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Novel pyrrole based triazole moiety as therapeutic hybrid: synthesis, characterization and anti-Alzheimer potential with molecular mechanism of protein ligand profile

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Abstract

As a springboard to explore novel potent inhibitors of cholinesterase enzymes (AChE and BChE) responsible for causing Alzheimer disorder, the current study was conducted to synthesize pyrrole derived triazole based Schiff base scaffolds by facile synthetic route. These compounds were validated by ^{1}HNMR, ^{13}CNMR and HREI-MS. All these scaffolds (1–16) were examined for their inhibitory activity against AChE and BChE in contrast to Donepezil (**10.20 ± 0.10 and 10.80 ± 0.20 µM**) and Allanzanthone (**12.40 ± 0.10 and 13.10 ± 0.10 µM**). All pyrrole derived triazole based Schiff base scaffolds (1–16) showed varied range of inhibitory potentials against acetylcholinesterase and butyrylcholinesterase enzymes with lowest inhibition concentration values ranging from **5.10 ± 0.40–27.10 ± 0.10 µM** (for AChE) and **5.60 ± 0.30–28.40 ± 0.30 µM** (for BChE). SAR analysis of these derivatives revealed analog 7 as lead molecule against targeted enzyme, while analog 6 and 11 were ranked as second and third most potent scaffolds. Binding affinity and selectivity of potent molecules against targeted enzymes were examined by molecular docking and obtained results showed that potent molecule have versatile significant binding interactions with stated enzymes. Furthermore, safety profiles of potent analogues were predicted via ADMET protocols.

Keywords Pyrrole, Triazole, Schiff Base, AChE & BChE, Molecular Docking, ADMET

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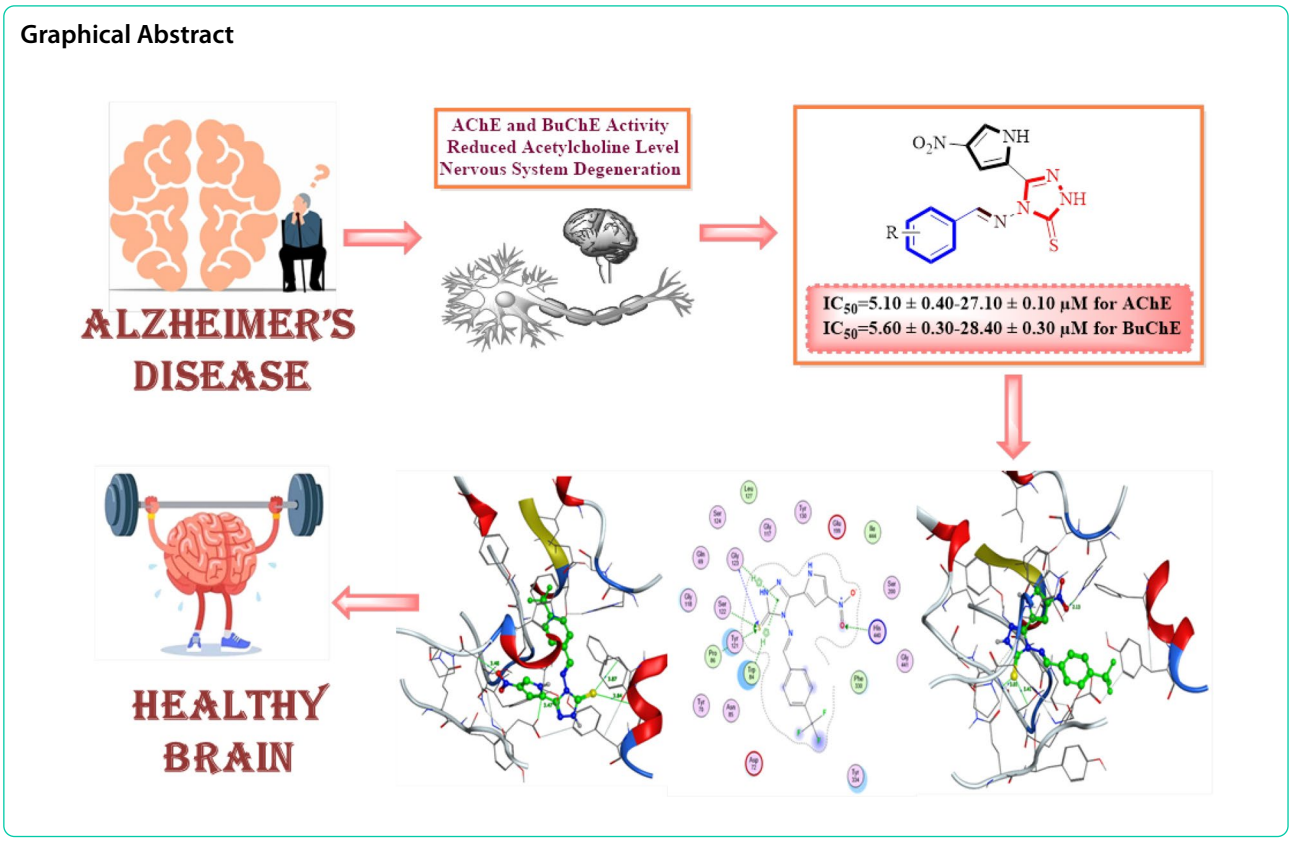
Graphical Abstract



