Results in Chemistry 10 (2024) 101717

Contents lists available at ScienceDirect

Results in Chemistry

journal homepage: www.sciencedirect.com/journal/results-in-chemistry

New Cholinesterase inhibitors based on 1,2,4-triazole bearing benzenesulfonohydrazide skeleton: Synthesis, *in vitro* and *in silico* studies

Mohamed S. Othman [a,b], Haseena Naz [c], Fazal Rahim [c,*], Hayat Ullah [d,*], Rafaqat Hussain [e], Muhammad Taha [f], Shoaib Khan [g], Mohamed A. Fareid [h,i], Shimaa M. Aboelnaga [j], Anas T. Altaleb [j], Rashid Iqbal [k,l], Syed Adnan Ali Shah [m]

[a] *Biochemistry Department, College of Medicine, University of Ha'il, Hail 2440, Saudi Arabia*
[b] *Faculty of Biotechnology, October University for Modern Science and Arts (MSA), Giza, Egypt*
[c] *Department of Chemistry, Hazara University, Mansehra 21120, Pakistan*
[d] *Institute of Chemistry, University of Okara, Okara 56300, Pakistan*
[e] *College of Biology, Hunan University Changsha, Hunan 410082, PR China*
[f] *Department of Clinical Pharmacy, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam 31441, Saudi Arabia*
[g] *Department of Chemistry, Abbottabad University of Science and Technology, Abbottabad 22500, Pakistan*
[h] *Department of Medical Laboratories, Faculty of Applied Medical Science, University of Ha'il, Hail 2440, Saudi Arabia*
[i] *Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo 11651, Egypt*
[j] *Basic Sciences Department, Deanship of Preparatory Year, University of Ha'il, Hail P.O. Box 2440, Saudi Arabia*
[k] *Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur 63100, Pakistan*
[l] *Department of Life Sciences, Western Caspian University, Baku, Azerbaijan*
[m] *Faculty of Pharmacy, Universiti Teknologi MARA Cawangan Selangor Kampus Puncak Alam, Bandar Puncak Alam, Selangor 42300, Malaysia*

ARTICLE INFO

Keywords:
Synthesis
1,2,4-Triazole
Benzenesulfonohydrazide
Acetylcholinesterase
Butyrylcholinesterase
Molecular Docking

ABSTRACT

We have synthesized 1,2,4-triazole bearing benzenesulfonohydrazide analogues (**1**–**21**), characterized through different spectroscopic techniques such as ^{1}HNMR, ^{13}CNMR, HREI-MS and were evaluated against Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) enzymes. All the newly synthesized analogues showed excellent to good inhibition potential with IC_{50} values ranged from 0.30 ± 0.050 to 15.21 ± 0.50 μM (against AChE) and 0.70 ± 0.050 to 18.27 ± 0.60 μM (against BuChE) as compared to the standard drug Donepezil (IC_{50} = 2.16 ± 0.12 and 4.5 ± 0.11 μM, respectively). Analogues 2 and 4 which were found inactive against these enzymes. However, analogues 17 (IC_{50} = 0.30 ± 0.050 and 0.70 ± 0.050 μM) and 13 (IC_{50} = 0.70 ± 0.05 and 1.70 ± 0.050 μM) were found to have potent inhibitory potentials against the targeted enzymes. Structure-activity relationship was carried out which mainly depends upon the nature, position and numbers of the substitution present on phenyl rings that may be electron withdrawing/donating. Molecular docking study was carried out to know about the binding mode of interaction of the most active site of the synthesized analogues with the targeted enzymes.

Introduction

Alzheimer's disease (AD) is a chronic disorder related to the central nervous system that usually occurs with aging and is the most common form of dementia. Due to the adverse effects of Alzheimer disease, the patient faces difficulties in recalling things, which will lead to long-term memory loss [1]. Before the diagnosis of Alzheimer's disease, a person faces reoccurring disturbances in sleep and mental behavior that led to severe anxiety [2]. The etiology of Alzheimer's disease remains unknown. However, the accumulation of Aβ (β-amyloid) in the cerebral cortex and intra-nerve filamentous neurons that have tau proteins is considered the major cause of Alzheimer's disease [3]. Aggregation of these toxic Aβ is treated by using AChE and BuChE enzymes that produce choline and acetic acid to facilitate proper function by minimizing the acetylcholine time period in the hippocampus and cortex [4]. The catalytic site of AChE will bring about the hydrolysis of acetylcholine, while

* Corresponding authors at: Department of Chemistry, Hazara University, Mansehra 21120, Pakistan (F. Rahim).
E-mail addresses: fazalstar@gmail.com (F. Rahim), hayatullah@uo.edu.pk (H. Ullah).

https://doi.org/10.1016/j.rechem.2024.101717
Received 2 July 2024; Accepted 7 August 2024
Available online 10 August 2024

the peripheral site of AChE will interact with Aβ which will lead to the Aβ-Acetylcholinesterase (Aβ-AChE) complex that causes neurotoxicity. AChE is present in cholinergic neurons, the brain, and muscles, while the liver, serum, kidney, heart, and lungs are the founding sites of BuChE [5,6]. Cholinesterase is used to break down derivatives that have esters as part of their structure. AChE is present in Central nervous system (CNS). In the brain of a person suffering from Alzheimer's disease, when acetylcholine does not function properly, BuChE becomes active. Hence Alzheimer's disease should be treated by synthesizing such drugs that show inhibition against AChE (AChE) and BuChE [7]. Different drugs have been synthesized by different administrations to cure Alzheimer's disease, like Galantamine and Donepezil for AChE, and Rivastigmine and Tacrine show inhibition for AChE and BuChE (Fig. 1) [8].

Triazole is an organic compound in which five-membered heterocyclic ring bears three nitrogen atoms at the 1, 2, and 4 positions of the ring. Triazole derivatives show a broad spectrum of biological activities, such as anti-cancer [9], anti-Alzheimer [10,11], and anti-diabetic [12].

Some potent drugs containing Triazole-containing compounds are Letrozole (antidiabetic) [13], Ribavirin (antiviral) [14], Tazobactum (antibacterial) [15], and Isavuconazole (antifungal) [16] (Fig. 2).

Our research group had published many papers in the field of heterocycles synthesis and studied their biological potential and already reported some derivatives as thymidine Phosphorylase, α-amylase, α-glucosidase, and anti-Alzheimer's inhibitors [17–21]. Literature survey revealed that hybridization of two or more heterocyclic moieties in the same compounds enhance the enzymatic activities. Based on these finding, current study was designed and synthesized hybrid compounds bearing triazole and sulphonamide moieties in the same compounds to further explore the inhibitory potential of both AChE and BuChE enzymes to cure Alzheimer's disease and result obtained corroborated that these hybrid compounds could be considered as potential anti-Alzheimer agent (Fig. 3).

Result and discussion

Chemistry

4-Nitrobenzoyl chloride (I, 1 mmol) was treated with thiosemicarbazide (II, 1 mmol) and refluxed for 6 h in Dimethylformamide (DMF) (10 ml) as a solvent in the presence of triethyl amine used as a catalyst to give 2-(4-nitrobenzoyl) hydrazine-1-carbothioamide as a first intermediate product (III). The cyclization of an intermediate product (III) was carried out in a 2 % aqueous solution of sodium hydroxide (NaOH), followed by neutralization with dil. HCl led to the formation of 1,2,4-triazole-3-thione as the second intermediate product (IV). Equivalent intermediate (IV) was then reacted with substituted phenacyl bromide in ethanol (10 ml) in the presence of triethyl amine used as a catalyst and refluxed for 3 h to give the third intermediate (V). In the next step, intermediate product (V) was treated with hydrazine hydrate (5 ml) in methanol (10 ml) followed by the addition of 2–3 drops of glacial acetic acid and refluxed for 4 h to give intermediate product (VI), which was finally treated with equivalent substituted benzenesulfonamide in Tetrahydrofuran (THF) (10 ml) and refluxed for 6 h to give the desired triazole based sulfonohydrazide derivatives (**1**–**21**) in a good yield (Scheme 1, Table 1). The structure of the newly synthesized analogues was characterized through different techniques such as NMR and HREI-MS.

In vitro Acetylcholinesterase and Butyrylcholinesterase activities

An *in vitro* study of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) was carried out in order to know the inhibitory potential of synthesized compounds (**1**–**21**). All the synthesized scaffolds display good to moderate inhibitory potential, with $IC_{50} = 0.30 \pm 0.050$ to 15.21 ± 0.50 μM (against AChE) and 0.70 ± 0.050 to 18.27 ± 0.60 μM (against BuChE), as compared to the standard drug Donepezil ($IC_{50} = 2.16 \pm 0.12$ and 4.5 ± 0.11 μM respectively). Among all synthesized derivatives, compounds 2 and 4 were inactive for AChE and BuChE (Table 1). Depending upon the number, position, and nature of substituents that may be either electron-donating or electron withdrawing attached to the ring, a structure–activity relationship (SAR) was observed. The synthesized analogues were split into various parts that are triazole, sulfonamide group, rings A, B, and C. Each part of the synthesized derivatives plays a key role in the inhibitory effect of AChE and BuChE. It was shown that the different positions of different substituents on different rings (B and C) caused variations in their inhibition for both AChE and BuChE.

Structure activity relationship (SAR) for inhibition of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE)

Analog 17, showing $IC_{50} = 0.30 \pm 0.050$ μM AChE and 0.70 ± 0.050 μM BuChE with di-substitution (3,4-dichloro) on ring B and mono-substitution (3-nitro) at ring C, shows high inhibition potency against AChE and BuChE enzymes. Different interactions of analog 17 were also studied under molecular docking study revealing the involvement of these moieties in hydrogen bond formation. In this way, substrate molecule cannot bind with the enzyme due to unavailability on its active site. The derivative 13 having di-substitution (3,4-dichloro) on ring B and mono-substitution (4-nitro) at ring C having $IC_{50} = 0.70 \pm 0.05$ μM (AChE) and $IC_{50} = 1.70 \pm 0.050$ μM (BuChE) was found to be the most potent inhibitors after 17. The strong inhibitory potential of the analog 13 was also investigated in molecular docking study and hydrogen bond, Pi-interactions and Van der Waal's interactions were revealed. The Cl and NO_2 groups engage the active site of enzymes which reduce the enzyme potential for substrate molecule. The potential of compounds depends upon the number of substitutions attached to rings B and C, such that greater numbers of electron-withdrawing groups like di-chloro and −nitro groups have the ability to form hydrogen bonding with amino acids in these derivatives that lead to an increase in the inhibitory effect for (AChE) and (BuChE). Due to the presence of these di-chloro and nitro groups, the electronic clouds of phenyl rings B and C were reduced, and they bound themselves with the active sites of (AChE) and (BuChE) enzymes to attain stability. However, compound 1, having a 2-hydroxy group at ring B and 4-(4-methyl) phenyl at ring C, with $IC_{50} = 15.21 \pm 0.50$ μM for (AChE) and $IC_{50} = 18.27 \pm 0.60$ μM for BuChE was found to be the least potent inhibitor. The presence of a bulkier group around the ring C leads to a reduction of the inhibitory effect (Table 1).

