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Synthesis of 5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-one's aryl Schiff base derivatives and investigation of carbonic anhydrase and cholinesterase (AChE, BuChE) inhibitory properties

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ABSTRACT

Carbonic anhydrase enzymes (EC 4.2.1.1, CAs) are metalloenzyme families that catalyze the rapid conversion of H_2O and CO_2 to HCO_3^- and H^+. CAs are found in different tissues where they participate in various significant biochemical processes such as ion transport, carbon dioxide respiration, ureagenesis, lipogenesis, bone resorption, electrolyte secretion, acid-base balance, and gluconeogenesis. In such processes, many CAs are significant therapeutic targets because of their inhibitory potentials especially in the treatment of some diseases such as edema, glaucoma, obesity, cancer, epilepsy, and osteoporosis. Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) inhibitors are also valuable compounds for different therapeutic applications including Alzheimer's disease. In this work, we report a fast and effective synthesis of 5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-one's aryl Schiff base derivatives and also their CA and cholinesterases inhibitory properties. Our findings showed that these Schiff base derivatives, with triazole ring, found as strong CA and cholinesterases inhibitors.

1. Introduction

Carbonic anhydrase isoenzymes (CAIs) are found in lots of tissues where they participate in several important biological processes [1–5]. Moreover, many CAIs are target molecules to be used in the treatment of diseases such as glaucoma, cancer, edema, obesity, epilepsy etc. [6–12].

Relations between various human CA isozymes and various organic compounds including different types of uracil, some salicylic acids, and some other derivatives have been investigated [13,14]. A recent study examined the inhibitory effect of some sulfonamides on mammalian carbonic anhydrase enzymes [15]. Their anti-inflammatory, anti-mutagenic, antiviral, anti-carcinogenic, antibacterial or anticancer activities were also reported [16,17].

Alzheimer's disease (AD) is defined as a neurodegenerative condition described by abnormal behavior, intellectual reduction, and it is one of the major public health problems, especially due to the increasing elderly population in developed countries [18,19]. In spite of the fact that AD pathogenesis has not been clarified yet, one of the most important theories was the "cholinergic hypothesis" [20]. The defect in the levels of acetylcholine (ACh) and butyrylcholine (BCh) was observed in the brains of patients with AD. The inhibition of AChE and BuChE enzymes that hydrolyze ACh and BCh neurotransmitters has become thus a treatment option of AD [20]. For this reason, many research groups have conducted investigations of the inhibitory activity for these enzymes involved in AD pathogenesis. AChE catalyzes the hydrolysis of ACh, which has an important role in cognition and memory. Because of the loss of cholinergic neurons in AD patients, ACh depletion observations constitute a strategy in treatment. Drugs such as donepezil, tacrine, galantamine, and rivastigmine are AChE enzyme inhibitors, mainly ACh inhibits hydrolysis and increases the amount of ACh [21]. While this treatment works in about half of the patients for several years, curative therapy continues to be an unachieved goal [21,22]. These drugs interact with the active site of the AChE: tacrine, without altering the structure of the enzyme (being a reversible inhibitor), whereas rivastigmine changes it [23,24]. The carbamoyl group of rivastigmine was found to covalently bound to AChE, with the rest of the drug in the catalytic site and with its phenol functional group exposed to the solvent [24–28].

In terms of their important potencies, especially in pharmaceutic

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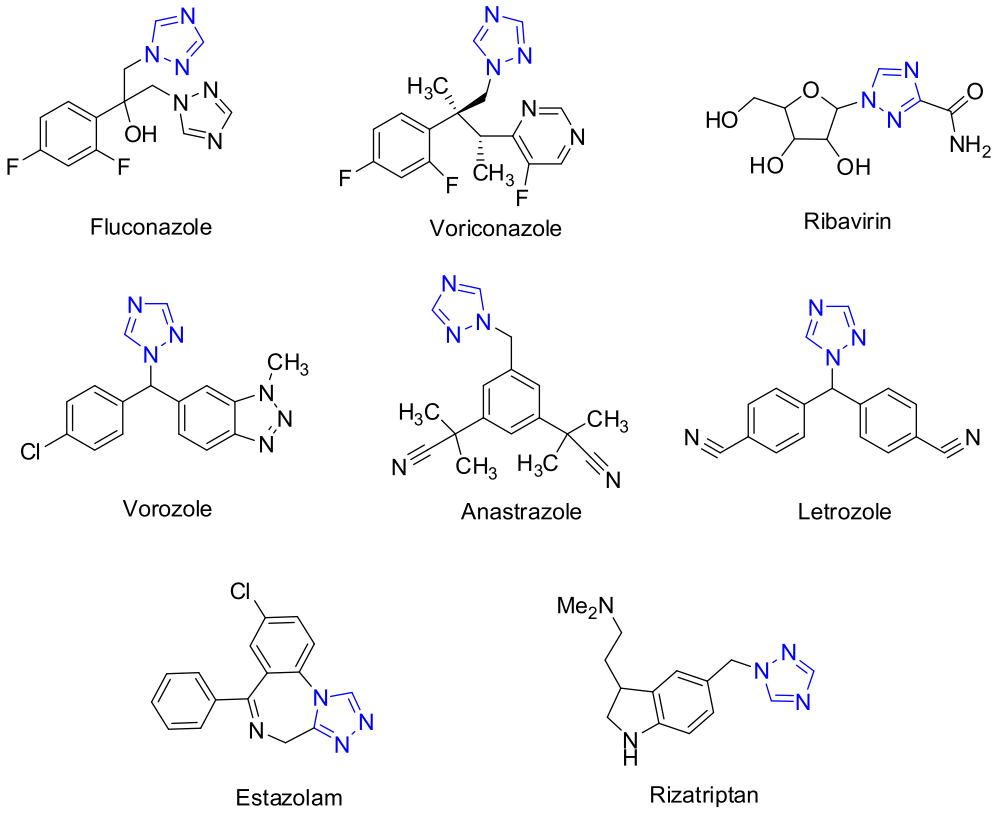


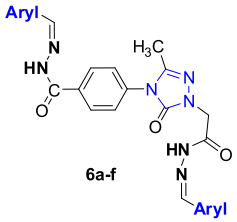
Fig. 1. Drugs with the inclusion of 1,2,4-triazole ring.

treatment as drug candidates, nitrogen-containing heterocycles linked Triazoles are widely synthesized and biologically tested [29–33]. 1,2,4-triazole rings are pharmaceutically well-known scaffolds. Some drugs having this ring have anticonvulsant [34], analgesic [34], antibacterial [35,36], anti-inflammatory [37], anticancer [38–40], antitumor [41], antifungal [42,43], antimicrobial [44,45], and antitubercular [46], effects. Known 1,2,4-triazole containing drug examples are shown in Fig. 1. It is understood that this heterocyclic system behaves as an attractive scaffold and promote chemical diversity for aimed purposes in medicinal chemistry.

In this researches in which organic syntheses are widely used, it is mostly preferred microwave irradiation technique as a more useful method in the recent years [47,48] because of their higher yields, easier work-up, better reaction rate, and so on. According to the eco-friendly approach, this method has unique benefits [49–51].

Within the scope of this study, we introduce a synthesis of triazole cycle, contained with congeneric groups, such as esters, hydrazides and Schiff base at N-2 and N-4 nitrogen atoms (see Fig. 2). The yields and reaction times of all reactions were compared between microwave and conventional methods.

To discover some novel CA, AChE and BChE inhibitors, new types of Schiff base derivatives were synthesized and evaluated in this study.



Comp.	Aryl	Comp.	Aryl
6a	-C_6H_5	6d	-C_6H_4(m-Br)
6b	-C_6H_4(p-Cl)	6e	-C_6H_4(p-OCH_3)
6c	-C_6H_4(p-Br)	6f	-C_6H_3(2-OH, 4-Br)

Fig. 2. Tested compounds' molecular structures.