Introduction

Alzheimer's disease is a complex and chronic neurodegenerative condition which is most common among old aged population [1, 2]. AD considered as a fatal disorder, as its symptoms appear after years of brain degeneration and cognitive disturbance [3, 4]. Mostly signs and clinical symptoms include memory loss, trouble in thinking, difficulties in decision making, working instability, and disturbed speaking and these symptoms becomes critical with the passage of time [5]. A key causative factor for AD is severe accumulation of aggregate of amyloid β-protein which leads to plaque formation [6] and also aggregation of hyperphosphorylated τ-protein complex [7], but still the cure of AD is not yet possible as it is a multifactorial disorder. Aβ and τ-proteins complex aggregation were characterized by maximum activity of acetylcholinesterase as well as butyrylcholinesterase enzymes. These enzymes were responsible for the conversion of acetylcholine neurotransmitter into choline and acetic acid. This leads to degeneration of muscarinergic neurons and ultimately reduced the acetylcholine level in the brain [8]. Tacrine, Galantamine, Rivastigmine and Donepezil were used as a treatment for AD. These marketed available drugs only control symptoms of AD upto some extent by inhibiting the activity of AChE and BChE enzymes but don't completely impede the AD as well as these drugs have mixed response as they caused certain critical side effects including abdominal pain, [9, 10]. Therefore, there is urgent need to design and synthesized potent inhibitors having enhanced biological potential with minimal side effects or complications.

Pyrrole is nitrogen containing heterocyclic compound, having a lot of importance in the field of medicinal chemistry. Scaffolds containing pyrrole moiety displayed diverse pharmacological characteristics due to their significant biological structure [11]. They were used as anticancer [12], anti-microbial [13], anti-histamine [14], and anti-inflammatory [15] agents. These scaffolds were also involved in the inhibition of many viruses responsible for causing different severe infections [16]. These moieties inhibit the activity of these viruses and prevent human body from critical infections (Fig. 1).

Hybrid analogues of triazole based scaffolds exhibit fungicidal [17], bactericidal [18], anti-viral [19], anticancer [20], anti-convulsant [21], anti-diabetic [22] as well as anti-tubercular [23] characteristics. Their unique and diverse biological nature leads to their significant biological characteristics [24–34]. Similarly, Schiff bases are also significant group in the field of pharmaceutical due to their biological properties. They

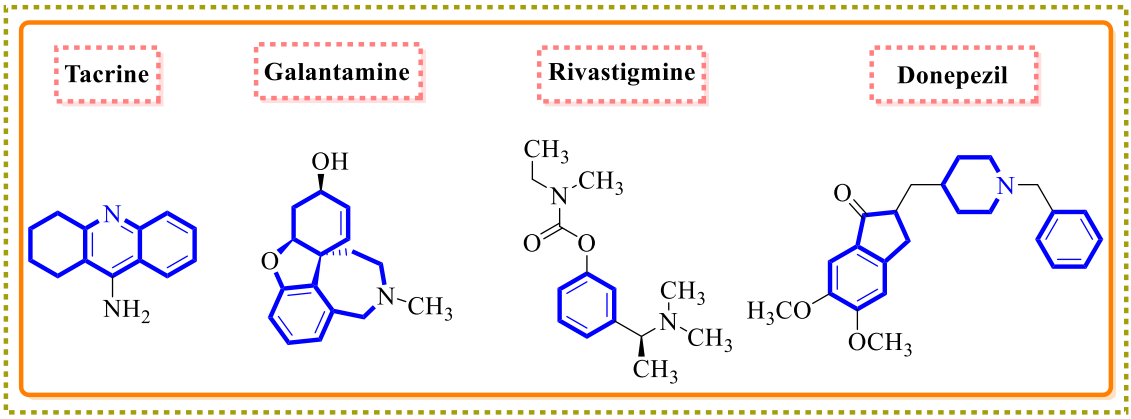


Fig. 1 Marketed available drugs for Alzheimer disease treatment

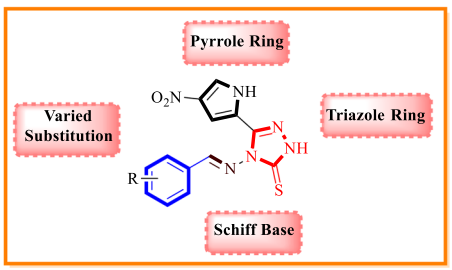


Fig. 2 General representation of synthesized pyyrole derived triazole based Schiff base analogues

were involved in the inhibition of various enzymes including AChE and BChE [35], α-amylase and α-glucosidase [36], β-glucoronidase [37], urease [38] and also used for the inhibition of various cancer cell lines growth [39]. Hybrid analogues of enhanced and cumulative inhibitory potential were designed by hybridizing two or more active moieties which individually possessed unique properties for the treatment of certain diseases. By this synthetic approach, various candidates have been reported by different researchers to cure and control the critical symptoms of AD [40, 41]. In current study, pyrrole, triazole as well as Schiff base moieties were hybridized to synthesized biological active compounds having maximum inhibitory potential (Fig. 2). These compounds were evaluated against AChE and BChE enzymes, to identify their anti-Alzheimer potency.