The derivatives **7**, **13**, **19** and **21** having nitro group at para position

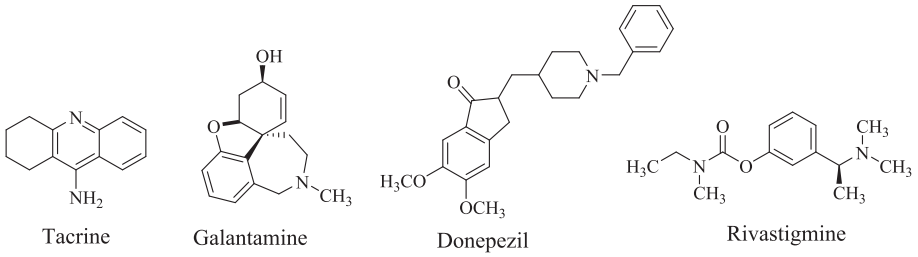


Fig. 1. Commercially used drugs for Alzheimer's disease.

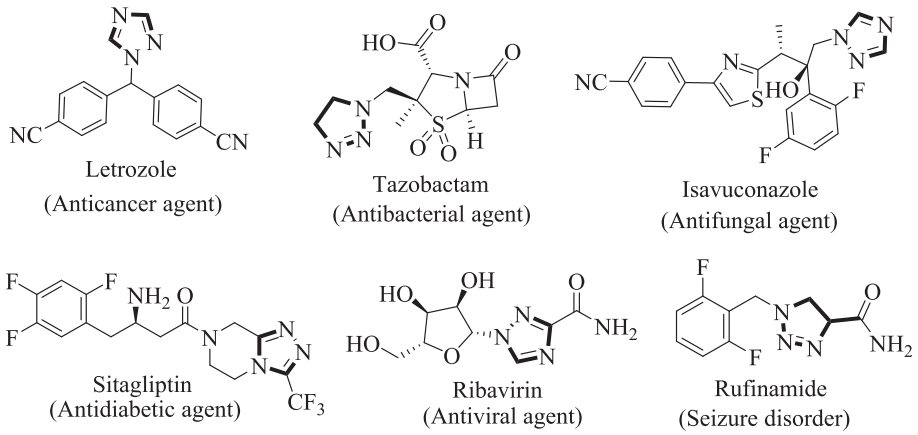


Fig. 2. Bioactive drugs containing Triazole moiety.

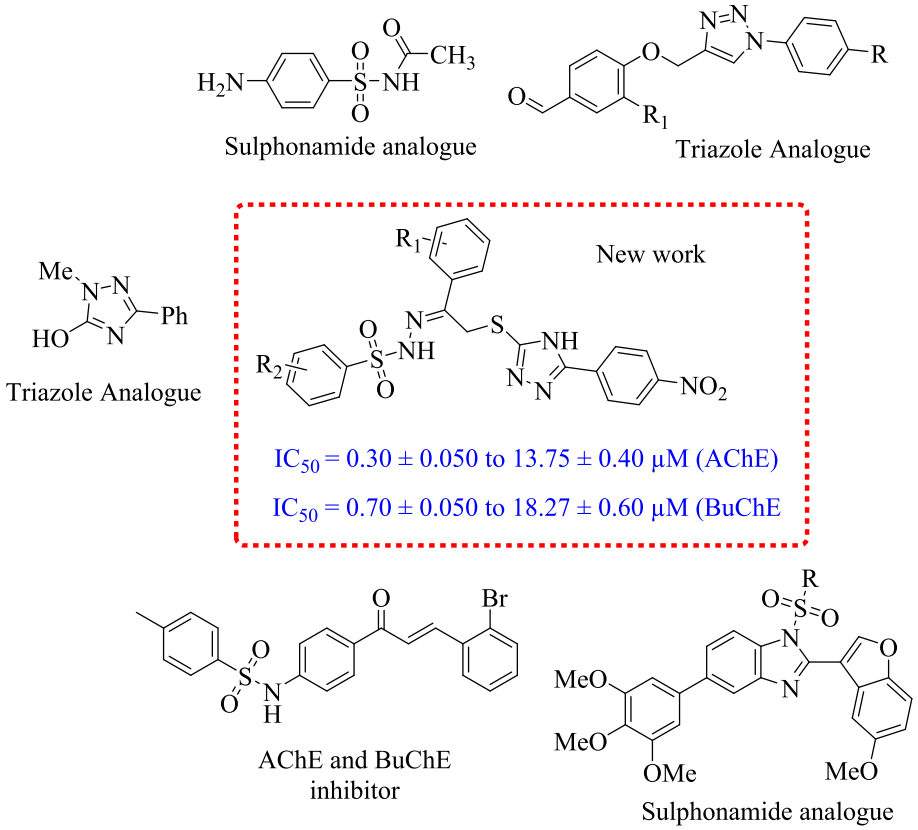
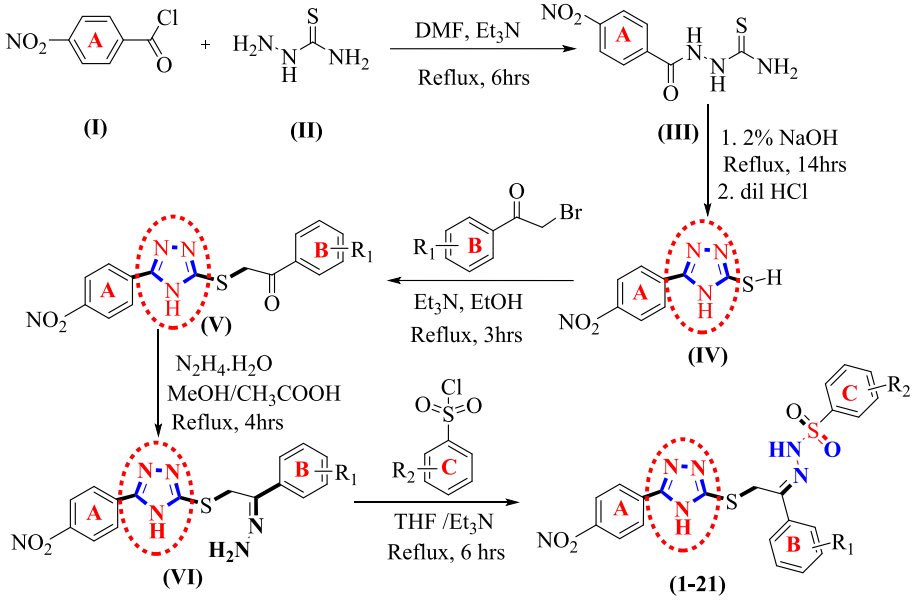


Fig. 3. Rational of current study.

of ring C and methoxy, nitro and di-chloro groups at different position of ring B showed improved inhibitory effect for AChE as well as BuChE enzymes. In the above mentioned **4** analogues, the analogue **13** shows high inhibition due to the presence of di-chloro group at ring B showing IC_{50} = 0.70 ± 0.05 μM for (AChE) and IC_{50} = 1.70 ± 0.050 μM for (BuChE) as compared to the compound **7** (IC_{50} = 2.10 ± 0.10 μM for AChE and IC_{50} = 4.30 ± 0.10 μM for BuChE) with di-substitution (3,6-di-methoxy) at ring B, compound **19** (IC_{50} = 1.60 ± 0.10 μM for AChE and IC_{50} = 2.65 ± 0.20 μM for BuChE) with mono substitution (2-nitro) at ring B and compound **21**(IC_{50} = 3.66 ± 0.20 μM for AChE and IC50 = 4.73 ± 0.30 μM for BuChE) having substitution at meta position (3-Methoxy)at ring B. These four derivatives contain same groups at same position of ring C but different substitution at different position at ring B. The variation in their inhibitory effect is due to the presence of different nature of substitution at ring B that effect in different manner with the targeted (AChE) and (BuChE). Moreover, derivatives **5** and **17** both have same substitution (3-nitro) at ring C but different substitution 3,4-dichloro at ring C in **17** and 3,6-di-methoxy at ring C of compound **5** cause variation in their inhibition activity (Table 1). The high potency of analog 17 was also validated via molecular docking and different interactions including hydrogen bond, pi-pi T-shaped, pi-sulfur etc were found which enhance the drug potential of analog 17.

Derivatives **1** (IC_{50} = 15.21 ± 0.50 and 18.27 ± 0.60 μM), **11** (IC_{50} = 11.72 ± 0.30 and 13.20 ± 0.30 μM) and **16** (IC_{50} = 4.60 ± 0.20 and 5.80 ± 0.20 μM), all these have same methyl substitution at para position of ring C but the inhibitory effect of **16** is high as compared to the rest of two. These difference in their potency might be due to the presence of various substitution at ring B. Compound **1** have hydroxyl group



Scheme 1. Synthesis of 1,2,4-Triazole bearing benzenesulfonohydrazide derivatives.

at ortho position of ring B while **11** have bromo group at para position and compound **16** have di-substitution at meta and para position of ring B. This increased inhibition potency of (AChE) and (BuChE) activities of **16** may be because of strong electron withdrawing chlorine group, which make ring B somewhat positive to bind with active site of enzyme (Table 1).

Comparing derivatives **3** (IC_{50} = 8.90 ± 0.30 and 9.46 ± 0.30 μM) that have nitro group at ortho position and two chlorine at para and *ortho*-position of ring C and bromo group at ortho position of ring B with analogue **8** (IC_{50} = 13.75 ± 0.40 and 16.83 ± 0.60 μM), having nitro at ortho and di chlorine groups at para and ortho position of ring C and phenyl at para position of ring B and **13** (IC_{50} = 4.60 ± 0.010 and 5.90 ± 0.10 μM) having nitro at ortho position and di chlorine groups at para and ortho position and nitro at ortho and methyl at para position of ring B. The variation in nature and position of substitutions at ring B leads to the difference in their inhibitory potential (Table 1). The inhibition of target enzyme by analog 13 was also studied in docking study and it was found that substitution of No_2 group gives rise to the formation of hydrogen bond between analog and the protein which plays significant role in the inhibition profile of this compound.

Comparing derivatives **7** (IC_{50} = 2.40 ± 0.10 and 4.70 ± 0.10 μM), **9** (IC_{50} = 9.10 ± 0.20 and 11.20 ± 0.30 μM), **12** (IC_{50} = 2.60 ± 0.20 and 3.79 ± 0.20 μM), **18** (IC_{50} = 1.90 ± 0.10 and 2.79 ± 0.20 μM) and **19** (IC_{50} = 2.43 ± 0.20 and 3.18 ± 0.20 μM), all these have substitutions with same nature and same position at ring C but substitution at ring B are totally different in each, that lead to change their inhibitory potential from each other (Table 1).

Molecular docking study

The molecular docking study was used for most active analogs **17, 13** and **20** in order to explore the binding mode of interactions of these highly active scaffolds with the active sites of targeted (AChE) and (BuChE) enzymes. Subsequently, the active analogs **17, 13 and 20** showed fit well binding mode with different affinities and correlate well with the *in vitro* study. All these active analogs **17, 13 and 20** have similar and related chemistry with slight modification of attached substituents in nature, number/s and position at both aryl part B and C respectively. These different functional moieties have found a wide difference in the interactions of these active analogs **17, 13 and 20** with the active sites of targeted (AChE) and (BuChE) enzymes. Different types of interactions adopted by these active analogs with the active sites of (AChE) and (BuChE) enzymes as given in Table 2. Optimized computational analysis and binding coordinates of the complexes between potent compounds and enzymes in Table 3.