2. Experimental and methods

2.1. Chemistry

2.1.1. General information

For melting point detection, it was used as a Büchi electric melting point appliance. The infrared spectra were recorded on a Spectrum 100 FTIR Spectrometer (Perkin Elmer) using potassium bromide pellets. The ^{1}H and ^{13}C NMR spectra were run at 400 and 100 MHz, respectively on an Agilent Premium spectrometer in deuterated DMSO with TMS as interior standard. The elemental microanalyses (C, H, and N) were executed on a Carlo-Erba 1106 CHN analyzer. The electrospray ionization mass spectra was verified on using Thermo Scientific Quantum (TSQ) mass spectrometer. Microwave-assisted reactions were executed in a monomode CEM-Discover device using at 300 W maximum power. The vial was chilled to 60 °C *via* air-jet cooling, after the completion of the reaction.

2.1.2. Synthesis of compound *4*

The corresponding compound **3** (2.47 g, 10 mmol) and an equivalent amount of sodium (0.23 g, 10 mmol) were mixed in absolute ethanol (15 mL). Then, the mixture was irradiated with microwaves at 75 °C for 3 min. After the acidic proton is released, 1.66 mL bromoacetic acid ethyl ester (15 mmol) was added to the mixture and irradiated for an additional 3 min at 75 °C. After the reaction was over (TLC, eluent Hexane - AcOEt, 1:3), the solvent was evaporated under vacuum. Finally, to obtain the solid product as pure, it was recrystallized from water. Yield 2.65 g (80%), mp 98–100 °C (lit. 98–100) [49].

2.1.3. Synthesis of compound *5*

Compound **4** (1.66 g, 5 mmol) and excess $NH_2NH_2{\cdot}H_2O$ (100% concentration) (9.52 mL, 200 mmol) were dissolved in absolute EtOH (10 mL) and the mixture was irradiated with microwaves at 60 °C for 15 min. When the reaction was ended (TLC, MeOH) the reaction

content was cooled to room temperature (RT). The separated solid was filtered and recrystallized twice from EtOH. Yield (1.38 g, 91%), mp 215–217 °C (lit. 215–217) [49].

2.1.4. General process for the preparation of Schiff base derivatives ***6a-f***

Conventional Method: The equimolar quantities of compound **5** (1 mmol) and the corresponding arom. aldehyde compound (1 mmol) were dissolved in 30 mL EtOH containing glacial acetic acid in catalytic amount. The reaction mixture was refluxed for 12 h. After the finalization of the reaction (by monitoring with TLC, ethylacetate: Hexane, 3:1), the reaction content was cooled to RT until a solid appeared. The resulting solid was recrystallized two times from EtOH to afford compounds **6a-f**.

Microwave Method: The mixture of compound **5** (1 mmol) and the corresponding arom. aldehyde (1 mmol) in EtOH (10 mL) containing catalytic amount of glacial acetic acid was taken in a microwave vessel. After the microwave irradiation of the mixture in the closed vessels with pressure control at 120 °C and 300 W for 5 min (hold time), the final procedures (work-up and purification) were executed as explained overhead.

4-(1-{2-[2-Benzylidenehydrazino]-2-oxoethyl}-3-methyl-5-oxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)-N'-[phenylmethylene]benzohydrazide **(6a):** Yield: 0.37 g, (76%) (for conv. method), 0.41 g, (86%), (for mw method), mp 256–258 °C; FTIR (KBr): 3236 (NH), 3059 (Ar−CH), 2942 (Alip-CH), 1715 (C=$O_{triazol}$), 1682, 1656 (C=$O_{hydrazide}$), 1592 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.18 (s, 3H, CH_3), 4.92 and 4.50 (s, 2H, CH_2, *cis* and *trans* conformers, *cis/trans* ratio 71/29), 7.42–7.73 (m, 12H, Ar−H), 8.01 (s, 1H, NCH), 8.06 (d, *J* = 8 Hz, 2H, Ar−H), 8.46 and 8.24 (s, 1H, N=CH, *E/Z* geometrical isomers, *E/Z* ratio 72/28), 11.71, and 11.67 (s, 1H, NH, *cis* and *trans* conformers, *cis/trans* ratio 70/30), 11.97 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.8 (CH_3), 46.7, and 47.4 (CH_2, *cis/trans*), 127.1, 127.2, 127.4, 127.6 129.2, 129.3, 130.5, 130.6, 133.7, 134.3, 134.7, 136.2 (Ar.C), 142.9 (C=N), 144.7, 148.0, and 148.6 (N=CH, *E/Z*), 153.4, 162.8, and 163.5 (C=O, *cis/trans*), 168.4 (C=O). Calcd for $C_{26}H_{23}N_7O_3$ %: C 64.85, H 4.81, N 20.36; found %: C 64.83, H 4.80, N 20.37; Mass spectrum, *m/z* (I_{rel}, %): 482.1 $[M + H]^+$ (100).

4-(1-{2-[2-(4-Chlorobenzylidene)hydrazino]-2-oxoethyl}-3-methyl-5-oxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)-N'-[(4-chlorophenyl)methylene] benzohydrazide **(6b):** Yield: 0.41 g, (74%) (for conv. method), 0.49 g, (89%), (for mw method), mp 268–270 °C; FTIR (KBr): 3192 (NH), 3060 (Ar−CH), 2941 (Alip-CH), 1723 (C=$O_{triazol}$), 1675, 1651 (C=$O_{hydrazide}$), 1589 (C=N), 829 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.18 (s, 3H, CH_3), 4.92 and 4.51 (s, 2H, CH_2, *cis* and *trans* conformers, *cis/trans* ratio 70/30), 7.47–7.77 (m, 10*H*, Ar−H), 8.00 (s, 1H, NCH), 8.05 (d, *J* = 8 Hz, 2H, Ar−H), 8.44 and 8.23 (s, 1H, N=CH, *E/Z* geometrical isomers, *E/Z* ratio 72/28), 11.76, and 11.74 (s, 1H, NH, *cis* and *trans* conformers, *cis/trans* ratio 68/32), 12.03 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.8 (CH_3), 46.7, and 47.3 (CH_2, *cis/trans*), 127.1, 127.2, 128.2, 129.1, 129.2, 129.3, 129.4, 133.3, 133.6, 134.9, 135.1, 136.2 (Ar.C), 143.0 (C=N), 143.4, 146.7, and 147.2 (N=CH, *E/Z*), 153.4, 162.8, and 163.6 (C=O, *cis/trans*), 168.5 (C=O). Calcd for $C_{26}H_{21}Cl_2N_7O_3$ %: C 56.74, H 3.85, N 17.81; found %: C 56.72, H 3.85, N 17.79; Mass spectrum, *m/z* (I_{rel}, %): 550.2 $[M+H]^+$ (100).

4-(1-{2-[2-(4-Bromobenzylidene)hydrazino]-2-oxoethyl}-3-methyl-5-oxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)-N'-[(4-bromophenyl)methylene] benzohydrazide **(6c):** Yield: 0.53 g, (83%) (for conv. method), 0.60 g, (94%), (for mw method), mp 253–255 °C; FTIR (KBr): 3214 (NH), 3066 (Ar−CH), 2988 (Alip-CH), 1717 (C=$O_{triazol}$), 1680, 1662 (C=$O_{hydrazide}$), 1591 (C=N), 685 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.18 (s, 3H, CH_3), 4.88 and 4.62 (s, 2H, CH_2, *cis* and *trans* conformers, *cis/trans* ratio 74/26), 7.61–7.70 (m, 10*H*, Ar−H), 7.98 (s, 1H, NCH), 8.05 (d, *J* = 8 Hz, 2H, Ar−H), 8.42 and 8.21 (s, 1H, N=CH, *E/Z* geometrical isomers, *E/Z* ratio 73/27), 11.76, and 11.74 (s, 1H, NH, *cis* and *trans* conformers, *cis/trans* ratio 70/30), 12.03 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.9 (CH_3), 46.4, and 47.0 (CH_2, *cis/trans*), 127.0, 127.4, 127.9, 128.7, 129.1, 129.4, 130.0, 133.1, 133.8, 134.6, 135.2, 137.4 (Ar.C), 143.6 (C=N), 144.4, 146.2, and 147.0 (N=CH, *E/Z*), 152.9, 163.1, and 163.4 (C=O, *cis/trans*), 167.9 (C=O). Calcd for $C_{26}H_{21}Br_2N_7O_3$ %: C 48.85, H 3.31, N 25.00; found %: C 48.82, H 3.31, N 25.02; Mass spectrum, *m/z* (I_{rel}, %): 638.2, $[M+H]^+$ (61), 640.1 $[M+H+2]^+$ (100), 642.3 $[M+H+4]^+$ (63).

