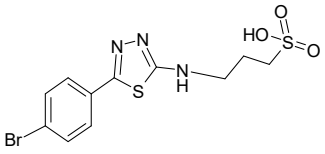
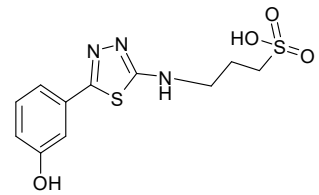
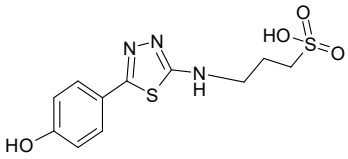
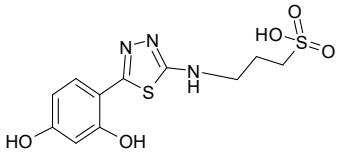
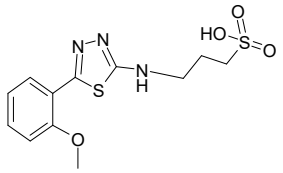
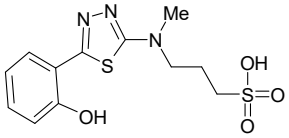
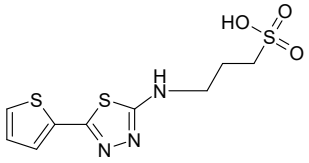
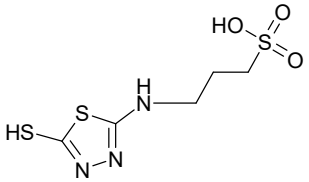
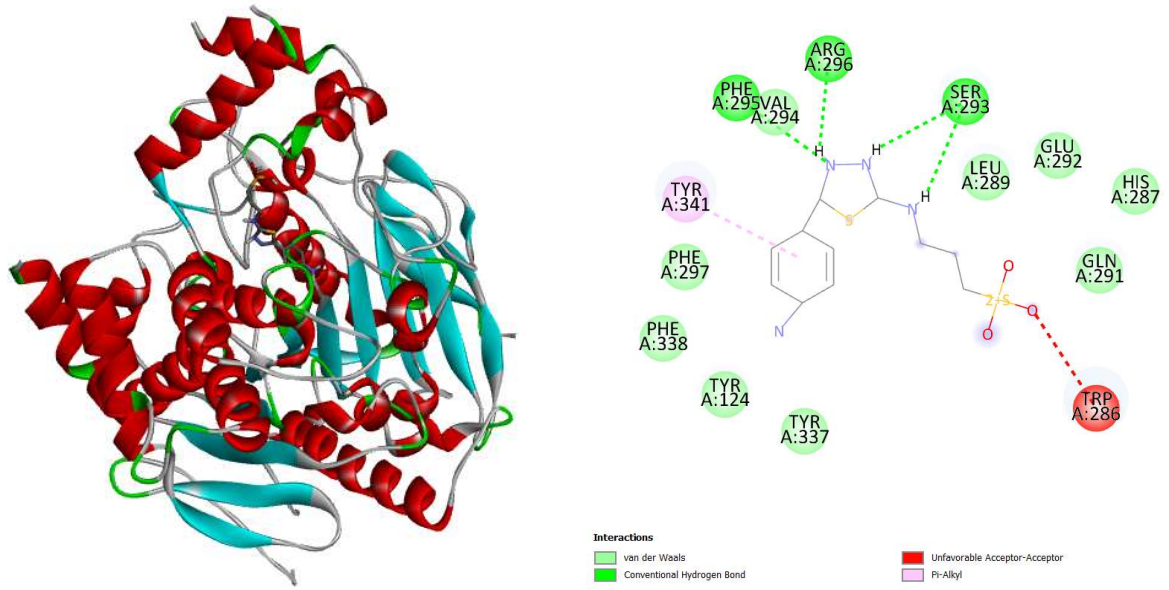
Entry	Products	MW	Hydrogen bond donor	Hydrogen bond acceptor	LogP
1c		299.36	2	6	2.1
2c		313.39	2	6	2.59
3c		315.36	3	5	1.71
4c		344.36	2	9	1.87
5c		344.36	2	9	1.87
6c		333.81	2	6	2.66
7c		333.81	2	6	2.66
8c		314.38	4	7	1.3
9c		329.39	2	7	1.98