Pyrrole, triazole as well as various azomethine (Schiff base) containing analogues were previously synthesized by different researchers and evaluated against AChE and BChE enzymes, (Fig. 3) represents rationale study of pyrrole [42], Schiff base [43] and trizaole [44] containing scaffolds and their biological potential as well as newly reported analogues and their biological profile.

Result and discussion

Chemistry

Pyrrole derived triazole based Schiff base scaffolds (1–16) were synthesized by facile synthetic pathway comprised of two steps reaction. In this procedure, first pyrrole bearing acid moiety (I) was allowed to react with hydrazine carbothiohydrazide in ethanol and reaction was catalyzed by treithylamine under refluxed for 18 h to obtain pyrrole derived triazole bearing amine and thione group as intermediate (II). After confirmation of reaction completion by TLC, intermediate II was treated with benzaldehyde having varied substitution in ethanol (solvent) and acetic acid (catalyst) under refluxed for 5 h to afford varied substituted pyrrole derived triazole based Schiff base analogues (1–16), as illustrated in (Scheme 1). Impurities were removed from product by using n-hexane solvent (washing) and then solvent was evaporated under reduced pressure and fine powdered products were collected. These synthesized products were characterized via ^{1}HNMR, ^{13}CNMR and HREI-MS.

Biological evaluation for AChE and BChE

Pyrrole derived triazole based Schiff base scaffolds (1–16) were synthesized and assess against cholinesterase enzymes (AChE and BChE), to explore the enhanced biological potential of pyrrole, triazole as well as Schiff base moieties in a single molecule. For establishing comparison criteria, Donepezil (**10.20 ± 0.10 and 10.80 ± 0.20 µM**) and Allanzanthone (**12.40 ± 0.10 and 13.10 ± 0.10 µM**) were used as standard drug candidates. Results showed that these analogues (1–16) hold good to moderate inhibitory activity due to their versatile attached substituents (Table 1). The zone of inhibition of these derivatives against both enzymes was (**5.10 ± 0.40–27.10 ± 0.10 µM**) for AChE and

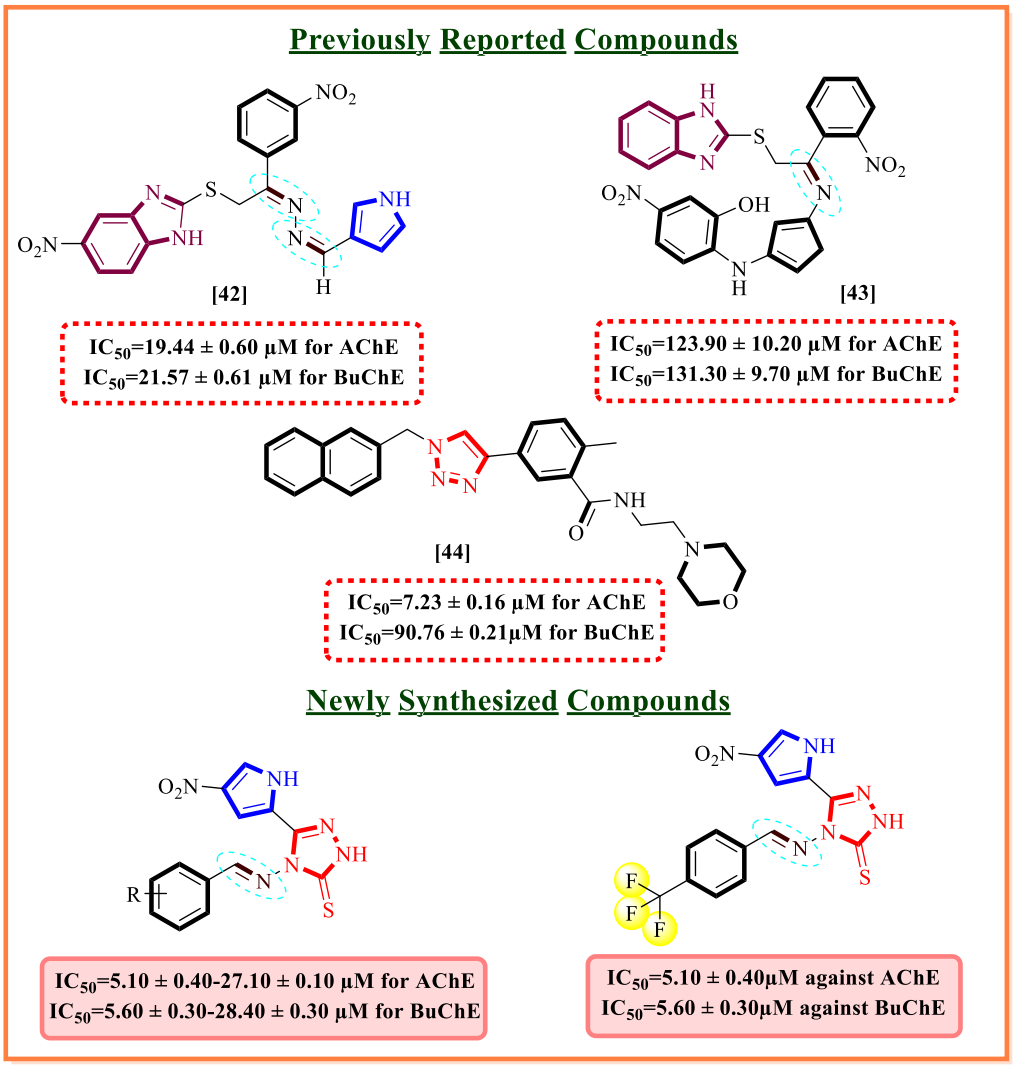
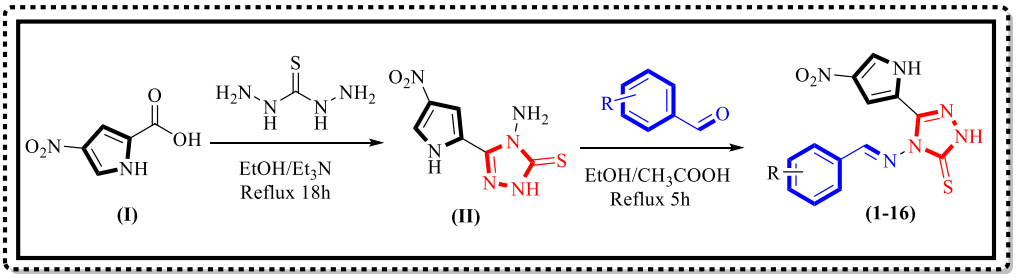