4-(1-{2-[2-(3-Bromobenzylidene)hydrazino]-2-oxoethyl}-3-methyl-5-oxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)-N'-[(3-bromophenyl)methylene] benzohydrazide **(6d):** Yield: 0.48 g, (76%) (for conv. method), 0.56 g, (88%), (for mw method), mp 222–224 °C; FTIR (KBr): 3212 (NH), 3066 (Ar−CH), 2984 (Alip-CH), 1716 (C=$O_{triazol}$), 1681, 1668 (C=$O_{hydrazide}$), 1584 (C=N), 689 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.17 (s, 3H, CH_3), 4.84 and 4.60 (s, 2H, CH_2, *cis* and *trans* conformers, *cis/trans* ratio 69/31), 7.54–7.72 (m, 10*H*, Ar−H), 7.92 (s, 1H, NCH), 8.02 (d, *J* = 8 Hz, 2H, Ar−H), 8.39 and 8.19 (s, 1H, N=CH, *E/Z* geometrical isomers, *E/Z* ratio 70/30), 11.74, and 11.70 (s, 1H, NH, *cis* and *trans* conformers, *cis/trans* ratio 73/27), 11.97 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.8 (CH_3), 47.3, and 47.9 (CH_2, *cis/trans*), 126.8, 127.3, 127.7, 128.6, 129.2, 129.7, 130.1, 130.9, 131.6, 132.4, 133.1, 133.7, 134.5, 135.7, 137.0, 137.8 (Ar.C), 143.5 (C=N), 144.6, 145.9, and 146.9 (N=CH, *E/Z*), 153.1, 162.8, and 163.3 (C=O, *cis/trans*), 167.4 (C=O). Calcd for $C_{26}H_{21}Br_2N_7O_3$ %: C 48.85, H 3.31, N 25.00; found %: C 48.84, H 3.30, N 25.01; Mass spectrum, *m/z* (I_{rel}, %): 638.4, $[M+H]^+$ (65), 640.3 $[M+H+2]^+$ (100), 642.1 $[M+H+4]^+$ (71).

4-(1-{2-[2-(4-Methoxybenzylidene)hydrazino]-2-oxoethyl}-3-methyl-5-oxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)-N'-[(4-methoxyphenyl)methylene]benzohydrazide **(6e):** Yield: 0.42 g, (77%) (for conv. method), 0.49 g, (91%), (for mw method), mp 244–246 °C; FTIR (KBr): 3213 (NH), 3018 (Ar−CH), 2981 (Alip-CH), 1708 (C=$O_{triazol}$), 1683, 1661 (C=$O_{hydrazide}$), 1588 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.19 (s, 3H, CH_3), 3.92 (s, 3H, CH_3), 3.94 (s, 3H, CH_3), 4.82 and 4.60 (s, 2H, CH_2, *cis* and *trans* conformers, *cis/trans* ratio 71/29), 6.74–7.82 (m, 12H, Ar−H), 8.01 (s, 1H, N=CH), 8.50 and 8.31 (s, 1H, N=CH, *E/Z* geometrical isomers, *E/Z* ratio 72/28), 11.54, and 11.58 (s, 1H, NH, *cis* and *trans* conformers, *cis/trans* ratio 70/30), 12.05 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.8 (CH_3), 45.6, and 47.4 (CH_2, *cis/trans*), 49.4, 49.6 (CH_3), 127.0, 127.5, 128.2, 128.7, 129.4, 129.9, 131.0, 133.2, 134.1, 134.8, 143.2, 143.6 (Ar.C), 144.5 (C=N), 145.1, 145.8, and 146.2 (N=CH, *E/Z*), 153.1, 162.8, and 163.2 (C=O, *cis/trans*), 168.2 (C=O). Calcd for $C_{28}H_{27}N_7O_5$ %: C 62.10, H 5.03, N 18.10; found %: C 62.06, H 5.02, N 18.10; Mass spectrum, *m/z* (I_{rel}, %): 542.3 $[M+H]^+$ (73), 564.0 $[M + Na]^+$(100) [52].

4-(1-{2-[2-(4-Bromo-2-hydroxybenzylidene)hydrazino]-2-oxoethyl}-3-methyl-5-oxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)-N'-[(4-bromo-2-ydroxyphenyl)methylene]benzohydrazide **(6f):** Yield: 0.47 g, (70%) (for conv. method), 0.58 g, (86%), (for mw method), mp 233–235 °C; FTIR (KBr): 3382 (OH), 3189 (NH), 3053 (Ar−CH), 2976 (Alip-CH), 1722 (C=$O_{triazol}$), 1672, 1668 (C=$O_{hydrazide}$), 1592 (C=N) 735 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.14 (s, 3H, CH_3), 4.82 and 4.73 (s, 2H, CH_2, *cis* and *trans* conformers, *cis/trans* ratio 71/29), 7.12–7.79 (m, 10*H*, Ar−H), 7.97 (s, 1H, N=CH), 8.21 and 8.10 (s, 1H, N=CH, *E/Z* geometrical isomers, *E/Z* ratio 73/27), 9.54 (s, 1H, OH), 9.56 (s, 1H, OH), 11.70, and 11.63 (s, 1H, NH, *cis* and *trans* conformers, *cis/trans* ratio 69/31), 11.94 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.7 (CH_3), 47.0, and 47.6 (CH_2, *cis/trans*), 113.4, 114.1, 120.5, 121.3, 123.7, 125.6, 126.2, 126.9, 127.1, 128.0, 131.8, 133.4, 134.6, 135.5, 149.6, 150.3 (Ar.C), 144.5 (C=N), 145.2, 146.4, and 146.9 (N=CH, *E/Z*), 154.1, 163.8, and 164.7 (C=O, *cis/trans*), 167.8 (C=O). Calcd for $C_{26}H_{21}Br_2N_7O_5$ %: C 46.52, H 3.15, N 14.61; found %: C 46.53, H 3.15, N 14.60; Mass spectrum, *m/z* (I_{rel}, %): 670.1, $[M+H]^+$ (65), 672.1 $[M+H+2]^+$ (100), 674.2 $[M+H+4]^+$ (67) [52].

2.2. Purification of hCA isozymes from erythrocytes by affinity chromatography

hCA I and II isoenzymes were purified from fresh human erythrocytes as described in the literature [6–8].

2.3. Esterase activity assay

hCA I and II isoenzyme activity assays were carried out according to the Verpoorte method [53]. In this part of the study, the inhibitory effects of **6a-f** derivatives were tested. These derivatives were examined in triplicate at each concentration used. Different concentrations were used for all inhibitors. In the absence of inhibitor, the control cuvette activity was accepted as 100%. Activity (%) - [Inhibitor] graphs were plotted for each inhibitor.

2.4. AChE and BChE activity

Inhibitory activities of the compounds on AChE and BuChE enzymes were determined by using the Ellman method [54]. Neostigmine was used as the reference drug in this study. The IC_{50} values obtained for compounds **6a-f** are summarized in Table 1.

The stock solutions of all the materials tested were prepared by dissolving in 1 mg/mL of dimethylsulfoxide. It was then diluted to different concentrations using deionized water. Six serial dilutions of these substances were measured to determine the inhibitory activity of AChE and BChE enzymes [55,56].