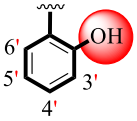
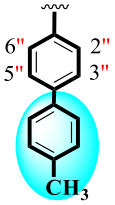
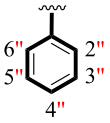
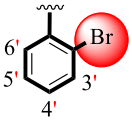
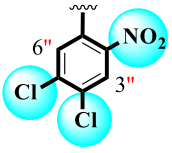
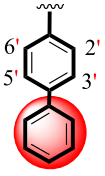
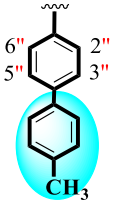
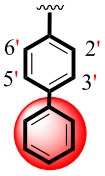
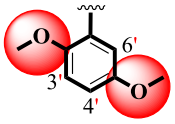
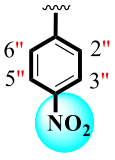
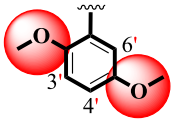
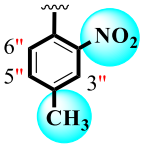
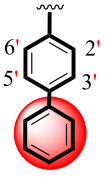
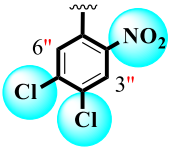
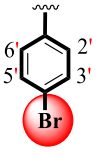
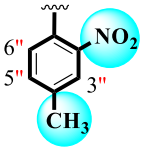
Analog **17,** when investigated against AChE for exploration of binding interactions via *in silico* molecular docking, a number of such interactions were found responsible for inhibiting the enzyme potential for its substrate i.e. acetylcholine. Different amino acids of target enzyme bind with the ligand molecule at different sites through varied interactions at a specific distance. These amino acids include HIS-201, LEU-162, TRP-62, ASP-197, HIS-101, ARG-195, HIS-299 and HIS-305 interacting through hydrogen bonding, Pi-Pi Stacked, Pi-Pi T Shaped, Pi-Anion and Pi-Sulphur. Besides these interactions, Van der Waal's forces also contribute to the inhibitory profile of the analog. These interactions are depicted in Fig. 4, differentiating the interactions through different colors (Fig. 4).

Similarly, analog **17** was also investigated against BuChE for exploration of binding interactions. Amino acids ASP-232, ARG-552, TRP-432, PHE-601, ASP-469, HIS-626, LYS-506, ALA-231, ILE-233, ASN-496 and SER-505 interact with the ligand molecule via hydrogen bonding, Pi-Pi Stacked, Pi-Anion, Pi- Alkyl and Pi-Sulphur. The 2D and 3D binding interactions are depicted in Fig. 5, differentiating the interactions through different colors (Fig. 5).

Analog **13** was investigated for its potential to inhibit acetylcholinesterase (AChE) through molecular docking simulations, which identified several binding interactions responsible for reducing the enzyme's activity towards acetylcholine. The ligand molecule interacted with multiple amino acids on the enzyme, including HIS-201, LEU-165, TYR-62, HIS-299, and TRP-59, through a variety of interactions such as hydrogen bonding, Pi-Pi stacking, Pi-Alkyl, Pi-sulphur, and Van der Waals forces. These interactions, depicted in Fig. 6 using different colors to highlight distinct binding modes, contribute to the inhibitory profile of analogue-13 (Fig. 6).

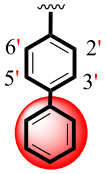
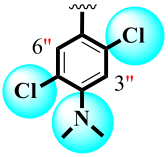
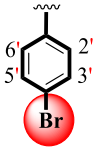
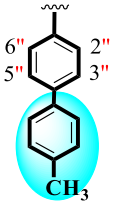
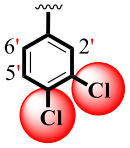
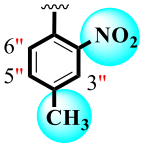
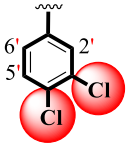
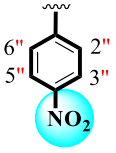
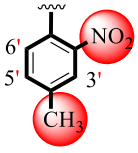
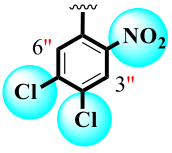
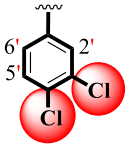
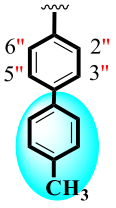
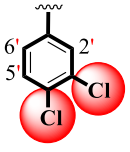
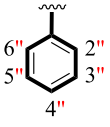
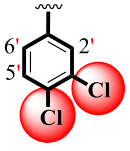
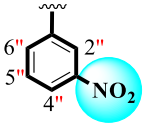
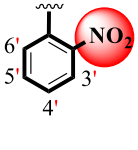
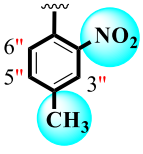
Analog **13** was further evaluated against (BuChE) to explore its binding interactions. Molecular docking simulations revealed that the ligand molecule forms bonds with several amino acids on the enzyme, including ARG-552, ALA-234, TRP-432, PHE-601, ASP-568, HIS-626, LYS-506, ASP-232, ALA-231, ILE-233 and SER-505, through various interactions such as hydrogen bonding, Pi-Pi T-shaped, Pi-anion, Pi-alkyl, Pi-sigma and Pi-sulfur. The 2D and 3D binding interactions of analog **13** are illustrated in Fig. 7.

Table 1
Different substituents and *in vitro* Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) inhibitory activities of triazole bearing Benzenesulfonohydrazide derivatives.

Compounds	Ring B	Ring C	AChE IC_{50} (μM)	BuChE IC_{50} (μM)
1			15.21 ± 0.50	18.27 ± 0.60
2	▲		N. A	N. A
3			8.90 ± 0.30	9.46 ± 0.30
4			N. A	N. A
5			4.70 ± 0.10	6.30 ± 0.10
6			2.10 ± 0.10	4.30 ± 0.10
7			2.40 ± 0.10	4.70 ± 0.10
8			13.75 ± 0.40	16.83 ± 0.60
9			9.10 ± 0.20	11.20 ± 0.30

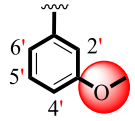
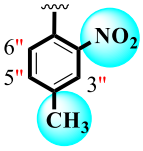
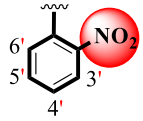
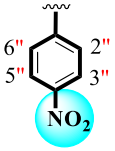
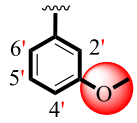
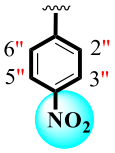
(*continued on next page*)

Table 1 (*continued*)

Compounds	Ring B	Ring C	AChE IC_{50} (μM)	BuChE IC_{50} (μM)
10			11.30 ± 0.30	12.30 ± 0.30
11			11.72 ± 0.30	13.20 ± 0.30
12			2.60 ± 0.20	3.79 ± 0.20
13			0.70 ± 0.05	1.70 ± 0.050
14			4.60 ± 0.010	5.90 ± 0.10
15			4.60 ± 0.20	5.80 ± 0.20
16			4.67 ± 0.20	5.88 ± 0.30
17			0.30 ± 0.050	0.70 ± 0.050
18			1.90 ± 0.10	2.79 ± 0.20

(*continued on next page*)

Table 1 (*continued*)

Compounds	Ring B	Ring C	AChE IC_{50} (μM)	BuChE IC_{50} (μM)
19			2.43 ± 0.20	3.18 ± 0.20
20			1.60 ± 0.10	2.65 ± 0.20
21			3.66 ± 0.20	4.73 ± 0.30
Standard drug Donepezil			2.16 ± 0.12	4.5 ± 0.11

Analog **20** was also found with strong inhibitory profile against AChE on the basis of both *in vitro* and *in silico* studies. The *in vitro* results were validated through *in silico* molecular docking study and were found in accordance with the biological activity results. Analog **20** has the potential to bind with different amino acids including HIS-201, ILE-285, LYS-200, ALA-198, TYR-151, HIS-299, ASP-300, GLN-63 and LEU-162. Different interactions were found between analog **20** and AChE enzyme which majorly include hydrogen bonding, Pi-cation, Pi-sigma and Pi-alkyl. The binding interactions were visualized as 2D and 3D and are illustrated in Fig. 8.

Analog 20 exhibited a potent inhibitory effect against BuChE, as confirmed by both *in vitro* and in silico studies. Analog 20 showed potential binding interactions with various amino acids on the BuChE enzyme, including ASP-568, ARG-552, TRP-432, ILE-233, LYS-506 and SER-505. The binding modes involved diverse interactions such as hydrogen bond, Pi-Pi T-shaped, Pi-anion, Pi-alkyl and Pi-sulfur. The 2D and 3D binding interactions providing a detailed visualization of the inhibitory mechanism of Analog 20 against BuChE are depicted in Fig. 9.

The reference compound donepezil was also docked against AChE and BuChE to study the mode of inhibition. Fewer interactions were observed against both enzymes revealing the lower potential of control drug in comparison to the potent analogs of the novel series. The binding interactions of donepezil against AChE and BuChE are shown in Fig. 10.

Experimental section

Material and methods

Analytical-grade reagents and solvents were purchased from Sigma-Aldrich and used as received. Thin-layer chromatography (TLC) was performed on precoated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). ^{1}H NMR and ^{13}C NMR spectra were recorded on Advance Bruker AM spectrometers, operating at 600 and 150 MHz, respectively. The chemical shift values are presented in ppm (δ), as compared to tetramethylsilane (TMS) as an internal reference standard, and coupling constants (J) are in Hz. TLC chromatograms were visualized under ultraviolet light at 254 and 366 nm. Mass spectra were recorded by electron impact (EI) on MAT 312 and MAT 113D mass spectrometers.

General procedure for the synthesis of triazole-based sulfonamide analogs

4-Nitrobenzoyl chloride (I) was treated with thiosemicarbazide (II) and refluxed for 6 h in Dimethylformamide (DMF) as a solvent and trimethylamine (catalyst) to give 2-(4-nitrobenzoyl) hydrazine-1-carbothioamide as a first intermediate product (III). The cyclization of an intermediate product (III) was carried out in a 2 % aqueous solution of sodium hydroxide, followed by neutralization with dil. HCl led to the formation of 1,2,4-triazole-3-thione as the second intermediate product (IV). The intermediate (IV) was then reacted with substituted phenacyl bromide in ethanol in the presence of triethylamine and refluxed for 3 h to give the third intermediate (V). In the next step, intermediate product (V) was treated with hydrazine hydrate in methanol, followed by the addition of glacial acetic acid, and refluxed for 4 h to give intermediate product (VI), which was finally treated with substituted sulphonamide in tetrahydrofuran (THF) and refluxed for 6 h to give the desired triazole-sulphonamide hybrid derivatives (1–21). The structure of the newly synthesized analogues was characterized through different techniques such as NMR and HREI-MS.