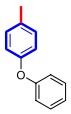
Fig. 3 Pyrrole, trizaole and Schiff base containing scaffolds and their biological potential as well as newly reported analogues and their biological potential (Rationale Study)



Scheme 1 Synthesis of pyrrole derived triazole based Schiff base scaffolds (1–16)

(**5.60 ± 0.30–28.40 ± 0.30 µM**) for BChE. They have varied biological potential due to different nature, different position of attachment and different numbers of attached substituents. Those substituents which have ring activating nature (EDG), or have high electronegativity, or attached on favorable position, they suppress maximum

Table 1 Varied Substitution of pyrrole derived triazole based Schiff base (1–16) and their biological profile

S/No	R	Acetylcholinesterase (AchE) IC_{50} = (μM) ± SEM	Butyrylcholinesterase (BChE) IC_{50} = (μM) ± SEM
1		8.20 ± 0.20	9.10 ± 0.50
2		14.20 ± 0.10	15.10 ± 0.10
3		15.10 ± 0.60	16.40 ± 0.20
4		13.20 ± 0.20	14.10 ± 0.20
5		19.30 ± 0.20	21.10 ± 0.10
6		6.20 ± 0.10	6.80 ± 0.20
7		5.10 ± 0.40	5.60 ± 0.30
8		13.60 ± 0.20	14.30 ± 0.10
9		8.80 ± 0.10	9.60 ± 0.10
10		22.90 ± 0.10	23.10 ± 0.20
11		7.10 ± 0.20	7.80 ± 0.30
12		9.20 ± 0.20	9.70 ± 0.20
13		15.50 ± 0.20	16.10 ± 0.20
14		25.10 ± 0.40	26.20 ± 0.40
15		27.10 ± 0.10	28.40 ± 0.30
16		21.20 ± 0.10	22.20 ± 0.10
Standard drug Donepezil Allanzanthone		10.20 ± 0.10\ 12.40 ± 0.10	**10.80 ± 0.20** **13.10 ± 0.10**

The bold values represent inhibitory values of standard compounds

enzymatic activity. Compound 7 was most potent scaffold and was found active against both enzymes in comparison to donepezil and Allanzanthone. Compound 6 and 11 also had better biological activity, while other analogs have good to moderate potency against AChE and BChE enzymes.

AChE and BChE biological assessment

*SAR a*nalysis for these synthesized derivatives showed that analog 7 substituted with trifluoromethyl ($-CF_3$) group on *para*-position of phenyl ring have better enzymatic activity profile (5.10 ± 0.40 µM, 5.60 ± 0.30 µM) against AChE and BChE enzymes (Fig. 4) and considered as the most potent analog of the synthesized series. The better activity of this analog might be due to attachment of $-CF_3$ group on favorable position, and spatial arrangement of this substituent in a molecule give rise to conformational rigidity and enhanced its binding affinity and selectivity towards targeted amino acids residues. At this position, by highly electronegative fluorine atoms, this $-CF_3$ group make maximum number of strong hydrogen bonding with targeted enzymes, and prevent these enzymes to catalyze normal biological reaction. Due to strong electron withdrawing property it withdraw electronic density from the ring and allow the ring as well as overall molecule to make effective binding interactions with anionic species present in the amino acids of targeted enzymes. $-CF_3$ group has the ability to improve the inhibitor potential and binding affinity to inhibit the activity of stated enzymes. This indicates that presence of $-CF_3$ substituent had greater impact on the activity of this analog, as it enhance its biological potential to suppress the enzymatic action of targeted enzymes.

Analog 6 having trihydroxy substituents were ranked as the second most active analog and have zone of inhibition (6.20 ± 0.10 µM, 6.80 ± 0.20 µM) as illustrated in (Fig. 5). This analog contain highly electron donating group (-OH) on both *ortho* and *para*-positions. These positions were considered as favorable position for electron donating group to donate electron towards ring and activate it. So, all these –OH group effectively activate phenyl rings, this highly electron rich phenyl ring interact with polar groups or polar moieties present in the amino acids of stated enzymes. These hydroxyl groups also have polar nature, and due to these three –OH groups, the overall polarity of the molecule increases which leads to its maximum binding affinity (polar interactions) towards targeted enzymes. This –OH group also have acidic nature and nucleophilic character, and these properties makes this analog highly potent against targeted enzymes. They attack on electrophilic region of amino acids due to lone pair of oxygen atoms, and inhibit their enzymatic activity. These –OH group also involved making multiple hydrogen bonds with amino acids of stated enzymes and enhanced the binding affinity or potency of this analog to inhibit their activity. These hydroxyl groups also influence the structural confirmation of this analog to completely fit into the active sites of targeted enzymes and inhibit their normal enzymatic potential.