3. Results

3.1. Chemistry

The synthetic route was started with producing compound 1. In the first step, to obtain compound **1**, the acetonitrile with EtOH and $HCl_{(g)}$ were reacted according to the Pinner method [57]. Later, the ethyl 2-(1-ethoxyethylidene)hydrazine carboxylate (**2**) was obtained from compound **1** and the ethyl carbazate at 0–5 °C [58]. Compound **2** reacted with 4-Aminobenzoic acid ethyl ester under solvent-free condition using with the microwave technique to produce ethyl 4-(3-methyl-5 oxo-1,5-dihydro-4*H*-1,2,4-triazole-4-yl)benzoate (**3**). In the next step, compound **3** was reacted with the Bromoacetic acid ethyl ester under both the conventional and microwave irradiation methods to obtained ethyl-4-[1-(2-ethoxy-2-oxoethyl)-3-methyl-5-oxo-1,5-dihydro-4H-1,2,4-triazol-4-yl]benzoate (**4**). To achieve aimed synchronous modification, compound **5,** which has two identical functional groups, was obtained from compound **4** with excess hydrazine hydrate as described in the literature [47].

Hydrazide compounds can be considered as a versatile group leading to the formation of different heterocycle systems such as 1,3,4-oxadiazole, 1,2,4-triazole, and 1,3,4-thiadiazole derivatives [49]. On the other hand, these compounds are very ambitious derivatives to reacted with aldehydes to obtained Schiff bases. In our earlier studies, Schiff bases derivatives which containing with 1,2,4-triazole-5-one ring system were prepared from aromatic amines and different aldehydes [35,58]. However, in this study to obtain the biologically active target compounds 4-[1-(2-{2-[4-(substitute)benzylidene] hydrazino}-2-oxoethyl)-3-methyl-5-oxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl]-N'-{[4-(substitute) phenyl]methylene}benzohydrazide (**6a-f**), compound **5** (hydrazide derivative) was performed with diverse aromatic aldehydes in concur with acetic acid as a catalyst. These reactions were in the moderate yield (70–83%) using conventional heating, and in high yield (86–94%) by means of the microwave as an energy source (Scheme 1). The reaction times also were shortened from 12 h to 5 min by using the microwave technique. When compared with the conventional (thermal) heating method, microwave irradiation heating put forwards more advantages such as simplicity in processing, low cost, reduced pollution, reduced reaction time, and high yield.

Table 1
hCA I, II, AChE and BChE inhibition data some compounds.

Inhibitor	IC_{50} (μM)			
	hCA I	hCA II	AChE	BChE
6a	0.1110	0.0780	0.0465	0.0702
6b	0.0769	0.0762	0.0574	0.0557
6c	0.1361	0.1243	0.0877	0.1253
6d	0.1401	0.1170	0.0966	0.0937
6e	0.0538	0.0514	0.0623	–
6f	0.0774	0.0717	0.0577	0.0486
AZA[a]	0.9857	0.4894	–	–
Neostigmine	–	–	0.136	0.084

Mean from at least three determinations. Errors in the range of ± 3% of the reported value (data not shown).
[a] From Ref. [67].

3.2. Biological studies

This study reported the synthesis of Schiff base derivatives (**6a-f**) and also determined the inhibitory effects of the compounds **6a-f** on human CA I and II isoenzymes and AChE and BChE enzymes.

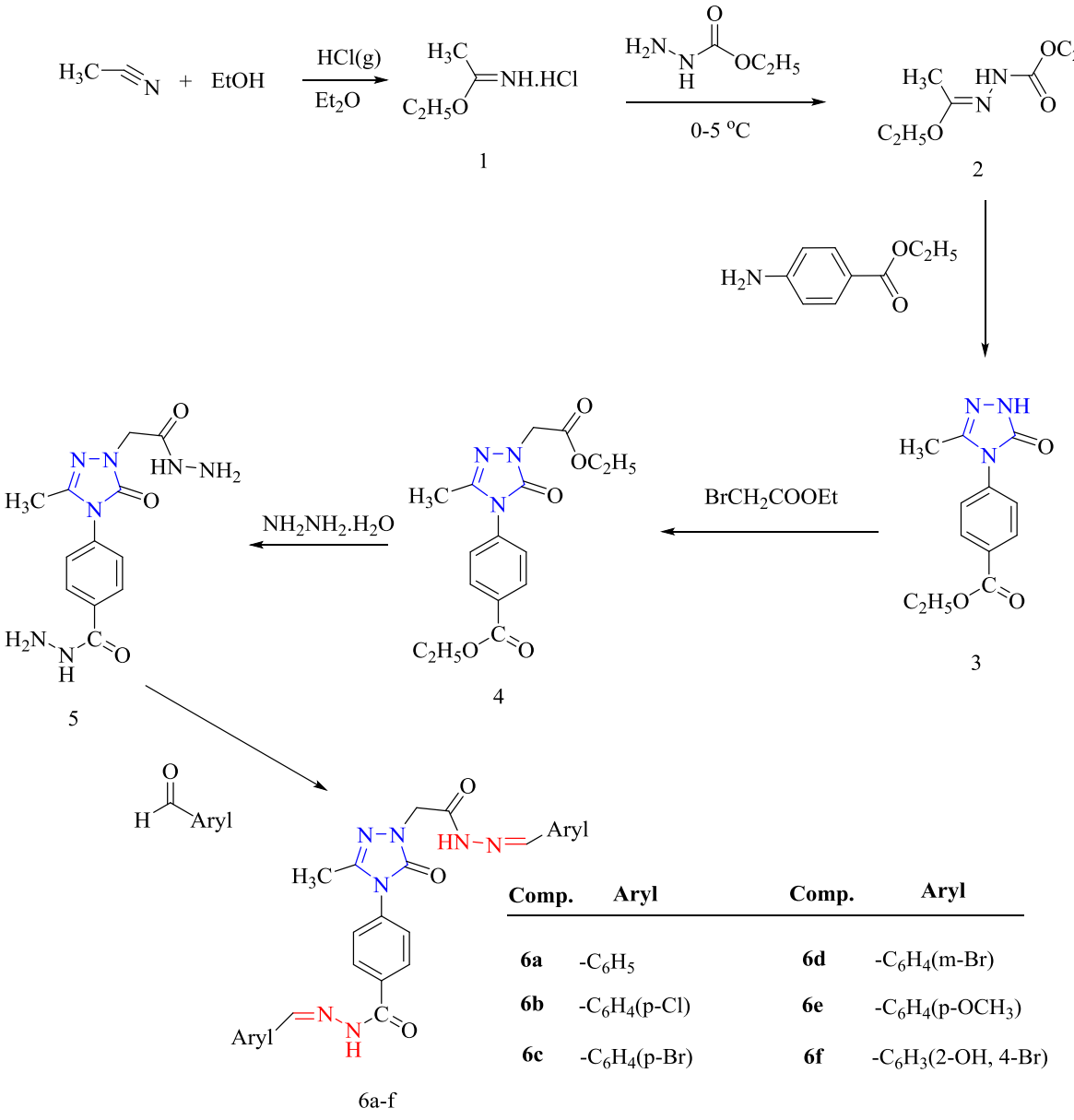
AChE and BChE enzymes are known to have very important roles in memory and cognition. These cholinergic enzymes catalyze the hydrolysis of ACh, which leads to a decrease in neural communication between nerve cells. This leads to the function of the brain and finally to the AD. Therefore, the treatment of AD depends to some extent on the rebalancing of the level of ACh [25–28]. In many pharmaceutical studies, cholinesterase inhibitors (ChEIs) have been targeted to treat cognitive disorders. Because of its disadvantages such as AD drugs, bioavailability, and gastrointestinal disorders, new ChEIs are being investigated continuously. This study thus screened derivatives **6a-f** for their inhibitory activity.