Spectral analysis

N'-(1-(2-hydroxyphenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)thio) ethylidene)-4′-methyl-[1,1′-biphenyl]-4-sulfonohydrazide (1)

Yield: 71 %; yellow solid; m.p: 190 – 191 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.95 (s, 1H, Triazole-H), 11.91 (s,1H, NH), 10.45 (s, 1H, OH), 8.88 (d, *J*=7.3 Hz, 1H, Ar-H), 8.81 (d, *J*=7.2 Hz, 2H, Ar-H), 8.57 (t, *J*=6.8 Hz, 1H, Ar-H), 7.58 (d, *J*=7.6 Hz, 1H, Ar-H), 6.96 (d, *J*=7.1 Hz, 2H, Ar-H), 6.76 (d, *J*=7.2 Hz, 2H, Ar-H), 6.74 (d, *J*=7.0 Hz, 1H, Ar-H), 6.73 (d, *J*=7.5 Hz, 4H, Ar-H), 6.61 (t, *J*=6.4 Hz, 2H, Ar-H), 3.18 (s, 2H, CH_2), 2.49 (s, 3H, CH_3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 171.7, 168.5, 147.7, 145.8, 142.5, 140.8, 137.4, 132.2, 129.4,129.3, 126.2,123.9, 123.1, 118.8, 109.2, 106.5, 104.6, 45.6, 10.9, 8.8; HREI-MS: *m/z* calcd for $C_{29}H_{24}N_6O_5S_2$ $[M]^+$600.1250; Found; 600.1245.

N'-(1-([1,1′-biphenyl]-4-yl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)benzenesulfonohydrazide (2)

Yield: 69 %; yellow solid; m.p: 194 – 195 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 12.09 (s, 1H, Triazole-H), 11.97 (s, 1H, NH), 8.82 (d, *J*=7.3 Hz, 2H, Ar-H), 8.03 (d, *J*=7.6 Hz, 2H, Ar-H), 8.00 (d, *J*=7.2 Hz, 2H, Ar-

Table 2
The different types of interactions between active analogs (**17, 13** and **20**) and interactive residues of amino acids of targeted (AChE) and (BuChE) enzymes with varied distance.

Active analogs	Targeted enzymes	Receptors	Interactions	Distance
Analog-**17**	AChE	HIS-201	HB	5.46 °A
		LEU-162	Pi-Pi Stacked	6.23 °A
		TRP-62	Pi-Pi T Shaped	4.74 °A
		ASP-197	Pi-Anion	7.56 °A
		HIS-101	Pi-Pi T shaped	7.24 °A
		ARG-195	HB	7.02 °A
		HIS-299	HB	5.36 °A
		HIS-305	HB	4.43 °A
		HIS-305	Pi-Sulphur	5.68 °A
	BuChE	ASP-232	HB	5.21 °A
		ARG-552	HB	7.12 °A
		ARG-552	HB	6.19 °A
		TRP-432	Pi-anion	6.07 °A
		PHE-601	Pi-Pi Stacked	7.24 °A
		ASP-469	Pi-anion	7.13 °A
		HIS-626	HB	5.36 °A
		LYS-506	Pi-Sulphur	6.66 °A
		ALA-231	Pi-alkyl	4.09 °A
		ILE-233	Pi-alkyl	5.35 °A
		ASN-496	HB	4.58 °A
		SER-505	HB	3.56 °A
Analog-**13**	AChE	HIS-201	HB	5.82 °A
		HIS-299	HB	5.32 °A
		TYR-62	Pi-Pi Stacked	4.72 °A
		LEU-165	Pi-alkyl	5.96 °A
		TRP-59	Pi-sulphur	4.39 °A
	BuChE	ARG-552	HB	5.14 °A
		ALA-234	HB	3.48 °A
		ILE-233	Pi-sigma	5.04 °A
		ILE-233	Pi-alkyl	4.25 °A
		SER-505	HB	4.83 °A
		LYS-506	HB	3.93 °A
		LYS-506	Pi-alkyl	6.53 °A
		ASP-232	HB	5.08 °A
		ASP-568	Pi-anion	7.68 °A
		PHE-601	Pi-sulphur	7.15 °A
		TRP-432	Pi-sulphur	6.09 °A
		TRP-432	Pi-Pi T shaped	6.79 °A
Analog-**20**	AChE	HIS-201	Pi-cation	6.25 °A
		ILE-285	Pi-sigma	5.35 °A
		LYS-200	Pi-alkyl	6.35 °A
		ALA-198	Pi-alkyl	5.38 °A
		TYR-151	HB	6.16 °A
		HIS-299	HB	5.84 °A
		ASP-300	HB	5.20 °A
		ASP-300	HB	4.71 °A
		GLN-63	HB	5.90 °A
		LEU-162	Pi-alkyl	5.05 °A
		ASP-568	Pi-anion	6.20 °A
		TRP-432	Pi-sulphur	7.62 °A
	BuChE	TRP-432	Pi-pi T shaped	6.87 °A
		ARG-552	HB	5.26 °A
		ILE-233	Pi-alkyl	4.27 °A
		LYS-506	HB	4.22 °A
		LYS-506	Pi-alkyl	6.41 °A
		LYS-506	Pi-alkyl	6.92 °A
		TRP-A-84	Pi-Pi- Stacked	3.94
		PHE-A-330	Pi-R	3.83
		TYR-A-334	Pi-R	4.99
		TYR-A-334	Pi-sigma	3.74
	AChE	ARG-A-289	HB	7.45
		SER-A-286	HB	5.58
Donepezil		TRP-A-279	Pi-Pi- Stacked	5.66
		TRP-A-279	Pi-R	5.56
		TRP-A-279	Pi-sigma	4.50
		TYR-A-70	Pi-R	5.54
	BuChE	TRP-A-82	Pi-Pi- Stacked	4.26
		HIS-A-438	Pi-cation	5.58
		PHE-A-329	Pi-R	5.24
		TYR-A-332	Pi-R	4.51

Table 3
Optimized computational analysis and binding coordinates of the complexes between potent compounds and enzymes.

Compound	Center			Size		
	x	y	z	x	y	z
Analog 13, 17 and 20 in AChE complex	18.221339	7.684984	49.559016	20	20	20
Analog 13, 17 and 20 in BuChE complex	−7.366378	−4.220829	−12.905963	20	20	20

H), 7.92 (d, *J*=7.6 Hz, 2H, Ar-H), 7.83 (d, *J*=7.6 Hz, 2H, Ar-H), 7.75 (d, *J*=7.7 Hz, 2H, Ar-H), 7.63 (t, *J*=6.8 Hz, 1H, Ar-Ar-H), 7.59 (t, *J*=6.9 Hz, 2H, Ar-H), 7.49 (t, *J*=6.4 Hz, 2H, Ar-H), 7.41 (t, *J*=6.3 Hz, 1H, Ar-H), 3.77 (s, 2H, CH_2), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 157.6, 155.6, 147.9, 143.1, 140.8, 138.9, 138.6, 132.9, 131.9, 129.7, 129.2, 129.2, 129.0, 129.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.9, 127.6, 127.3, 127.0, 127.0, 124.4, 124.4, 31.2.; HREI-MS: *m/z* calcd for $C_{28}H_{22}N_6O_4S_2$ $[M]^+$570.1144; Found; 570.1139.

N'-(1-(2-bromophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)thio) ethylidene)-4,5-dichloro-2-nitrobenzenesulfonohydrazide (3)

Yield: 67 %; yellow solid; m.p: 189 – 190 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 12.01 (s, 1H, Triazole-H), 11.93 (s, 1H, NH), 8.82 (s, 1H, ArH), 8.53 (d, *J*=7.3 Hz, 2H, Ar-H), 8.15 (d, *J*=7.3 Hz, 3H, Ar-H), 7.99 (dd, *J*=6.8, 1.3 Hz, 2H, Ar-H), 7.58 (s, 1H, Ar-H), 6.62 (t, *J*=7.1 Hz, 1H, Ar-H), 3.99 (s, 2H, CH_2), ^{13}C NMR (150 MHz, DMSO-d_6): δ 165.4, 155.5, 137.6, 132.2, 125.1, 123.9, 123.0, 122.5, 121.2, 120.9, 118.6, 112.8, 112.5, 112.3, 109.7, 46.1, 9.2; HREI-MS: *m/z* calcd for $C_{22}H_{14}BrCl_2N_7O_6S_2$ $[M]^+$ 570.1144; Found; 570.1139.

N'-(1-([1,1'-biphenyl]-4-yl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-4'-methyl-[1,1'-biphenyl]-4-sulfonohydrazide (4)

Yield: 73 %; white solid; m.p: 196 – 197 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.12 (s, 1H, Triazole-H), 10.5 (s, 1H, NH0, 8.27 (d, *J*=7.2 Hz, 2H, Ar-H), 8.03 (d, *J*=7.4 Hz, 2H, Ar-H), 8.00 (d, *J*=7.1 Hz, 2H Ar-H), 7.92 (d, *J*=7.7 Hz, 2H, Ar-H), 7.88 (d, *J*=7.8 Hz, 4H, Ar-H), 7.75 (d, *J*=7.2 Hz, 2H, Ar-H), 7.49 (t, *J*=6.8 Hz, 2H, Ar-H), 7.41 (t. *J*=6.3 Hz, 1H, Ar-H), 7.33 (d, *J*=7.3 Hz, 2H, Ar-H), 7.15 (d, *J*=7.6 Hz, 2H, Ar-H), 3.77 (s, 2H, CH_2), 2.34 (s, 3H, CH_3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 157.6, 155.6, 147.9, 144.9, 143.1, 140.8, 138.6, 137.8, 133.0, 132.9, 130.6, 129.7, 129.7, 129.5, 129.5, 129.2, 129.2, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.6, 127.1, 127.1, 127.0, 127.0, 124.4, 124.4, 31.2, 21.3.; HREI-MS: *m/z* calcd for $C_{35}H_{28}N_6O_4S_2$ $[M]^+$660.1613; Found; 660.1609.

N'-(1-([1,1'-biphenyl]-4-yl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-3-nitrobenzenesulfonohydrazide (5)

Yield: 64 %; yellow solid; m.p: 201 – 202 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.87 (s, 1H, Triazole-H), 11.56 (s, 1H, NH), 9.71 (d, *J*=7.2 Hz, 1H, Ar-H), 9.24 (s, 1H, Ar-H), 9.13 (d, *J*=7.3 Hz, 1H, Ar-H), 8.77 (d, *J*=7.6 Hz, 2H, Ar-H), 8.49 (d, *J*=7.2 Hz, 2H, Ar-H), 8.29 (t, *J*=6.8 Hz, 1H, Ar-H), 7.57 (d, *J*=7.0 Hz, 2H, Ar-H), 7.27 (d, *J*=7.0 Hz, 2H, Ar-H), 6.95 (d, *J*=7.0 Hz, 1H, Ar-H), 6.80 (d, *J*=7.8 Hz, 2H, Ar-H), 6.59 (t, *J*=6.5 Hz, 2H, Ar-H), 2. (s, 2H, CH_2), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.6, 157.4, 155.6, 148.3, 147.9, 143.1, 140.8, 140.6, 138.5, 133.3, 132.8, 129.8, 129.7, 129.7, 129.1, 129.1, 128.0, 128.0, 127.7, 127.7, 127.5, 127.5, 127.1, 127.0, 127.0, 124.3, 124.3, 123.1, 31.2.; HREI-MS: *m/z* calcd for $C_{28}H_{21}N_7O_6S_2$ $[M]^+$615.0995; Found; 615.0990.