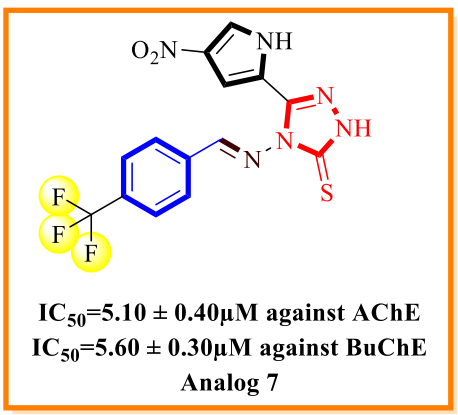


Fig. 4 Therapeutic potential of analog 7

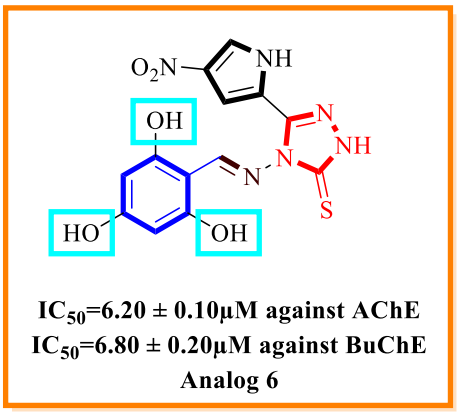


Fig. 5 Therapeutic potential of analog 6

Analog 11 was recognized as third most potent analog (7.10 ± 0.20 µM, 7.80 ± 0.30 µM) and contain –OH at *ortho*-position and -F on *para*-position (Fig. 6). The better biological profile of this analog might be attributed to these attached substituents. -OH moiety due to its electron donating effect, activate phenyl ring and this ring interact with receptor residues of targeted enzymes. –OH group have capability to make multiple hydrogen bonds (donor as well as acceptor hydrogen bond interactions), with targeted enzyme and increased the binding affinity of this analog. Moreover, -F group were also involved in hydrogen bonding as it is present on favorable *para*-position. The overall molecule has less steric effect on this –F

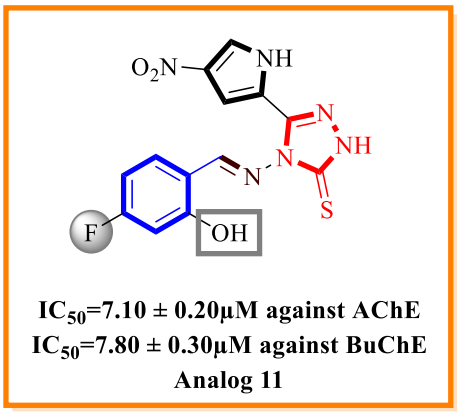


Fig. 6 Therapeutic potential of analog 11

group and it easily undergoes binding interactions with targeted enzymes. Both –OH and –F groups contributes to the selectivity and binding affinity of analog to inhibit the enzymatic action of AChE and BChE enzymes.

Similarly, analog 9 and 12 also have same substituents (-OH and –F) as of analog 11 but on different positions, and they are also potent analogues in comparison to donepezil and allanzanthone drugs. Analog 9 holds –OH as well as –F on *meta*-positions and have few folds more biological potential (8.80 ± 0.10 μM, 9.60 ± 0.10 μM) than analog 12 having –OH group on *meta* and –F on *para*-position (9.20 ± 0.20 μM, 9.70 ± 0.20 μM) (Fig. 7). Analog 9 has good biological profile due to involvement of both attached substituents in hydrogen bond formation. By these stable hydrogen bonds with targeted amino acids, they inhibit the activity of these stated enzymes. At these positions, both groups were spatially arranged, and this makes the molecule more flexible and selective to bind with receptor residues of targeted enzymes. Due to polar nature of –OH group, this analog makes dipole interactions with targeted enzymes and also it attacks on electrophilic sites of targeted amino acids due to its nucleophilic character. But when position of –F group was replaced to *para*-position as in analog 12, its binding affinity was slightly reduced in comparison to analog 9. At *para*-position –F group might get involved in making hydrogen bond with hydrogen atom of neighboring –OH group, and reduced its ability to inhibit the activity of targeted enzymes. But still this analog was found potent in comparison to donepezil and allanzanthone, as it still has capability to make several interactions with amino acid residues of targeted enzymes. Similarly, when –OH group was replaced with $–NO_2$ moiety at *meta*-position in analog 13, the biological activity was much declined. The additional nitro group might get involved in making interactions within the molecule. Moreover, in contrast to -OH group (which is EDG and ring activator), $-NO_2$ is electron withdrawing group and deactivates the phenyl ring.