Here, we first report the inhibition effects of the newly synthesized 6a-f derivatives using the esterase activity of human CA I and CA II isoenzymes. In Table 1, the inhibition values determined by the esterase activity of 6a-f compounds for hCA I and hCA II isoenzymes are shown below [53], with 4-NPA (4-nitrophenylacetate):

(i) Against the hCA I isozyme, the compounds **6a-f** behaved as good inhibitors, with the values of IC_{50} in the range of 0.0538–0.1401 μM, similar to structurally related compounds *N*-benzyl-2-(2-hydroxybenzoyl) hydrazine carbothioamide (IC_{50} of 0.834 μM). In this study, compounds 6b, 6e, and 6f show much better hCA I inhibition effect than 6a, 6c, and 6d. For compounds 6a-f, it was found that many previous kinetic studies showed similar results to phenolic compounds, sulphonamide derivatives, uracil derivatives, natural products, and anions [6–17,59–63].

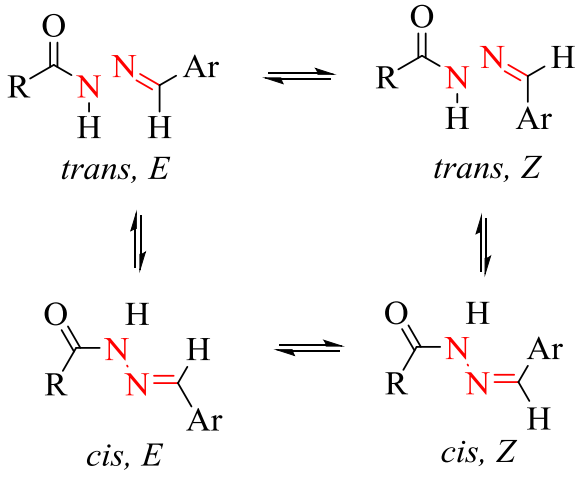
(ii) In this study, it was determined that **6a-f** derivatives showed better inhibitory activity for h CA II isoenzyme compared to hCA I isoenzyme (Table 1). If we analyze the structure-activity relationship for **6a-f** compounds, The compounds **6c** and **6d** which containing Br are lower effective than other compounds. The best hCA II inhibitor in the **6a-f** derivatives is the **6e** containing the methoxy group in the para position in the structure, the IC_{50} of this substance being determined as 0.0514 μM. For hCA II isoenzyme, 6a, 6b, and 6f yielded very close to each other in the range 0.0717 to 0.780 μM. As human CA I and II isoenzymes exhibit poor esterase activity, IC_{50} values are mostly in micromolar range when measured according to the esterase method for these enzymes [63].

(iii) Derivative **6d** (IC_{50} = 0.0966 μM) showed the weakest inhibitory against AChE. Decreasing one para position chloride group on the aromatic ring showed an improvement of the IC_{50} values obtained, **6a** (0.0465 μM) with p-Cl group demonstrated a 1.23 fold decrease of inhibition activity while **6b** (0.0574 μM) with para position bromide



Comp.	Aryl	Comp.	Aryl
6a	-C_6H_5	**6d**	-C_6H_4(m-Br)
6b	-C_6H_4(p-Cl)	**6e**	-C_6H_4(p-OCH_3)
6c	-C_6H_4(p-Br)	**6f**	-C_6H_3(2-OH, 4-Br)

Scheme 1. The synthetic route for the 1,2,4-triazole derivatives.



Scheme 2. *cis/trans* isomers and *E/Z* geometrical isomers of compound **6a-f**.

group **6c** showed a 1.89 fold decrease of inhibition activity compared to compound **6a**. However, compound **6a** (0.0465 μM) possessing only the benzene group showed better inhibitory activity compared to the other five molecules. The difference between the other tested compounds and **6a** is that this molecule is a more voluminous derivative. The fact that only the benzene group containing derivative **6a** has a stronger inhibitory capacity as a functional group may indicate that its geometry interacts with the enzyme active site is more potent. According to these results obtained for AChE enzyme and **6a-f** derivatives, it can be indicated that the electronegative groups in the molecule have a great effect on the inhibition value according to the position. Larger research will be conducted to explain this situation. To find out the effect of the benzene group on the inhibitory activity as a functional group, five other compounds (**6b-f**) were compared with compound **6a**. Adding a 2-hydroxyl and 4 bromide group (**6f**) to just benzene group-containing compound (**6a**) the inhibitory activity decreased 1.24 fold, it only suggests that the benzene group had a significant inhibitory effect on AChE enzyme activity. In the structure, a chlorine-containing compound **6b** (0.0574 μM) in the para position appears to have an inhibitor value of 1.23 times less than the **6a** molecule. In addition, in our study,