N'-(1-(2,5-dimethoxyphenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-4-nitrobenzenesulfonohydrazide (6)

Yield: 65 %; yellow solid; m.p: 203 – 204 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.12 (s, 1H, Triazole-H), 10.5 (s, 1H, NH), 8.38 (d, *J*=7.2 Hz, 2H, Ar-H), 8.27 (d, *J*=7.4 Hz, 2H, Ar-H), 8.05 (d, *J*=7.0 Hz, 2H, Ar-

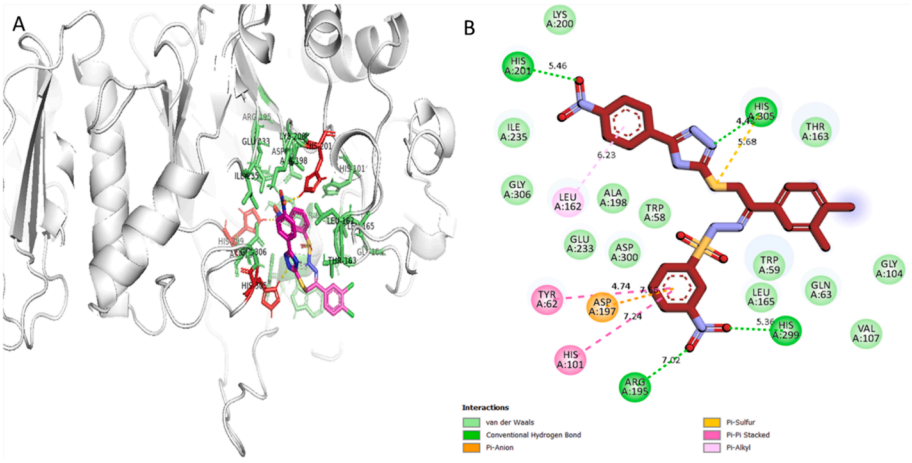


Fig. 4. Represent the PLI profile of most active analog-**17** against AChE enzyme and its 3D (A) and 2D (B) diagram.

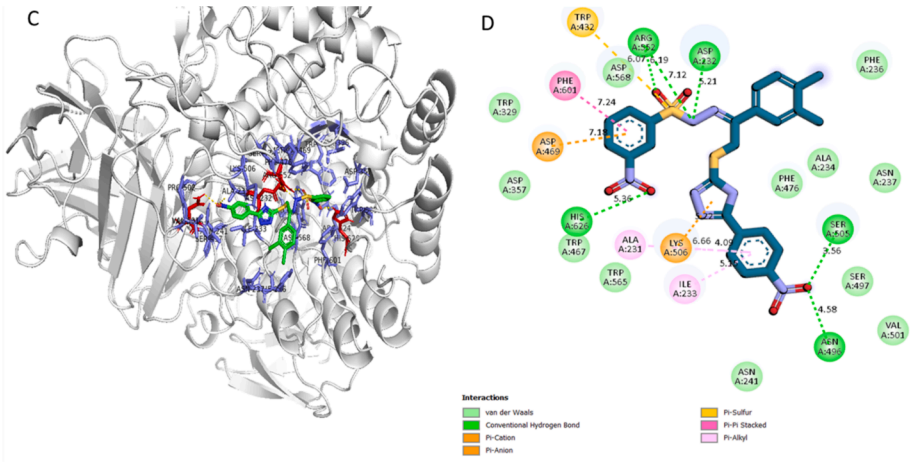


Fig. 5. Represent the PLI profile of most active analog-**17** against BuChE enzyme and its 3D (C) and 2D (D) diagram.

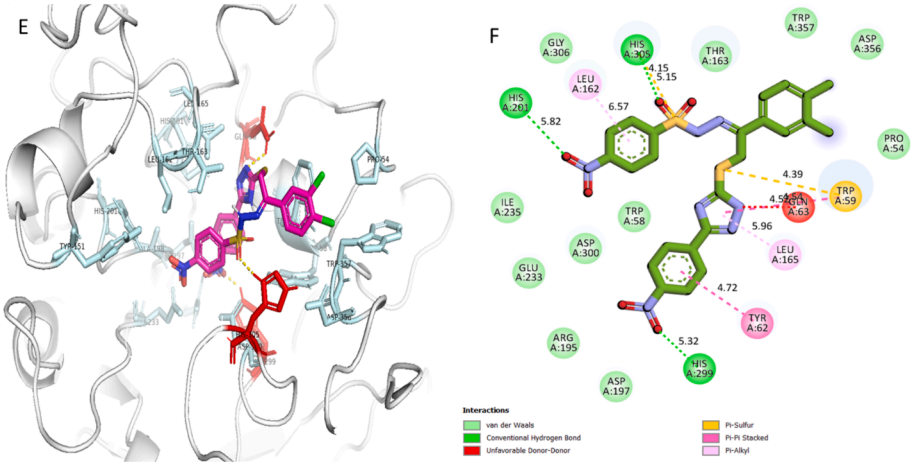


Fig. 6. Represent the PLI profile of 2nd most active analog-**13** against AChE enzyme and its 3D (E) and 2D (F) diagram.

H), 8.03 (d, *J*=7.5 Hz, 2H, Ar-H), 7.40 (s, 1H, Ar-H), 7.04 (d, *J*=7.1 Hz, 2H, Ar-H), 3.90 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.75 (s, 2H, CH_2), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 157.8, 155.6, 153.0, 152.7, 152.1, 151.1, 147.9, 138.6, 128.2, 128.2, 127.0, 127.0, 124.4, 124.4, 124.1, 124.1, 118.2, 117.6, 114.3, 55.8, 55.8, 31.5, 15.4.; HREI-MS: *m/z* calcd for $C_{24}H_{21}N_7O_8S_2$ $[M]^+$ 615.0995; Found; 615.0990.

N'-(1-(2,5-dimethoxyphenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-4-methyl-2-nitrobenzenesulfonohydrazide (7)

Yield: 66 %; white solid; m.p: 186 – 187 °C; ^1H NMR (600 MHZ, DMSO-d_6): δ 11.12 (s, 1H, Triazole-H), 10.3 (s, 1H, NH), 8.34 (s, 1H, Ar-H), 8.27 (d, *J*=7.0 Hz, 2H, Ar-H), 8.03 (d, *J*=7.2 Hz, 2H, Ar-H), 8.01 (d, *J*=7.1 Hz, 1H,Ar-H), 7.73 (d, *J*=7.5 Hz, 1H, Ar-H), 7.40 (s, 1H, Ar-H),

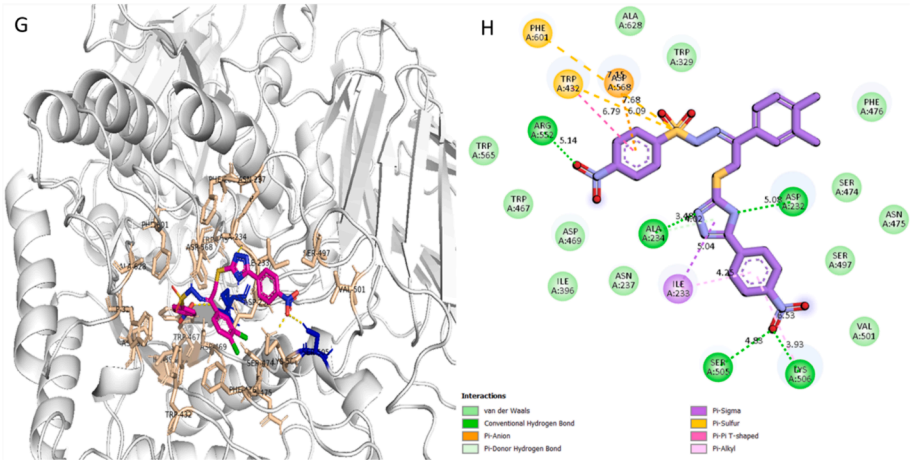


Fig. 7. Represent the PLI profile of 2nd most active analog-**13** against BuChE enzyme and its 3D (G) and 2D (H) diagram.

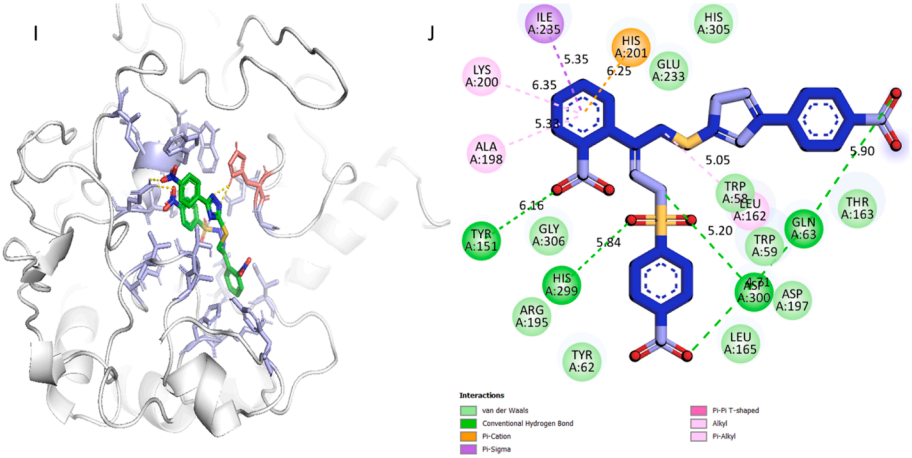


Fig. 8. Represent the PLI profile of 3rd most active analog-**20** against AChE enzyme and its 3D (I) and 2D (J) diagram.

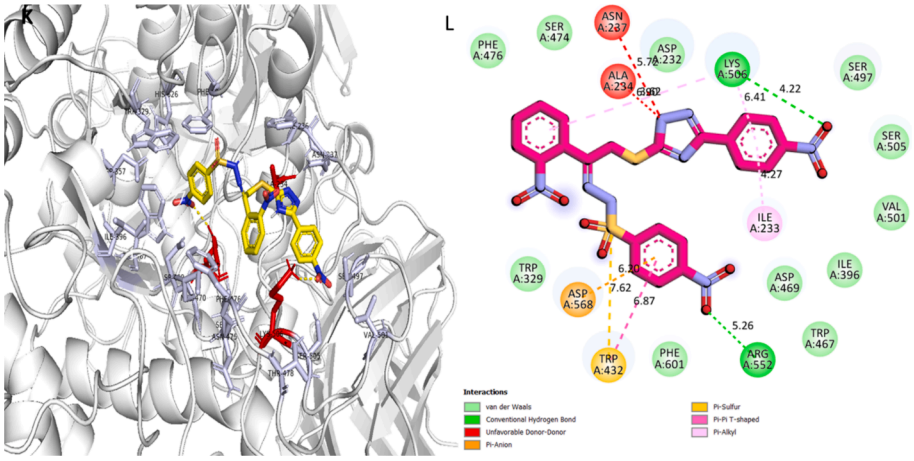


Fig. 9. Represent the PLI profile of 3rd most active analog-**20** against BuChE enzyme and its 3D (K) and 2D (L) diagram.

7.04 (d, *J*=7.6 Hz, 2H, Ar-H), 3.90 (s, 3H, OCH_3), 3.77 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.8, 158.6, 155.7, 152.0, 152.7, 147.9, 147.1, 142.5, 138.6, 135.4, 131.4, 128.1, 127.0, 127.0, 125.7, 124.4, 24.4, 118.2, 117.6, 114.3, 55.8, 55.8, 31.5, 20.3, 15.4.; HREI-MS: *m/z* calcd for $C_{25}H_{23}N_7O_8S_2$ $[M]^+$ 613.1050; Found; 613.1046.