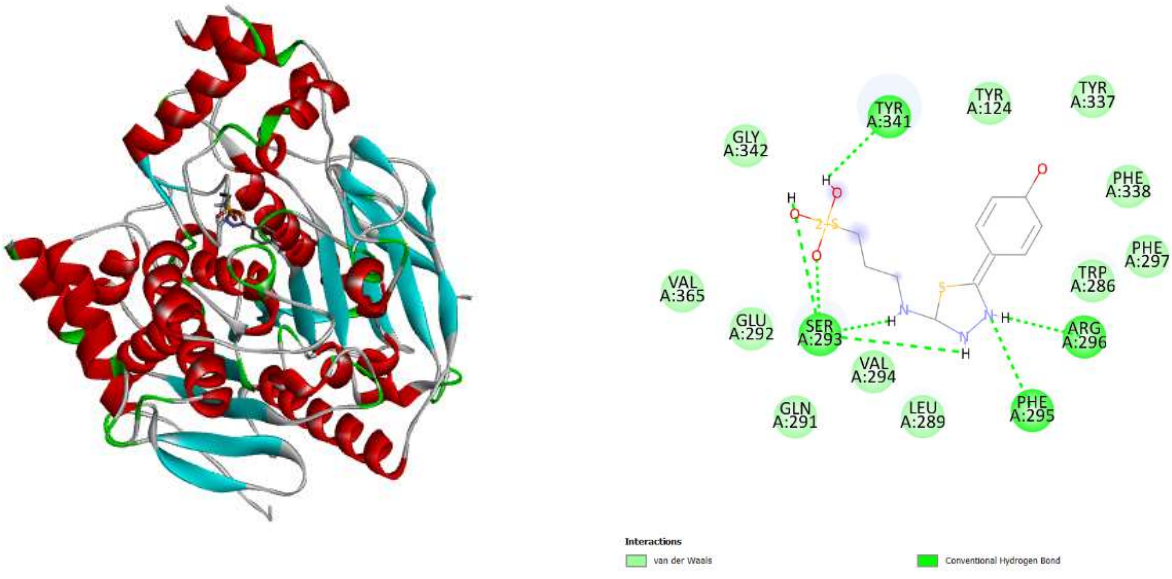
Table 1. Continued

10c		378.26	2	6	2.39
11c		315.36	3	7	1.71
12c		315.36	3	7	1.71
13c		331.36	4	8	1.32
14c		329.39	2	7	1.98
15c		329.39	2	7	2.5
16c		305.39	2	6	2.09
17c		255.33	3	6	1.22



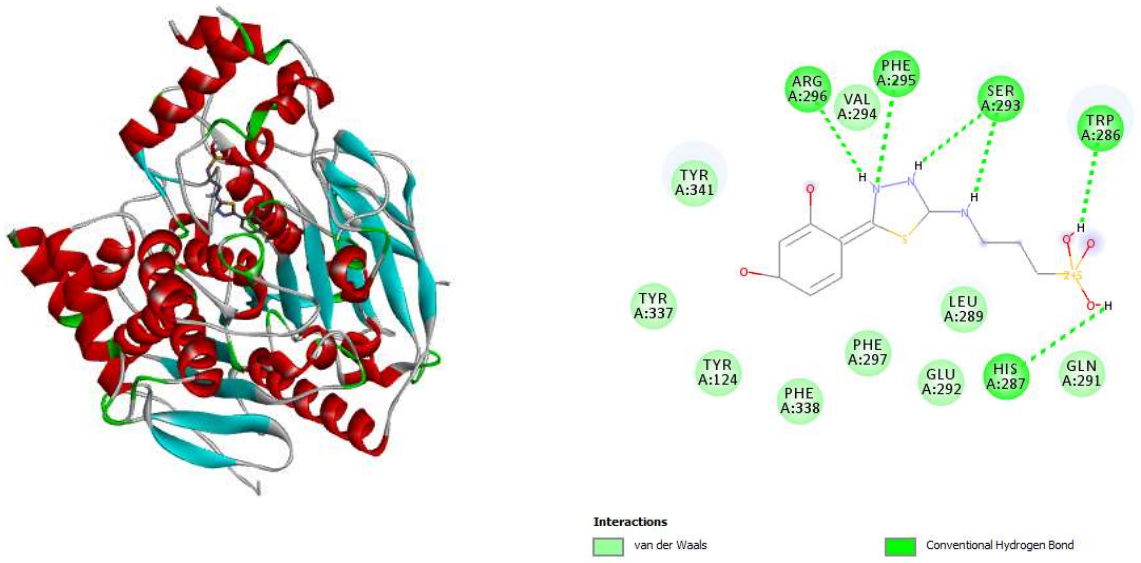
(a) **(b)**

Fig. 1. (a) compound 8c docked against AChE (b) predicted model of interactions compound 8c with AChE.