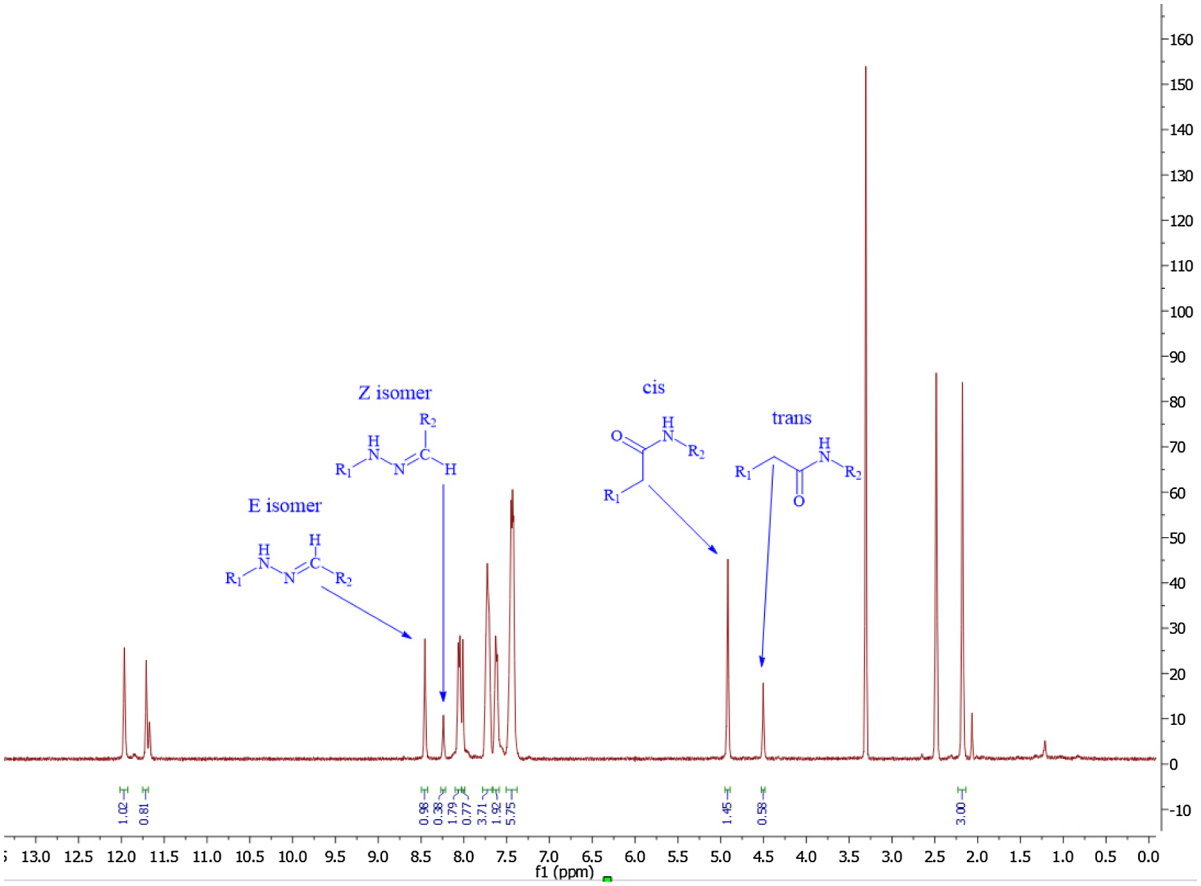


Fig. 3. ^{1}H NMR Spectrum of compound **6a.**

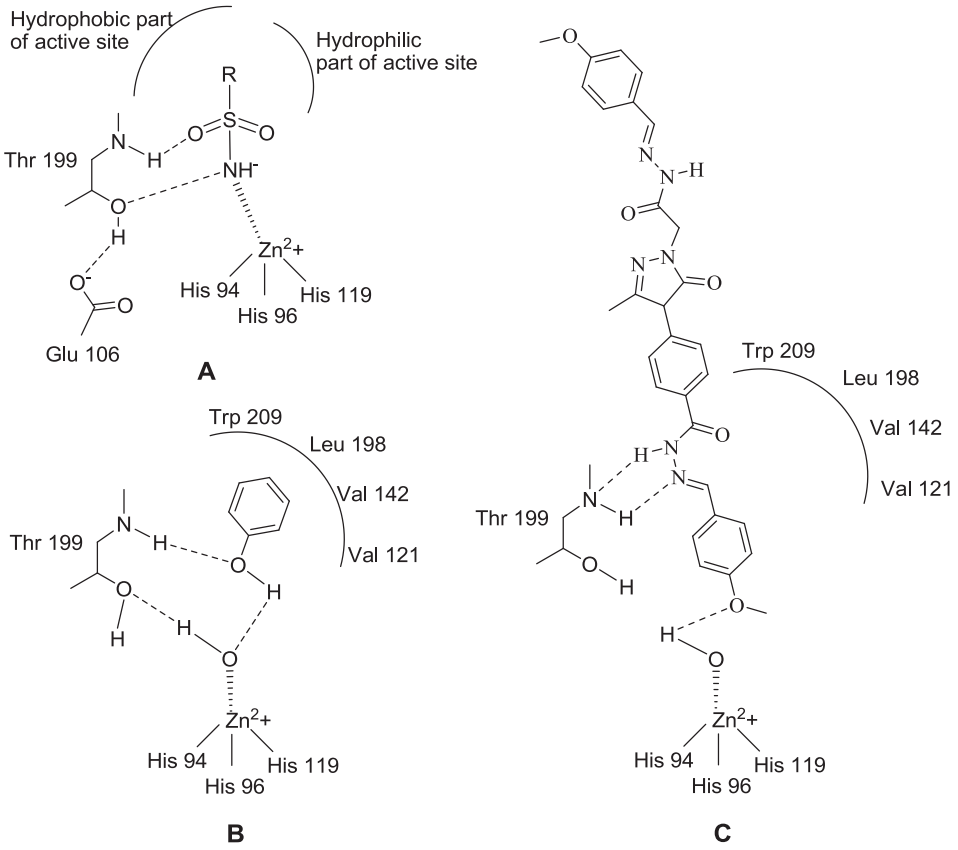


Fig. 4. CA inhibition with zinc binders such as sulphonamides (A) and phenol (B) and respected compound **6e** (C). Figures represent distances (in Å), as determined by X-ray crystallographic techniques [12,63]. Hydrogen bonds are represented as dashed lines. All these binding modes have been proven by means of X-ray crystallography on enzyme-inhibitor adducts [63].

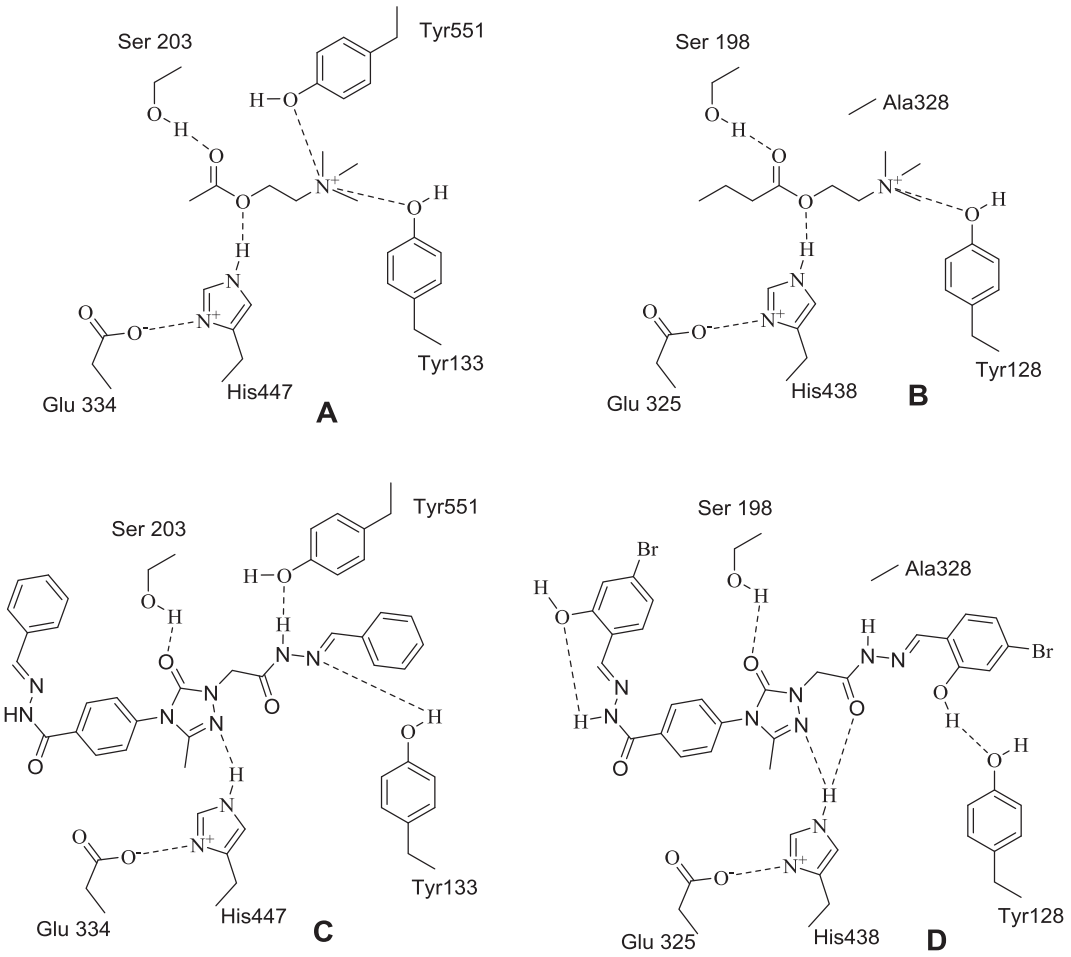


Fig. 5. A: the binding of acetylcholine with the active region of the hAChE enzyme (pdb: 4ey4) [19], B: the binding pattern of butyrylcholine with the active site of the hBChE enzyme (pdb: 1p0i) [19], C: the estimated AChE binding form of **6a**, D: the predicted BChE binding pattern of **6f**.

the values (0.0465–0.0966 μM) obtained for AChE enzyme are better when they are shortened by the reference molecule, neostigmine (0.136 μM).

(iv) Derivative **6c** (IC_{50} = 0.1253 μM) showed the weakest inhibitory effect against the BChE enzyme. Decreasing one para position bromide group on the aromatic ring showed an improvement of the IC_{50} values obtained, **6f** (0.0486 μM) with 2-OH and 4-Br groups demonstrated a 2.58 fold decrease of inhibition activity while **6c** (0.1253 μM) with para position bromide group **6c** showed a 1.93 fold decrease of inhibition activity compared to compound **6f**. However, compound **6f** (0.0486 μM) possessing 2 hydroxy and 4 bromide group showed better inhibitory activity compared to the other five molecules. The difference between the other tested compounds and **6f** is that this molecule is a more voluminous derivative. The fact that 2 hydroxy and 4 bromide groups containing derivative **6f** has a stronger inhibitory capacity as a functional group may indicate that its geometry interacts with the enzyme active site is more potent. According to these results obtained for BChE enzyme and **6a-f** derivatives, it can be indicated that the electronegative groups in the molecule have a great effect on the inhibition value according to the position. Larger research will be conducted to explain this situation. To find out the effect of the benzene group on the inhibitory activity as a functional group, five other compounds (**6a-e**) were compared with compound **6f**. In the structure, a chlorine-containing compound **6b** (0.0557 μM) in the para position appears to have an inhibitor value of 1.15 times less than the **6f** molecule. In addition, in our study, it was observed that the IC_{50} values (0.0486–0.1253 μM) obtained for BChE enzyme were close or better when they were shortened by neostigmine (0.0840 μM) as the reference molecule.