N'-(1-([1,1'-biphenyl]-4-yl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-4,5-dichloro-2-nitrobenzenesulfonohydrazide (8)

Yield: 61 %; white solid; m.p: 196 – 197 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.11 (s, 1H, triazole-H), 10.3 (s, 1H, NH), 8.43 (s, 1H, Ar-H), 8.42 (s, 1H, Ar-H), 8.27 (d, *J*=7.2 Hz, 2H, Ar-H), 8.03 (d, *J*=7.2 Hz, 2H, Ar-H), 8.00 (d, *J*=7.3 Hz, 2H, Ar-H), 7.92 (d, *J*=7.6 Hz, 2H, Ar-H),

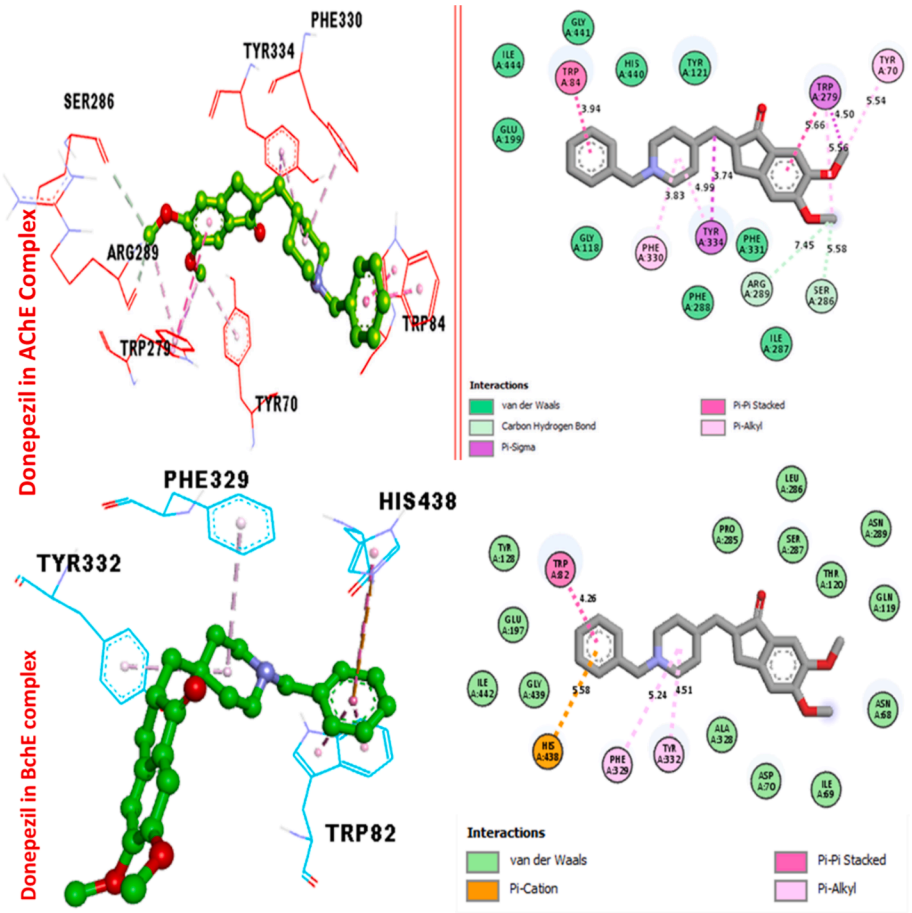


Fig. 10. Represent the 2D and 3D PLI profile of standard compound Donepezil against AChE and BuChE enzymes.

7.75 (d, *J*=7.6 Hz, 2H, Ar-H), 7.49 (t, *J*=6.8 Hz, 2H, Ar-H), 7.41 (t, *J*=7.6 Hz, 1H, Ar-H), 3.77 (s, 2H, CH_2), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 157.6, 155.6, 147.9, 146.7, 143.1, 140.8, 139.8, 138.6, 133.9, 133.9, 132.9, 129.7, 129.7, 129.3, 129.3, 128.9, 128.4, 128.4, 127.6, 127.6, 127.3, 127.3, 127.0, 125.9, 124.3, 124.3, 31.2.; HREI-MS: *m/z* calcd for $C_{28}H_{19}Cl_2N_7O_6S_2$ [M]$^+$683.0215; Found; 683.0210.

N'-(1-(4-bromophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)thio) ethylidene)-4-methyl-2-nitrobenzenesulfonohydrazide (9)

Yield: 68 %; brown solid; m.p: 198 – 199 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.13 (s, 1H, triazole-H), 10.2 (s, 1H, NH), 8.35 (s, 1H, Ar-H), 8.27 (d, *J*=7.8 Hz, 2H, Ar-H), 8.03 (d, *J*=7.0 Hz, 2H, Ar-H), 8.01 (d, *J*=7.1 Hz, 1H, Ar-H), 7.73 (d, *J*=7.4 Hz, 1H, Ar-H), 7.70 (d, *J*=7.6 Hz, 2H, Ar-H), 7.64 (d, *J*=7.1 Hz, 2H, Ar-H), 3.77 (s, 2H, CH_2), 2.30 (s, 3H, CH_3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 157.5, 154.6, 147.9, 147.1, 142.5, 138.6, 133.0, 135.4, 131.7, 131.7, 128.6, 128.6, 128.1, 128.1, 127.0, 127.0, 125.7, 125.4, 124.4, 124.4, 31.2, 20.3.; HREI-MS: *m/z* calcd for $C_{23}H_{18}BrN_7O_6S_2$ [M]$^+$630.9943; Found; 630.9939.

N'-(1-([1,1'-biphenyl]-4-yl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-2,5-dichloro-4-(dimethylamino)benzenesulfonohydrazide (10)

Yield: 72 %; white solid; m.p: 202 – 204 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.10 (s, 1H, Triazole-H), 10.6 (s, 1H, NH), 8.27 (d, *J*=7.0 Hz, 2H, Ar-H), 8.01 (d, *J*=7.5 Hz, 2H, Ar-H), 8.00 (d, *J*=7.6 Hz, 2H, Ar-H), 7.98 (s, 1H, Ar-H), 7.90 (d, *J*=7.3 Hz, 2H, Ar-H), 7.75 (d, *J*=6.9 Hz, 2H, Ar-H), 7.49 (t, *J*=6.5 Hz, 2H, Ar-H), 7.41 (t, *J*=6.7 Hz, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 3.01 (s, 6H, $N(CH_3)_2$), 3.77 (s, 2H, CH_2), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.9, 158.9, 157.6, 147.8, 143.1, 140.6, 138.4, 132.5, 130.5, 13-0.2, 129.5, 129.1, 129.1, 128.6, 128.0, 128.0, 127.6, 127.6, 127.3, 127.0, 127.0, 124.3, 124.3, 121.5, 116.4, 40.8, 40.8, 31.2.; HREI-MS: *m/z* calcd for $C_{30}H_{25}Cl_2N_7O_4S_2$ [M]$^+$ 630.9943; Found; 630.9939.

N'-(1-(4-bromophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)thio) ethylidene)-4'-methyl-[1,1'-biphenyl]-4-sulfonohydrazide (11)

Yield: 59 %; light brown solid; m.p: 192 – 194 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.12 (s, 1H, triazole-H), 10.2 (s, 1H, NH), 8.27 (d, *J*=7.2 Hz, 2H, Ar-H), 8.01 (d, *J*=7.2 Hz, 2H, Ar-H), 7.88 (d, *J*=7.6 Hz, 4H, Ar-H), 7.70 (d, *J*=7.1 Hz, 1H, Ar-H),7.64 (d, *J*=7.0 Hz, 2H, Ar-H), 7.33 (d, *J*=7.1 Hz, 2H, Ar-H), 7.15 (d, *J*=7.0 Hz, 2H, Ar-H), 3.77 (s, 2H, CH_2), 2.34 (s, 3H, CH_3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 157.6, 155.6, 147.9, 144.9, 138.6, 137.8, 133.0, 133.0, 131.7, 131.7, 130.6, 129.5, 129.5, 128.6, 128.6, 127.8, 127.8, 127.8, 127.8, 127.1, 127.1, 127.0, 125.4, 124.4, 124.4, 31.2, 21.3.; HREI-MS: *m/z* calcd for $C_{29}H_{23}BrN_6O_4S_2$ [M]$^+$662.0406; Found; 662.0401.

N'-(1-(3,4-dichlorophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-4-methyl-2-nitrobenzenesulfonohydrazide (12)

Yield: 65 %; white solid; m.p: 205 – 207 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.12 (s, 1H, Triazole-H), 10.5 (s, 1H, NH), 8.35 (s, 1H, Ar-H), 8.27 (d, *J*=7.1 Hz, 2H, Ar-H), 8.27 (d, *J*=7.0 Hz, 2H, Ar-H), 8.03 (d, *J*=7.2 Hz, 2H, Ar-H), 8.01 (d, *J*=7.1 Hz, 1H, Ar-H), 7.86 (d, *J*=7.1 Hz, 1H), 7.85 (s, 1H, Ar-H), 7.73 (d, *J*=7.6 Hz, 1H, Ar-H), 7.69 (d, *J*=7.0 Hz, 1H, Ar-H), 3.77 (s, 2H, CH_2), 2.30 (s, 3H, CH_3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.1, 157.5, 155.4, 147.6, 147.1, 142.5, 138.6, 135.7, 135.4, 133.5, 133.5, 131.4, 130.6, 130.3, 128.1, 127.0, 127.0, 125.3, 125.3, 124.4, 124.4, 131.2, 20.3.; HREI-MS: *m/z* calcd for $C_{23}H_{17}Cl_2N_7O_6S_2$ [M]$^+$621.0059; Found; 621.0054.

N'-(1-(3,4-dichlorophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-4-nitrobenzenesulfonohydrazide (13)

Yield: 67 %; yellow solid; m.p: 187 – 188 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.13 (s, 1H, Triazole-H), 10.4 (s, 1H, NH), 8.38 (d, *J*=7.0 Hz, 2H, Ar-H), 8.27 (d, *J*=7.4 HZ, 2H, Ar-H), 8.05 (d, *J*=7.1 HZ, 2H, Ar-H), 8.03 (d, *J*=7.3 HZ, 2H, Ar-H), 7.86 (d, *J*=7.6 Hz, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 7.69 (d, *J*=7.1 Hz, 1H, Ar-H), 3.77 (s, 2H, CH_2), ^{13}C NMR (150 MHz, DMSO-d_6): δ 157.8, 156.6, 155.5, 152.0, 147.8, 138.4, 135.6, 132.5, 132.5, 130.5, 130.5, 127.2, 127.2, 126.9, 126.9, 126.3, 124.2, 124.4, 124.2, 124.2, 31.2.; HREI-MS: *m/z* calcd for $C_{23}H_{17}Cl_2N_7O_6S_2$ [M]$^+$ 606.9902; Found; 606.9900.