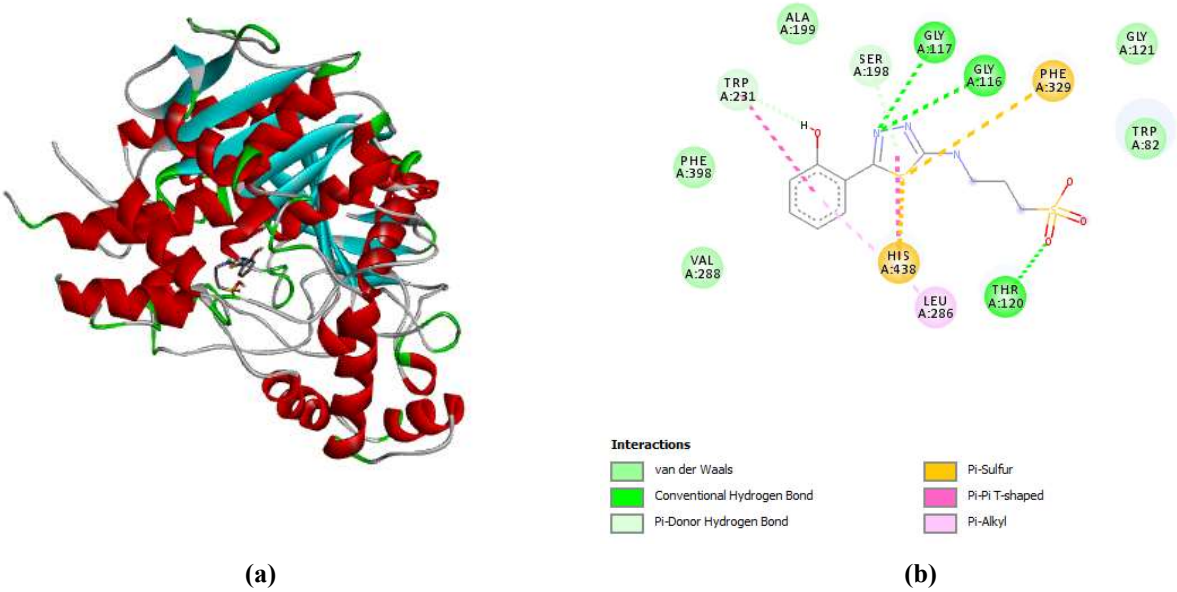
(a) **(b)**

Fig. 2. (a) compound 12c docked against AChE (b) predicted model of interactions compound 12c with AChE.