Flouro substituted analog 1 also has more inhibitory potency than both reference drugs (8.20 ± 0.20 μM, 9.10 ± 0.50 μM) (Fig. 8). This -F at favorable position was capable of making stable and effective hydrogen bonding to inhibit the activity of stated enzymes and contributes to anti-Alzhiemer potential of this analog. It also enhance the selectivity, specificity and binding affinity of this molecule to completely get fit into the active sites of targeted enzymes, and prevent its to bind with substrate. On the other hand, analog 14 have –OMe substituent on same *para*-position, but have poor biological profile (25.10 ± 0.40 μM, 26.20 ± 0.40 μM) against AChE and BChE in comparison with donepezil as well as allanzanthone. This might be due to steric hindrance caused by methoxy group due to involvement of oxygen in hyper conjugation with methyl group. So, oxygen atom has less capability to make effective hydrogen bond. It reduced the overall binding affinity of molecule to bind with enzyme.

Analog 4 and 3 have same $–NO_2$ and –Cl groups on different position, and among them analog 4 having –Cl group on *para*-position and $–NO_2$ group on *meta*-position have moderate biological potential (13.20 ± 0.20 μM, 14.10 ± 0.20 μM) (Fig. 9) in contrast to reference drugs. At *para*-position –Cl moiety donate electron towards ring and this activated phenyl rings make possible anionic interactions with receptor sites, as well as both nitrogen and oxygen atoms of $–NO_2$ group might get involved in

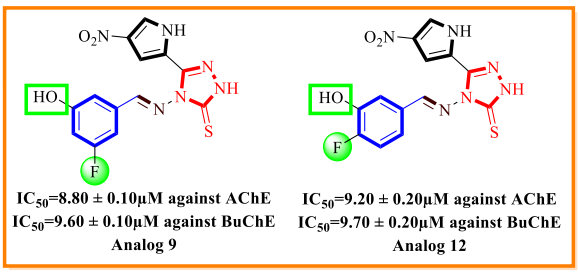


Fig. 7 Therapeutic potential of analog 9 and 12

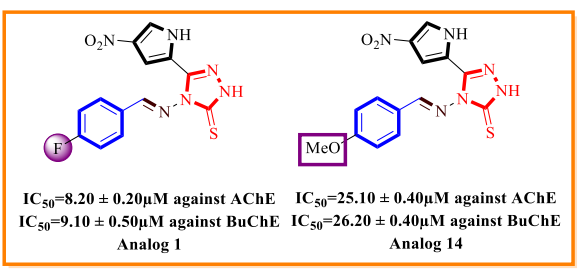


Fig. 8 Therapeutic potential of analog 1 and 14

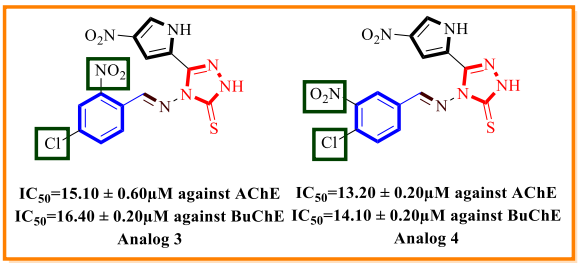


Fig. 9 Therapeutic potential of analog 3 and 4

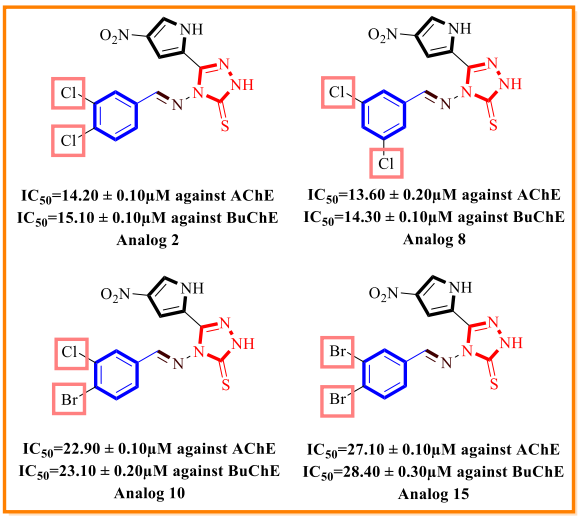


Fig. 10 Therapeutic potential of analog 2,8,10 and 15

hydrogen bond formation. But when $-NO_2$ moiety position was changed to *ortho* in analog 3, its activity was declined. At this position it might get involved in making binding interactions (hydrogen bond) with other atoms of the molecule and reduced the overall molecule selectivity towards targeted enzymes.

Among analog 2, 8 and 10, analog 8 having di-Cl groups on both *meta*-positions have comparable enzymatic potential in comparison to reference drugs (Fig. 10). This might be due to ring activating capability of these chlorine atoms by donating maximum electronic density towards the ring and contributes to the overall spatial arrangement of molecule and its binding affinity and selectivity. However, when one –Cl group was replaced with –Br group and place at *para*-position, its activity was reduced which observed in analog 10. This might be due to large size of bromine atom, which hinder the activity of –Cl group to activate ring and also caused severe steric hindrance around the phenyl ring and overall molecule, which leads to reduced binding affinity of this analog. Subsequently, when both –Cl group were replaced with –Br groups its biological activity was declined upto maximal level. As both –Br groups have bulky nature and sterically hinder the activity of analog 15 to bind with enzyme and prevent its normal enzymatic activity.