In many recent studies, it has been found that simple phenolic compounds show good inhibition as sulfonamides, which are potent CA inhibitors [16]. This may provide a new class of inhibitors that is advantageous for the treatment of patients with sulfonamide allergy [13–17]. Sulphonamides are the best known CA inhibitors, but many studies in recent years indicate that molecules containing different functional groups (especially those containing electronegative atoms) may exhibit as good inhibition as sulfonamides [6–15,59–64]. In addition, it is critical to investigate more potent CAI classes to identify substances that may exhibit a different inhibition profile as compared to sulfonamides and to find different applications for inhibitors of CA isoenzymes.

4. Discussion

The molecular structures of newly obtained compounds were demonstrated in details on the strength of different spectral analyses and all data are compatible with our proposed structures. According to the FTIR results for all compounds, it is seen a C=N signal at about 1588 cm^{-1} and C=O signals at about 1685 cm^{-1} for hydrazide group and at about 1715 cm^{-1} for triazole ring. On the other hand, the compounds **6a-f** had no $-NH_2$ signal because of the fact that the imine constitution and new C=O signals were a shift from 1657 cm^{-1} to 1685 cm^{-1}.

In the ^1H NMR spectral data, N–H signal has been shown at about 12.03 ppm and 11.74 ppm for compounds **6a-f**. When ^1H NMR data of **6a-f** have been checked, it was seen that these compounds' some protons have 2 sets of the signal at the different ppm. Arylidene-hydrazide

structure in the compounds which exist as *E/Z* geometrical isomer from C=N double bond and *cis/trans* amide conformers at the CO−NH single bond. As seen in the essay [65,66] the compounds which have C=N double bond would rather *E* geometrical isomer in DMSO-d_6 and *Z* isomers can be preferred in less fewer solvents by an intramolecular hydrogen bond. All ^{1}H NMR spectra the −NH, −N=CH, and N−CH_2 groups showed 2 sets of signals because of *cis/trans* conformers. The −NH signal was observed as 2 sets due to trans/cis amide conformers at about 12.03 ppm and about 11.74 ppm for compounds **6a-f**. On the other hand, 2 sets of signals at about 8.42 and about 8.20 ppm were interested into the −N=CH bond of the *E* and *Z* geometrical isomers, respectively. As seen in the essay, the downfield lines of the imine proton were attributed to the E isomer of the amide structure, while the upfield lines were assigned to the *Z* isomer of compounds **6a-f**. Thirdly, the N−CH_2 signal was monitored again as 2 sets as a result of *trans/cis* amide conformers at about 4.55 ppm and at about 4.88 ppm for compounds **6a-f**. The proportion has been calculated by using ^{1}H NMR and ^{13}C NMR data in each case. *E/Z* geometrical isomer and cis/trans conformer of compounds **6a-f** and selected ^{1}H NMR spectrum of compound **6a** were given in Scheme 2 and Fig. 3, respectively. Furthermore, compounds **6a-f** gave elemental analysis data and mass spectra coherent with the appointed structures.

The **6a-f** compounds used in this study showed different inhibition effects for hCA I and hCA II isoenzymes due to the presence of different functional groups (Cl, Br, OH, OCH_3, and Benzyl) found in their molecular structures. The results obtained in this study show that, in addition to the sulfamates/sulfonamides known as potent inhibitors in CA inhibition, there are possible new classes of CAIs. In fact, these synthesized compounds were determined using the esterase method in which they were highly effective hCAI and hCA II inhibitors at low micromolar concentrations. The data obtained in this study show that these synthesized compounds can potentially be used to produce strong inhibitors that target AChE and BChE enzymes, as well as other CA isoforms that have not yet been tested (in Figs. 4 and 5).

Considering all these remarkable results, the findings may the findings may lead to interesting interpretations and may help to develop new and effective drugs to slow down or stop AD. In this study, in addition to comparing these synthesized molecules with other compounds, it will further expand targets for determining the structure-activity relationship of **6a-f** derivatives. These newly synthesized compounds can also be used as drug precursors or building blocks in the preparation of more effective drug molecules.

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Conflict of interest

The authors declare no conflict of interest.