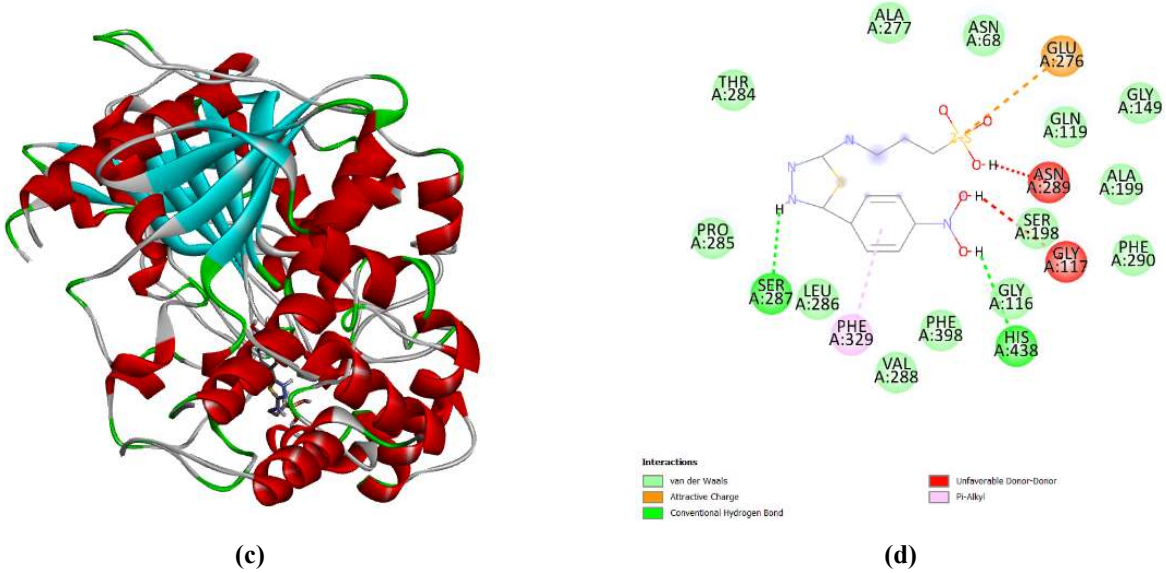
(a) **(b)**

Fig. 3. (a) compound 13c docked against AChE (b) predicted model of interactions compound 13c with AChE.



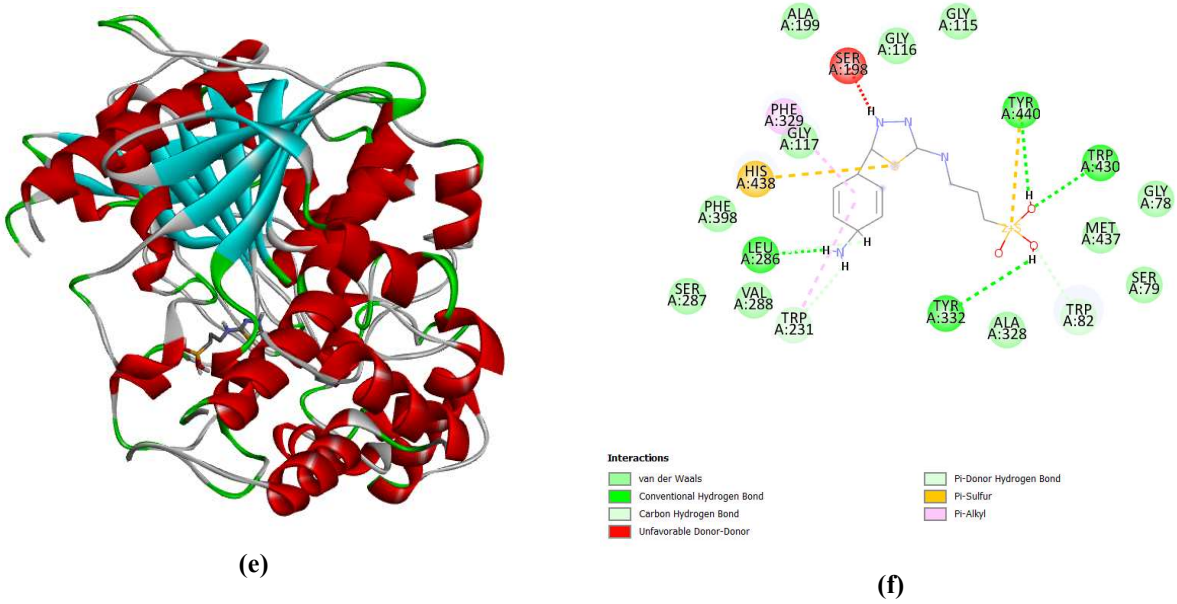
(a) **(b)**

Fig. 4. (a) compound 3c docked against BuChE (b) interactions of 3c with BuChE.



(c) **(d)**

Fig. 5. (c) compound 5c docked against BuChE (d) interactions of 5c with BuChE.



(e) **(f)**

Fig. 6. (e) compound 8c docked against BuChE (f) interactions of 8c with BuChE as hydrogen bounds, π-sulfur, π-π, π-alkyl, and van der waals.

First, the exponential growth phase culture became trypsinization, then 95 µl cells (1×10^6 cells/ml) were seeded into 96-well plates. The plates were incubated in a condition of the humidified atmosphere at 37 °C with 5% CO_2. Compounds solution in 0.5% concentration in DMSO prepared. Then 5 µl of each diluted compound was added per well in triplicate. Three control wells without any test compound and three blanks well with 0.5% DMSO were applied to check cell viability. The medium was removed after 48 h, and a mixture of 200 µl phenol red-free medium with 1 mg ml^{-1} MTT was added to each well. After 4 h incubation, culture medium was replaced with 100 µl of DMSO, and the absorbance of each well was measured by using a microplate reader at 570 nm (GEN5, EPOCH, Bio Tek, America) [46].