The overall discussion revealed these analogs activity was greatly influenced by attached substituents nature, their favorable positions and their number. It was noted that the replacement of these substituents and change in their position leads to change in binding affinity of these synthesized analogues.

Molecular docking

All these pyrrole derived triazole based Schiff base scaffolds have versatile biological profile and most of these analogues inhibit the activity of targeted enzymes up to larger extent in comparison to donepezil and allanzanthone. This strong inhibitory profile of these analogues was due to various types of interactions between these potent ligands and the targeted enzymes. These interactions were investigated by molecular docking study. To conduct molecular docking study both the proteins were retrieved from the online protein source RCSB protein data bank (PDB) using the code 1ACl for AChE and 1P0P for BChE. After retrieving, these proteins were prepared by removing water molecules and adding Kollman and Gasteiger charges. Polar hydrogen atoms were also added. Similarly, ligand molecules were also prepared by lowering their energy. After preparing proteins and ligands, both were docked to investigate the binding interactions between the two. This study revealed the binding affinity of potent molecule against targeted enzymes. It showed that how active analogue effectively fit into the active sites of targeted enzyme by making versatile interaction as illustrated in Figs. (11, 12, 13, 14, 15, 16). Various analogues docking were performed according to the specific docking parameters [45–51], and herein current study the docking tool Molecular Operational Environment (MOE) of Version-2019 was used. Results showed that these analogues have maximum binding interactions and have docking score with highly negative value (−14 to −10), respectively. This indicates that these analogues have maximum binding affinity and they have ability to bind strongly with enzymes active sites by making effective binding interactions. All these compounds have strong and effective binding interactions with different amino acids of targeted enzyme, due to their different attached substituents. As for analog 6, NO_2 moiety on pyrrole ring has capability to make side chain acceptor interactions. Sulfone group on triazole ring also involved in making side chain acceptor interactions, as well as hydrogen atom of hydroxyl substituted phenyl ring bind with arene moiety present in amino acids of