References

[1] C.T. Supuran, Nat. Rev. Drug Discov. 7 (2008) 168–181.
[2] M. Hilvo, L. Baranauskiene, A.M. Salzano, A. Scaloni, D. Matulis, A. Innocenti, A. Scozzafava, S.M. Monti, A. Di Fiore, G. De Simone, J. Biol. Chem. (2008).
[3] C.T. Supuran, A. Scozzafava, Bioorg. Med. Chem. 15 (2007) 4336–4350.
[4] C.T. Supuran, A. Scozzafava, Expert Opin. Ther. Pat. 12 (2002) 217–242.
[5] J.R. Casey, Biochem. Cell Biol. 84 (2006) 930–939.
[6] S. Isik, D. Vullo, S. Durdagi, D. Ekinci, M. Senturk, A. Cetin, E. Senturk, C.T. Supuran, Bioorg. Med. Chem. Lett. 25 (2015) 5636–5641.
[7] R. Yaseen, D. Ekinci, M. Senturk, A.D. Hameed, S. Ovais, P. Rathore, M. Samim, K. Javed, C.T. Supuran, Bioorg. Med. Chem. Lett. 26 (2016) 1337–1341.
[8] T. Arslan, Z. Biyiklioglu, M. Senturk, RSC Adv. 8 (2018) 10172–10178.
[9] H.T. Balaydin, M. Senturk, A. Menzek, Bioorg. Med. Chem. Lett. 22 (2012) 1352–1357.
[10] H.T. Balaydin, S. Durdagi, D. Ekinci, M. Senturk, S. Goksu, A. Menzek, J. Enzyme Inhib. Med. Chem. 27 (2012) 467–475.
[11] H.T. Balaydın, H. Soyut, D. Ekinci, S. Göksu, Ş. Beydemir, A. Menzek, E. Şahin, J. Enzyme Inhib. Med. Chem. 27 (2012) 43–50.
[12] S. Durdagi, M. Senturk, D. Ekinci, H.T. Balaydın, S. Goksu, O.I. Kufrevioglu, A. Innocenti, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. 19 (2011) 1381–1389.
[13] E. Bayram, M. Senturk, O.I. Kufrevioglu, C.T. Supuran, Bioorg. Med. Chem. 16 (2008) 9101–9105.
[14] D. Ekinci, M. Senturk, O.I. Kufrevioglu, Expert Opin. Ther. Pat. 21 (2011) 1831–1841.
[15] D. Ekinci, M. Senturk, E. Senturk, Acta Physiol. 215 (2015) 99.
[16] A. Innocenti, D. Vullo, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. Lett. 18 (2008) 1583–1587.
[17] A. Innocenti, M. Hilvo, A. Scozzafava, S. Parkkila, C.T. Supuran, Bioorg. Med. Chem. Lett. 18 (2008) 3593–3596.
[18] H. Ahmad, S. Ahmad, M. Ali, A. Latif, S.A.A. Shah, H. Naz, N. Rahman, F. Shaheen, A. Wadood, H.U. Khan, M. Ahmad, Bioorg. Chem. 78 (2018) 427–435.
[19] J.D. Ulrich, D.M. Holtzman, ACS Chem. Neurosci. 7 (2016) 420–427.
[20] M. Kratki, S. Stepankova, K. Vorcakova, J. Vinsova, Bioorg. Chem. 68 (2016) 23–29.
[21] M.A. Telpoukhovskaia, B.O. Patrick, C. Rodríguez-Rodríguez, C. Orvig, Bioorg. Med. Chem. Lett. 26 (2016) 1624–1628.
[22] L.S. Schneider, Clinics in Geriatric Med. 17 (2001) 337–358.
[23] F. Mangialasche, A. Solomon, B. Winblad, P. Mecocci, M. Kivipelto, Lancet Neurol. 9 (2010) 702–716.
[24] E. Giacobini, Ann N Y Acad. Sci. 920 (2000) 321–327.
[25] R.M. Dawson, Neurosci Lett. 118 (1990) 85–87.
[26] E. Senturk, M. Senturk, Anadolu J. Agr. Sci. 33 (2018) 237–240.
[27] X.H. Gao, C. Zhou, H.R. Liu, L.B. Liu, J.J. Tang, X.H. Xia, J. Enzym Inhib. Med. Chem. 32 (2017) 146–152.
[28] B. Feng, X. Li, J. Xia, S. Wu, J. Enzym. Inhib. Med. Chem. 32 (2017) 968–977.
[29] H.G. Hahn, J.S. Choi, H.K. Lim, K.I. Lee, I.T. Hwang, Pestic. Biochem. Physiol. 125 (2015) 78–83.
[30] Y. Ünver, K. Sancak, F. Çelik, E. Birinci, M. Küçük, S. Soylu, N.A. Burnaz, Eur. J. Med. Chem. 84 (2014) 639–650.
[31] I.A. Al-Masoudi, Y.A. Al-Soud, N.J. Al-Salihi, N.A. Al-Masoudi, Chem. Heterocycl. Compd. 42 (2006) 1377–1403.
[32] A. Rastegari, H. Nadri, M. Mahdavi, A. Moradi, S.S. Mirfazli, N. Edraki, F.H. Moghadam, B. Larijani, T. Akbarzadeh, M. Saeedi, Bioorg. Chem. 83 (2019) 391–401.
[33] S. Akin, H. Ayaloglu, E. Gultekin, A. Colak, O. Bekircan, M. Yildirim Akatin, Bioorg. Chem. 83 (2019) 170–179.
[34] A. Almasirad, S.A. Tabatabai, M. Faizi, A. Kebriaeezadeh, N. Mehrabi, A. Dalvandi, A. Shafiee, Bioorg. Med. Chem. Lett. 14 (2004) 6057–6059.
[35] B. Kahveci, M. Özil, E. Menteşe, O. Bekircan, K. Buruk, Russ. J. Org. Chem. 44 (2008) 1816–1820.
[36] E. Nagaradja, G. Bentabed-Ababsa, M. Scalabrini, F. Chevallier, S. Philippot, S. Fontanay, R.E. Duval, Y.S. Halauko, O.A. Ivashkevich, V.E. Matulis, Bioorg. Med. Chem. 23 (2015) 6355–6363.
[37] R. Paprocka, M. Wiese, A. Eljaszewicz, A. Helmin-Basa, A. Gzella, B. Modzelewska-Banachiewicz, J. Michalkiewicz, Bioorg. Med. Chem. Lett. 25 (2015) 2664–2667.
[38] M. Genc, Z.K. Genc, S. Tekin, S. Sandal, M. Sirajuddin, T. Ben Hadda, M. Sekerci, Acta Chim. Slov. 63 (2016) 726–737.
[39] Y.A. Al-Soud, M.N. Al-Dweri, N.A. Al-Masoudi, Farmaco 59 (2004) 775–783.
[40] N. Kulabaş, E. Tatar, Ö. Bingöl Özakpınar, D. Özsavcı, C. Pannecouque, E. De Clercq, İ. Küçükgüzel, Eur. J. Med. Chem. 121 (2016) 58–70.
[41] F. A. Hassan, K. W. Younus, A. H. AL–Qaisi, Aust. J. Basic Appl. Sci. 7 (2013) 133–140.
[42] W. Zhang, G. Sui, Y. Li, M. Fang, X. Yang, X. Ma, W. Zhou, Chem. Pharm. Bull. (Tokyo). 64 (2016) 616–624.
[43] R.S. Upadhayaya, N. Sinha, S. Jain, N. Kishore, R. Chandra, S.K. Arora, Bioorg. Med. Chem. 12 (2004) 2225–2238.
[44] M. Madhu Sekhar, U. Nagarjuna, V. Padmavathi, A. Padmaja, N.V. Reddy, T. Vijaya, Eur. J. Med. Chem. 145 (2018) 1–10.
[45] N. Gümrükçüoğlu, Karadeniz Fen Bilim. Derg. 6 (2016) 89–98.
[46] K. Walczak, A. Gondela, J. Suwiński, Eur. J. Med. Chem. 39 (2004) 849–853.
[47] N. Kaur, Synth. Commun. 45 (2015) 432–457.
[48] C.O. Kappe, Angew. Chemie-International Ed. 43 (2004) 6250–6284.
[49] M. Özil, O. Bodur, S. Ülker, B. Kahveci, Chem. Heterocycl. Compd. 51 (2015) 88–96.
[50] M. Özil, M. Canpolat, Polyhedron 51 (2013) 82–89.
[51] B. Kahveci, E. Menteşe, M. Özil, S. Ülker, M. Ertürk, Monatshefte fur Chemie 144 (2013) 993–1001.
[52] H.T. Balaydin, M. Ozil, M. Senturk, Arch. Pharm. (Weinheim). 351 (2018) 1800086.
[53] J.A. Verpoorte, S. Mehta, J.T. Edsall, J. Biol. Chem. 242 (1967) 4221–4229.
[54] G.L. Ellman, K.D. Courtney, VJr Andres, R.M. Featherstone, Biochem. Pharmacol. 7 (1961) 88-95.
[55] K. Zilbeyaz, N. Stellenboom, M. Guney, A. Oztekin, M. Senturk, J. Biochem. Mol. Toxicol. (2018) e22210.
[56] K. Cavusoglu, H. Celik, M. Senturk, D. Ekinci, Acta Physiol. 218 (2016) 57.
[57] A. Pinner, Die Imidoether Und Ihre Derivative, Auflage, Berlin, 1982.

[58] B. Kahveci, M. Özil, M. Serdar, Heteroat. Chem. 19 (2008) 38–42.
[59] T. Arslan, G. Çelik, H. Çelik, M. Senturk, N. Yaylı, D. Ekinci, Arch. Pharm. (Weinheim). 349 (2016) 741–748.
[60] D. Ekinci, M. al-Rashida, G. Abbas, M. Senturk, C.T. Supuran, J. Enzyme Inhib. Med. Chem. 27 (2012) 744–747.
[61] M. Guney, H. Cavdar, M. Senturk, D. Ekinci, Bioorg. Med. Chem. Lett. 25 (2015) 3261–3263.
[62] C.T. Supuran, Curr. Pharm. Des. 14 (2008) 641–648.
[63] S.K. Nair, P.A. Ludwig, D.W. Christianson, J. Am. Chem. Soc. 116 (1994) 3659–3660.
[64] T.L. Rosenberry, X. Brazzolotto, I.R. Macdonald, M. Wandhammer, M. Trovaslet-Leroy, S. Darvesh, F. Nachon, Molecules. 22 (2017) E2098, https://doi.org/10.3390/molecules22122098 29.
[65] B. Kahveci, E. Menteşe, M. Özil, F. Yilmaz, M. Serdar, J. Heterocycl. Chem. 53 (2016) 975–980.
[66] B. Hakan, A. Demirbaş, N. Demirbaş, Ş.A. Karaoglu, Turkish J. Chem. 34 (2010) 165–180.
[67] E. Mete, B. Comez, H.I. Gul, I. Gulcin, C.T. Supuran, J. Enzyme Inhib. Med. Chem. 31 (2016) 1–5.