The effect of cytotoxicity of all synthesized compounds 1-13 on HDF was evaluated. Up to a concentration of 100 mM, none of the samples showed cytotoxicity against HDF, indicating their non-toxicity. Cytotoxicity was assessed against HDF by MTT colorimetric assay.

In vitro AChE and BuChE inhibitory assay. To measure the potency of synthesizing compounds in inhibiting AChE and BuChE. The modified Ullman's method was used to evaluate the inhibitory activity of synthetic substances by AChE from electric eel (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, 1000 unit) and BuChE from equine serum (BuChE, E.C. 3.1.1.8). Compounds 1c-17c were dissolved in a mixture of DMSO (5 ml) and MeOH (5 ml). The resultant solution was added to the potassium phosphate buffer with pH 8.00. Then 50 µl of potassium phosphate buffer, 25 µl of the prepared compound, and 25 µl solution of the intended enzyme in a buffer (0.22 u/ml) were poured into each well. For 15 min at room temperature, the plates were pre-incubated. Then 125 µl of 3 mM solution of DTNB and 25 µl of acetylcholine aqueous solution (3 mM) was added per wall. The changes in absorbance were checked out at 405 nm every 15 min under the same condition, the blank solution without (inhibitor) was prepared and tested. After withdrawing the inhibition cure, the IC_{50} values were obtained.

The modified Ullman's method was used to evaluate the inhibitory activity of synthetic substances by AChE from electric eel (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, 1000 unit) and BuChE from equine serum (BuChE, E.C. 3.1.1.8). The amount of activity was measured based on the inhibitory concentration of the synthesized material at 50% inhibitory (IC_{50}) for all synthesized materials (Table 2).

Aromatic rings that are substituted in the meta or para position (compounds 2c, 5c, 7c- 13c) do not have biological activity. Perhaps the reason is the creation of a spatial barrier and the lack of complete establishment of the compound in the enzyme's active site. Only aromatic compounds without substitution (compound 1c) or with hydroxyl substitution in the ortho position (compound 3c) show biological activity against the enzyme. Replacing the hydrogen of the hydroxyl group with the methyl group (object 14c) deactivates this compound. It seems that the presence of a large methyl group in comparison with hydrogen prevents the complete absorption of the compound in the active site of the enzyme. Also, the presence of bulky groups such as chlorine (object 6c) in the position of 2 in aromatic rings prevents the biological activity of the compound. Therefore, the presence of relatively acidic hydrogen of the hydroxyl group, which is likely to form a hydrogen bond, is necessary for biological activity.

Amino hydrogen in the position of 2 thiadiazole rings is required for biological activity. For example, in compound 15c, the replacement of amino hydrogen with the methyl group inactivates the compound.

CONCLUSION

In this work, we designed and synthesized novel 1,3,4-thiadiazoles derivatives in good yield. To prepare in silico models and analyze protein-ligand interactions, we used AutoDock Vina software and studied their inhibitory activity against AChE and BuChE. Based on the molecular docking results, *in vitro* enzyme tests on AchE and BuChE have

Table 2. *In Silico* Study of Inhibitory Activity and Biological IC_{50} Results as Inhibition Effect against AChE and BuChE and Theoretical Results as Binding Affinity to AChE and BuChE (kcal mol^{-1}) of Compounds 1c-17c