4,5-dichloro-N'-(1-(4-methyl-2-nitrophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)thio)ethylidene)-2-nitrobenzenesulfonohydrazide (14)

Yield: 61 %; yellow solid; m.p: 195 – 198 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.12 (s, 1H, Triazole-H), 10.5 (s, 1H, NH), 8.43 (s, 1H, Ar-H), 8.42 (s, 1H, Ar-H), 8.27 (d, *J*=7.2 Hz, 2H, Ar-H), 8.03 (d, *J*=7.3 Hz, 2H, Ar-H), 8.02 (d, *J*=7.0 HZ, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 7.66 (d, *J*=7.6 Hz, 1H, Ar-H), 2.30 (s, 3H, CH_3), 3.77 (s, 2H, CH_2),^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 156.7, 155.6, 147.8, 146.7, 141.6, 139.8, 138.6, 135.2, 133.9, 133.9, 132.4, 130.0, 128.9, 127.0, 127.0, 125.9, 125.0, 124.4, 124.4, 30.2, 20.3.; HREI-MS: *m/z* calcd for $C_{23}H_{16}Cl_2N_8O_8S_2$ [M]$^+$ 665.4490; Found; 665.4486.

N'-(1-(3,4-dichlorophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-4′-methyl-[1,1′-biphenyl]-4-sulfonohydrazide (15)

Yield: 68 %; Light green solid; m.p: 193 – 195 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11. 12 (s, 1H, Triazole-H), 10.5 (s, 1H, NH), 8.27 (d, *J*=7. 3 Hz, 2H, Ar-H), 8.03 (d, *J*=7.0 Hz, 2H, Ar-H), 7.88 (d, *J*=7.6 HZ, 4H, Ar-H), 7.86 (d, *J*=7.0 Hz, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 7.69 (d, *J*=7.4 Hz, 1H, Ar-H), 7.33 (d, *J*=7.5 Hz, 2H, Ar-H), 7.15 (d, *J*=6.9 Hz, 2H, Ar-H), 3.77 (s, 2H, CH_2), 2.34 (s, 3H, CH_3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 157,6, 155.6, 144.9, 138.6, 137.8, 135.7, 133.5, 133.0, 130.6, 130.6, 130.3, 129.5, 129.5, 127.8, 127.8, 127.8, 127.8, 127.1, 127.1, 127.0, 127.0, 126.3, 124.4, 124.4, 31.2, 21.3.; HREI-MS: *m/z* calcd for $C_{29}H_{22}Cl_2N_6O_4S_2$ [M]$^+$652.0521; Found; 652.0518.

N'-(1-(3,4-dichlorophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)benzenesulfonohydrazide (16)

^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.12 (s, 1H, Triazole-H), 10.5 (s, 1H, NH), 8.27 (d, *J*=7.1 Hz, 2H, Ar-H), 8.01 (d, *J*=7.1 Hz, 2H, Ar-H), 7.83 (d, *J*=7.5 Hz, 1H, Ar-H), 7.81 (s, 1H, Ar-H), 7.69 (d, *J*=7.2 Hz, 1H, Ar-H), 7.63 (t, *J*=6,8 Hz, 1H, Ar-H),7.59 (t, *J*=6.9 Hz, 2H, Ar-H), 3.77 (s, 2H, CH_2), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 157.6, 155.6, 147.9, 138.9, 138.6, 135.7, 133.5, 133.5, 131.9, 130.6, 130.3, 129.0, 129.0, 127.3, 127.3, 127.0, 127.0, 126.3, 124.4, 124.4, 31.2.; HREI-MS: *m/z* calcd for $C_{22}H_{16}Cl_2N_6O_4S_2$ [M]$^+$562.0051; Found; 562.0046.

N'-(1-(3,4-dichlorophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-3-nitrobenzenesulfonohydrazide (17)

Yield: 73 %; white solid; m.p: 201 – 203 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.12 (s, 1H, Triazole-H), 10.4 (s, 1H, NH), 8.54 (d, *J*=7.3 Hz, 1H, Ar-H), 8.43 (s, 1H, Ar-H), 8.27 (d, *J*=7.1 Hz, 2H, Ar-H), 8.22 (d, *J*=7.6 Hz, 1H, Ar-H), 8.03 (d, *J*=7.1 Hz, 2H, Ar-H), 7.97 (t, *J*=6.4 Hz, 1H, Ar-H), 7.86 (d, *J*=6.3 Hz, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 7.69 (d, *J*=7.2 Hz, 1H, Ar-H), 3.77 (s, 2H, CH_2), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.6, 157.4, 155.4, 148.1, 147.7, 140.4, 138.4, 135.5, 133.4, 133.4, 133.2, 130.6, 130.3, 129.7, 127.1, 127.0,127.0, 126.3, 124.4, 124.4, 123.1, 31.2.; HREI-MS: *m/z* calcd for $C_{22}H_{15}Cl_2N_7O_6S_2$ [M]$^+$606.9902; Found; 606.9900.

4-Methyl-2-nitro-N'-(1-(2-nitrophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)thio)ethylidene)benzenesulfonohydrazide (18)

Yield: 69 %; brown solid; m.p: 189 – 192 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.12 (s, 1H, Triazole-H), 10.3 (s, 1H, NH), 8.35 (s, 1H, Ar-H), 8.27 (d, *J*=7.2 Hz, 2H, Ar-H), 8.07 (d, *J*=7.0 Hz, 1H, Ar-H), 8.03 (d, *J*=7.1 Hz, 2H, Ar-H), 8.01 (d, *J*=7.5 Hz, 1H, Ar-H), 7.98 (d, *J*=7.3 Hz, 1H, Ar-H), 7.89 (t, *J*=6.8 Hz, 1H, Ar-H), 7.73 (d, *J*=7.3 Hz, 2H, Ar-H), 7.59 (t, *J*=6.7 Hz, 1H, Ar-H), 3.77 (s, 2H, CH_2), 2.30 (s, 3H, CH_3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 157.6, 155.6, 147.9, 147.1, 142.5, 138.6, 135.4, 135.4, 134.2, 131.9, 131.8, 131.4, 128.1, 127.0,127.0, 126.4, 125.7, 125.4, 124.4, 124.4, 30.2, 20.3.; HREI-MS: *m/z* calcd for $C_{23}H_{18}N_8O_8S_2$ [M]$^+$598.0689; Found; 598.0684.

N'-(1-(3-methoxyphenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-4-methyl-2-nitrobenzenesulfonohydrazide (19)

Yield: 68 %; white solid; m.p: 194 – 196 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.12 (s, 1H, Triazole-H), 10.2 (s, 1H, NH), 8.35 (s, 1H, Ar-H), 8.27 (d, *J*=7.2 Hz, 2H, Ar-H), 8.03 (d, *J*=7.2 Hz, 2H, Ar-H), 8.01 (d, *J*=7.1 Hz, 1H, Ar-H), 7.73 (d, *J*=7.1 Hz, 1H, Ar-H), 7.62 (d, *J*=7.7 Hz, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 7.31 (t, *J*=6.8 Hz, 1H, Ar-H), 7.07 (d, *J*=7.1 Hz, 1H, Ar-H), 3.77 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 171.8, 168.5, 147.7, 145.8, 142.5, 140.8, 137.4, 132.2, 130.8, 129.4, 126.2, 125.7, 123.9, 123.1, 118.8, 114.0, 113.7, 109.2, 106.5, 104.6, 55.6, 45.6, 11.6, 8.9; HREI-MS: *m/z* calcd for $C_{24}H_{21}N_7O_2S_2$ [M]$^+$583.0944; Found; 583.0939.

4-Nitro-N'-(1-(2-nitrophenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)benzenesulfonohydrazide (20)

Yield: 66 %; brown solid; m.p: 197 – 199 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.10 (s, 1H, Triazole-H), 10.1 (s, 1H- NH), 8.38 (d,*J*=7.3 Hz,2H, Ar-H), 8.27 (d, *J*=7.1 Hz, 2H, Ar-H), 8.07 (d, *J*=7.8 Hz, 1H, Ar-H), 8.05 (d, *J*=7.6 Hz, 2H, Ar-H), 8.03 (d, *J*=7.2 Hz, 2H, Ar-H), 7.98 (d, *J*=7.0 Hz, 1H, Ar-H), 7.89 (t, *J*=6.8 Hz, 1H, Ar-H), 7.59 (t, *J*=6.5 Hz, 1H, Ar-H), 3.77 (s, 2H, CH_2), ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.8, 157.6, 155.6, 152.1, 151.1, 147.9, 138.6, 135.4, 134.3, 131.9, 131.8, 128.2, 127.0, 127.0, 126.4, 125.4, 124.4, 124.4, 124.2, 124.2, 30.2; HREI-MS: *m/z* calcd for $C_{22}H_{16}N_8O_8S_2$ [M]$^+$584.0533; Found; 584.0528.

N'-(1-(3-methoxyphenyl)-2-((5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) thio)ethylidene)-4-nitrobenzenesulfonohydrazide (21)

Yield: 72 %; yellow solid; m.p: 201 – 203 °C; ^{1}H NMR (600 MHZ, DMSO-d_6): δ 11.13 (s, 1H, Triazole-H), 10.5 (s, 1H, NH), 8.38 (d, *J*=7.2 Hz, 2H, Ar-H), 8.27 (d, *J*=7.6 Hz, 2H, Ar-H), 8.05 (d, *J*=7.1 Hz, 2H, Ar-H), 8.03 (d, *J*=7.0 HZ, 2H, Ar-H), 7.62 (d, *J*=7.1 Hz, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 7.31 (t, *J*=6.7 Hz, 1H, Ar-H), 7.07 (d, *J*=7.0 Hz, 1H, Ar-H), 3.77 (s, 2H, CH_2), 3.76(s, 3H, OCH_3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.7, 158.8, 157.6, 155.4, 152.0, 151.1, 147.7, 138.4, 134.9, 129.6, 128.0, 126.9, 126.9, 124.3, 124.3, 124.1, 124.1, 120.4, 116.2, 113.1, 55.6, 31.1.; HREI-MS: *m/z* calcd for $C_{23}H_{19}N_7O_7S_2$ [M]$^+$ 569.0787; Found; 569.0783.

Acetylcholinesterase activity assay protocol

Based on previously described methods, *in vitro* study of acetylcholinesterase inhibitory profile was calculated [22,23]. The compounds being analyzed were subjected to dimethyl sulfoxide (DMSO) in order to make the stock solution (1 mg/mL). In addition, the working solutions were also prepared by using serial dilution (1–100 μg /mL). The solution of acetylcholinesterase enzyme (20 μL; 0.1 U/mL), analogues being tested with different concentration and buffer of sodium-phosphate (150 μL; pH 8.0; 0.1 M) were pre-incubated appropriately for some time at 25 °C. The process was initiated as 5,5′-dithio-*bis*-(2-nitrobenzoic acid) (DTNB) (10 mM; 10 μL) and acetylcholinesterase inhibitor (AChEI) (14 mM; 10 μL) were accumulated, thus the resulting residue was mixed (by using cyclomixer) and put on incubation for 10 min at 25 °C. Instead of compounds being tested having 10 μL dimethyl sulfoxide, the absorbance against blank-reading was calculated by using microplate-reader at 410 nm. By using the given formula, % inhibition and IC_{50} values were measured in comparison to donepezil (0.01–100 μg/mL) as reference standard.

$$\%Inhibition = (Absorbance\ of\ control - Absorbance\ of\ compound) \times 100/A_{Control}$$

The IC_{50} was calculated by constructing a non-linear regression graph between % inhibition vs concentration, using Graph Pad prism software (version 5.3).