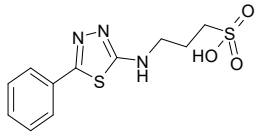
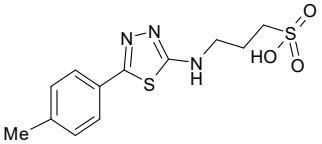
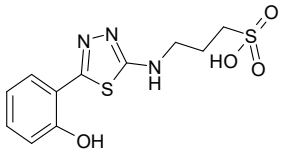
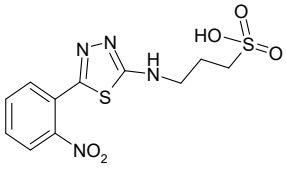
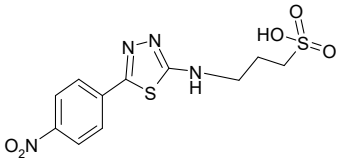
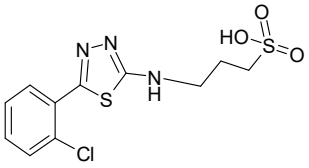
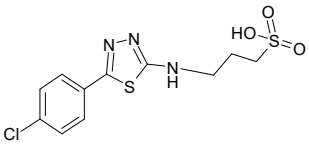
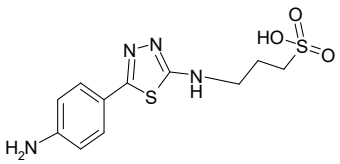
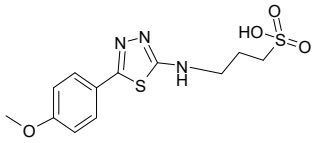
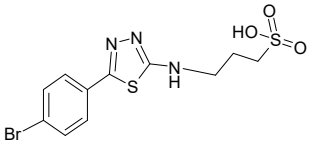
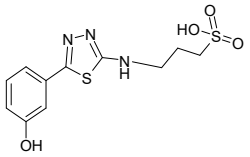
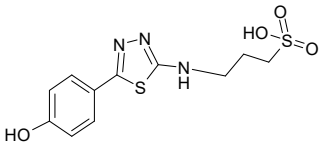
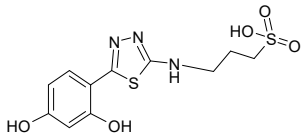
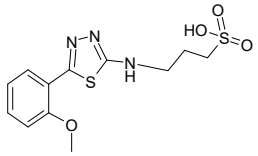
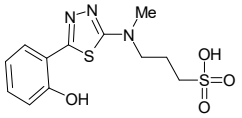
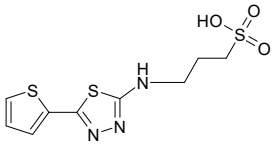
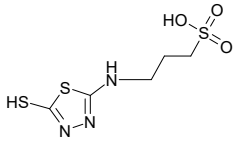
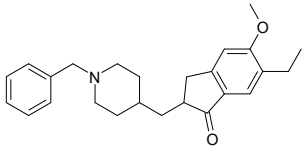
No.	Compound	AChE (IC_{50}, μM)[a]	BuChE (IC_{50}, μM)	Binding affinity for AChE (kcal mol^{-1})	Binding affinity for BuChE (kcal mol^{-1})
1c		>100	18.34	-8.4 to -6.4	-8.7 to -7.1
2c		>100	>100	-8.3 to -5.9	-8.0 to -7.2
3c		>100	10.45	-6.8 to -6.1	-8.9 to -6.9
4c		>100	>100	-7.7 to -6.5	-8.2 to -7.2
5c		>100	>100	-9.2 to -7.2	-8.9 to -7.5
6c		>100	>100	-6.5 to -5.8	-8.5 to -6.9
7c		>100	>100	-8.0 to -6.3	-8.0 to -7.2
8c		>100	>100	-9.3 to -6.9	-8.9 to -8.7

Table 3. Continued

9c		>100	>100	-8.7 to -6.6	-8.2 to -7.2
10c		>100	>100	-7.0 to -6.0	-6.9 to -6.4
11c		>100	>100	-7.5 to -5.5	-8.0 to -6.9
12c		>100	>100	-9.3 to -6.9	-8.6 to -7.8
13c		>100	>100	-9.3 to -7.0	-8.6 to -7.2
14c		>100	>100	-8.7 to -6.2	-8.6 to -7.3
15c		>100	>100	-6.8 to -5.7	-7.5 to -6.7
16c		>100	>100	-7.3 to -6.1	-7. 7 to -6.7
17c		>100	>100	-5.0 to -4.2	-5.8 to -5.2
Donepezil		0.079	5.20	-8.4 to -7.1	-10.1 to -7.4

[a]Inhibition concentration (mean ± SD of three experiments) required for 50% inhibition of AChE and BuChE.

demonstrated that 3-((5-phenyl-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid and 3-((5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl) amino) propane-1-sulfonic acid have selective inhibitory activity against BuChE.

ACKNOWLEDGMENTS

Iranian organization for science and technology (IROST) provided research facility and financial support for this work.

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