Butyrylcholinesterase activity assay protocol

In order to explore the inhibition profile of *in vitro* BuChE enzyme, the similar procedure was adopted with little bit alteration. For measurement of BuChE activity, the solution containing BuChE enzyme was used [22,23].

Assay protocol for docking study

To triangulate the results of *in vitro* and in silico investigation, Auto Dock Vina software programme was utilized in molecular docking to ascertain how synthesized analogues interact with both targeted enzymes (AChE and BuChE). The crystal structures for both targets were obtained from the RCSB protein databank using the PDB codes 1ACL for (AChE) and 1P0P for (BuChE). Using the standard MOE-Dock module parameters, the crystallographic structures and all synthesized analogues were protonated, producing analogue structures and an optimized enzyme. Following that, a docking investigation was carried out with the structures of the analogues and the optimized enzyme. All of the specific details of the docking method are contained in previously reported studies [24,25].

Conclusion

1,2,4-Triazole bearing sulfonohydrazide derivatives (**1–21**) were synthesized and evaluated against AChE and BuChE enzymes. All the synthesized compounds show a good to excellent inhibitory potential with IC_{50} values ranged from 0.30 ± 0.050 to 15.21 ± 0.50 μM (against AChE) and 0.70 ± 0.050 to 18.27 ± 0.60 μM (against BuChE) as compared to the reference drug Donepezil (IC_{50} = 2.16 ± 0.12 and 4.5 ± 0.11 μM, respectively). Derivatives **2** and **4** were found totally inactive. Analogues 17 (IC_{50} = 0.30 ± 0.050 and 0.70 ± 0.050 μM respectively) was found the most potent analogue against both AChE and BuChE enzymes. A molecular docking study was carried out to show the binding interaction of the active site of the synthesized analogues with the targeted enzyme.

CRediT authorship contribution statement

Mohamed S. Othman: Writing – review & editing. **Haseena Naz:** Methodology. **Fazal Rahim:** . **Hayat Ullah:** Formal analysis, Conceptualization. **Rafaqat Hussain:** . **Muhammad Taha:** . **Shoaib Khan:** . **Mohamed A. Fareid:** Writing – review & editing. **Shimaa M. Aboelnaga:** Writing – review & editing. **Anas T. Altaleb:** . **Rashid Iqbal:** . **Syed Adnan Ali Shah:** Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was funded by Scientific Research Deanship at University of Ha'il—Saudi Arabia through project number RG-23 161.

References

[1] A. kumar, A. singh ekavali. A review on Alzheimer's disease pathophysiology and its management: an update. J. Pharmacol. Rep. 67 (2015) 195-203.
[2] A. Atri, The Alzheimer's Disease Clinical Spectrum, Med. Clin. n. Am. 103 (2019) 263–293.
[3] S. Khan, H. Ullah, R. Hussain, M. Khan, A. Sattar, M.S. Khan, Synthesis, *in Vitro* Bio-evaluation, and Molecular Docking Study of Thiosemicarbazone-based Isatin/bis-Schiff base Hybrid Analogues as Effective Cholinesterase Inhibitors, J. Mol. Struct. 135351 (2023).
[4] S. Khan, Y. Khan, A.M. Al-Qaaneh, R. Hussain, T. Iqbal, H. Ullah, M. Akif, S. Al Zahrani, S. Aghayeva, Exploring effective diagnosis of Alzheimer disease: Experimental and computational analysis of hybrid benzimidazole based thiazolidinone derivatives, Results in Chemistry. 9 (2024) 101663.
[5] F. Rahim, H. Ullah, M. Taha, R. Hussain, M. Sarfraz, M.A. Abdelaziz, F.S. Alatawi, N. Zidan, I. Jafri, K.M. Khan, Synthesis of new triazole based thiosemicarbazone derivatives as anti-Alzheimer's disease candidates: Evidence-based on in vitro study, Molecules 28 (2023) 21.
[6] a) M. Elmusa, S. Elmusa, S. Mert, R. Kasımoğulları, F. Türkan, M. N. Atalar, E. Bursal. One-pot three-component synthesis of novel pyrazolo-acridine derivatives and assessment of their acetylcholinesterase inhibitory properties: An in vitro and in silico study. Journal of Molecular Structure. 1274 (2023) 134553. b) A. Cetin, E. Bursal, F. Türkan. 2-methylindole analogs as cholinesterases and glutathione S-transferase inhibitors: Synthesis, biological evaluation, molecular docking, and pharmacokinetic studies. Arabian Journal of Chemistry. 14 (2021) 103449.
[7] M. Yadov, K.A. Kumar, D. Kumar, Indole-chalcone linked 1,2,3-triazole hybrid: Facile synthesis, anti-microbial evaluation and docking studies as potential anti-microbial agents, J. Mol. Struct. 1261 (2022) 132867.
[8] B. Wang, D. Chu, Y. Feng, Y. Shen, M. Aoyagi-Scharber, L.E. Post, Discovery and Characterization of (8 S, 9 R)-5-Fluoro-8-(4-fluorophenyl)-9-(1-methyl-1 H-1, 2, 4-triazol-5-yl)-2, 7, 8, 9-tetrahydro-3 H-pyrido[4, 3, 2-de]phthalazin-3-one (BMN 673, Talazoparib), a Novel, Highly Potent, and Orally Efficacious Poly (ADP-ribose) Polymerase-1/2 Inhibitor, as an Anticancer Agent, J. Med. Chem. 59 (2016) 335–357.
[9] S. Khan, R. Hussain, H. Ullah, Y. Khan, H.S. Almoallim, M.J. Ansari, Identification of 1,2,4-triazole-bearing Bis-Schiff base hybrid scaffolds: In vitro and in silico insights to develop promising anti-urease and anti-cancer agents, Results in Chemistry. 7 (2024) 101540.
[10] S. Khan, T. Iqbal, R. Hussain, Y. Khan, H. Ullah, U. Jamal, S. Alfarraj, S.A. Alharbi, Structure based design, synthesis of novel fused triazole-thiazole derivatives and their comparative study on Alzheimer disease along with in silico modeling, Results in Chemistry. 8 (2024) 101590.
[11] H. Medetalibeyoğlu, F. Türkan, S. Manap, E. Bursal, M. Beytur, A. Aras, H. Yüksek, Synthesis and acetylcholinesterase enzyme inhibitory effects of some novel 4,5-Dihydro-1*H*-1,2,4-triazol-5-one derivatives; an *in vitro* and *in silico* study, J. Biomol. Struct. Dyn. 41 (10) (2022) 4286–4294.
[12] F. Rahim, H. Ullah, R. Hussain, M. Taha, S. Khan, M. Nawaz, F. Nawaz, S.J. Gilani, M.N.B. Jumah, Thiadiazole based triazole/hydrazone derivatives: Synthesis, in vitro α-glucosidase inhibitory activity and in silico molecular docking study, J. Mol. Struct. 1287 (2023) 135619.
[13] H.R. Obayes, A. Ahmed, A.A. Thamer, M. Abu Bakar, Sulphonamides as corrosion inhibitor: experimental and DFT studies, J. Mol. Struct. 1138 (2017) 27–34.
[14] W. Ullah, F. Rahim, S. Hayat, H. Ullah, M. Taha, S.A.A. Shah, K.M. Khan, Synthesis of Indole Based Sulfonamide Derivatives as potent inhibitors of α-glucosidase and α-amylase in management of type-II diabetes, Chem. Data Collect. 50 (2024) 101122.
[15] S. Maddila, R. Pagadala, S.B. Jonnalagadda, Triazole: A review of synthetic approach and the biological activity, Lett. Org. Chem. 10 (2013) 693–714.
[16] T. Arslan, M. Senturk, L. Karagoz, Y. Karagoz, D. Ekinci, A. Efe, E.A. Türkoğlu, F. Uras, Synthesis, *in vitro* and in silico Biological Studies of Sulfonamide Chalcones as Esterase Inhibitors, Chemistry Select. 7 (2022) e202202993.
[17] H. Ullah, F. Rahim, H. Zada, S. Hayat, F. Khan, M.S. Khan, Synthesis, molecular docking, and bioactivity study of isoquinoline-sulfonamide hybrid analogues: A promising acetylcholinesterase and butyrylcholinesterase inhibitor candidate, Chem. Data Collect. 46 (2023) 101048.
[18] H. Ullah, F. Khan, M. Taha, M. Ullah, New Thiazole-Bearing Oxadiazole Derivatives: Synthesis, Thymidine Phosphorylase Inhibitory Potential and Molecular Docking Study, Russ. J. Org. Chem. 57 (12) (2021) 1993–2001.
[19] S. Khan, H. Ullah, M. Taha, F. Rahim, M. Sarfraz, R. Iqbal, M.A. Abdelaziz, F. S. Alatawi, K.M. Khan, Synthesis, DFT Studies, Molecular Docking and Biological Activity Evaluation of Thiazole-Sulfonamide Derivatives as Potent Anti-Alzheimer's Inhibitors, Molecules 28 (2023) 559.
[20] F. Rahim, H. Ullah, M. Taha, R. Hussain, N. Iqbal, S. Khan, Synthesis of New Triazole-Based Thiosemicarbazone Derivatives as Anti-Alzheimer's Disease Candidates: Evidence-Based *In Vitro* Study, Molecules 28 (2023) 21.
[21] R. Hussain, H. Ullah, F. Rahim, M. Sarfraz, W. Rehman, S. Khan, Multipotent Cholinesterase Inhibitors for the Treatment of Alzheimer's Disease: Synthesis, Biological Analysis and Molecular Docking Study of Benzimidazole-Based Thiazole Derivatives, Molecules 27 (2022) 6087.
[22] F. Rahim, H. Ullah, M. Taha, R. Hussain, M. Sarfraz, R. Iqbal, N. Iqbal, S. Khan, S. A. Ali Shah, M.A. Albalawi, M.A. Abdelaziz, Synthesis of New Triazole-Based Thiosemicarbazone Derivatives as Anti-Alzheimer's Disease Candidates: Evidence-Based In Vitro Study, Molecules 28 (1) (2023) 21.
[23] S. Khan, H. Ullah, M. Taha, F. Rahim, M. Sarfraz, R. Iqbal, N. Iqbal, R. Hussain, S. A. Ali Shah, K. Ayub, M.A. Albalawi, Synthesis, DFT Studies, Molecular Docking

and Biological Activity Evaluation of Thiazole-Sulfonamide Derivatives as Potent Alzheimer's Inhibitors, Molecules 28 (2) (2023) 559.

[24] S. Khan, H. Ullah, R. Hussain, Y. Khan, M.U. Khan, M. Khan, A. Sattar, M.S. Khan, Synthesis, in vitro bio-evaluation, and molecular docking study of thiosemicarbazone-based isatin/bis-Schiff base hybrid analogues as effective cholinesterase inhibitors, J. Mol. Struct. 1284 (2023) 135351.

[25] R. Hussain, H. Ullah, F. Rahim, M. Sarfraz, M. Taha, R. Iqbal, W. Rehman, S. Khan, S.A.A. Shah, S. Hyder, M. Alhomrani, Multipotent Cholinesterase Inhibitors for the Treatment of Alzheimer's Disease: Synthesis, Biological Analysis and Molecular Docking Study of Benzimidazole-Based Thiazole Derivatives, Molecules 27 (18) (2022) 6087.