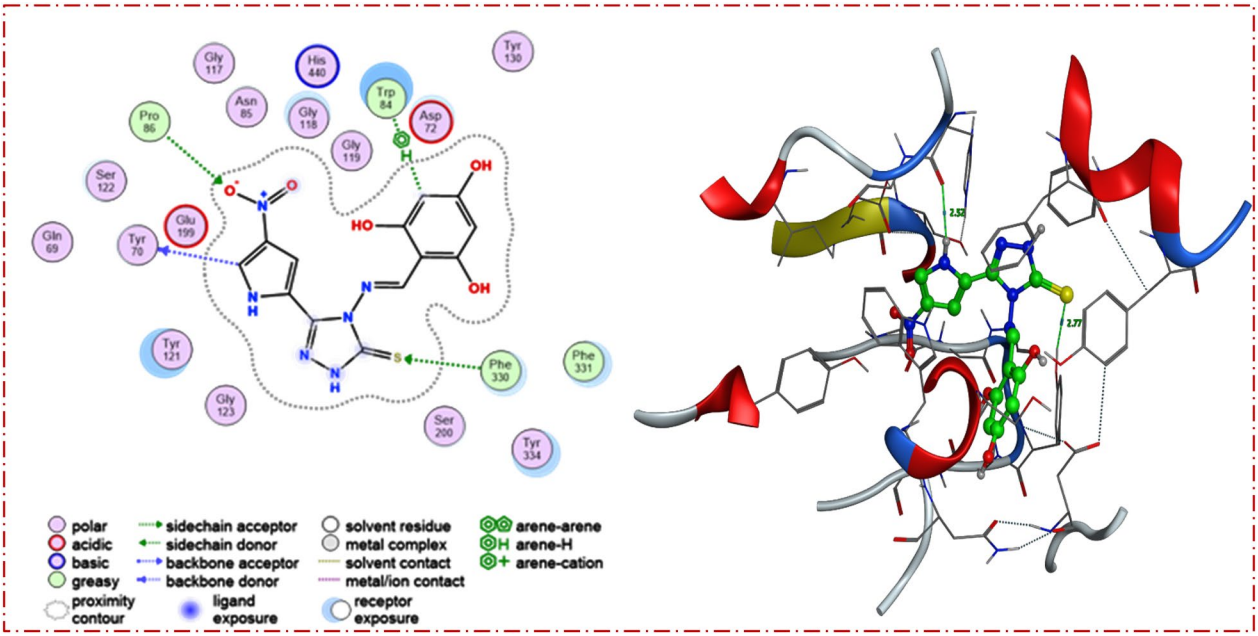


Fig. 11 Analog 6 binding affinity profile against AChE enzyme

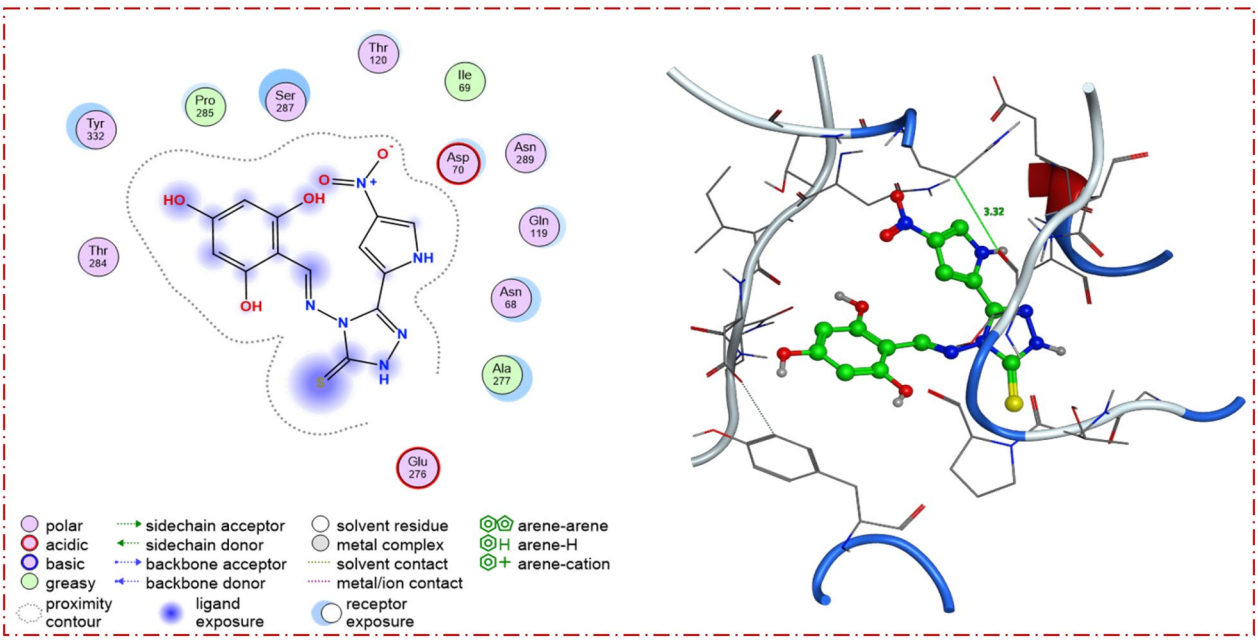


Fig. 12 Analog 6 binding affinity profile against BChE enzyme

targeted enzymes. Similarly, for BChE analog 6 have maximum ligand exposure region, due to presence of highly electronegative atoms, as they have maximum charge density and make stable interactions to complete fit into the active sites of targeted enzyme. Similarly, analog 7 also make different side chain acceptor and backbone acceptor interactions by triazole ring, as well as they also make arene-H interactions with different amino acids. The overall molecule has maximum proximity contour. Analog 11 makes arene-arene interactions by substituted phenyl ring. Oxygen of nitro group makes side chain acceptor and backbone acceptor interactions. –OH group makes backbone donor interactions and inhibit the activity of AChE enzyme.

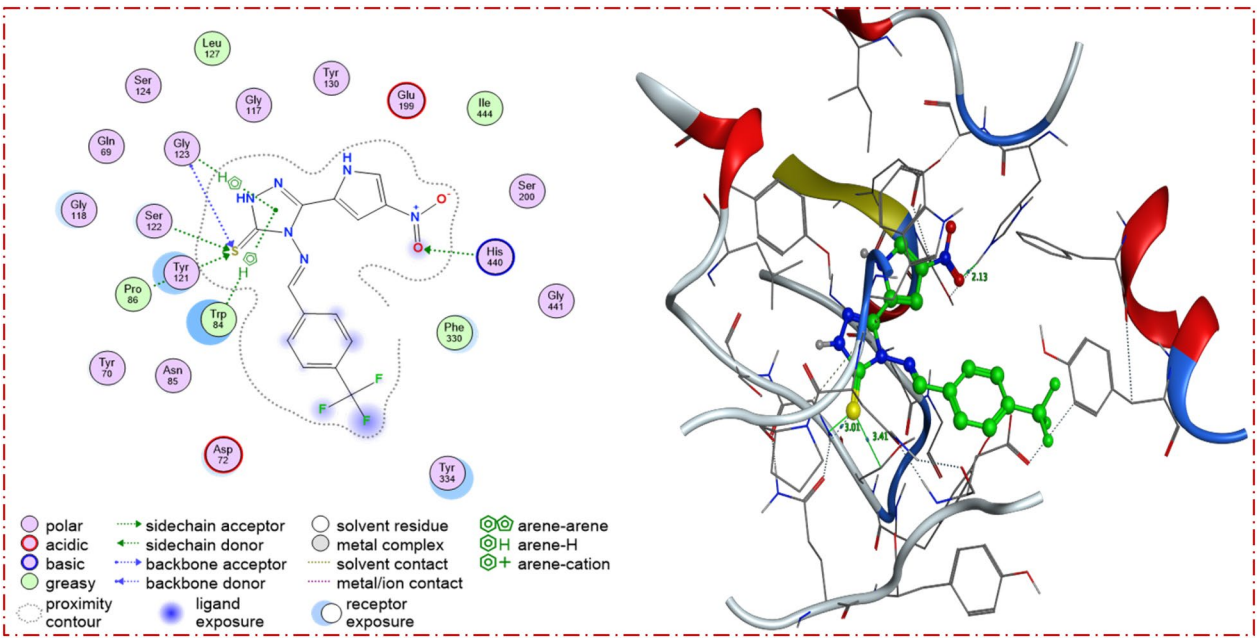


Fig. 13 Analog 7 binding affinity profile against AChE enzyme

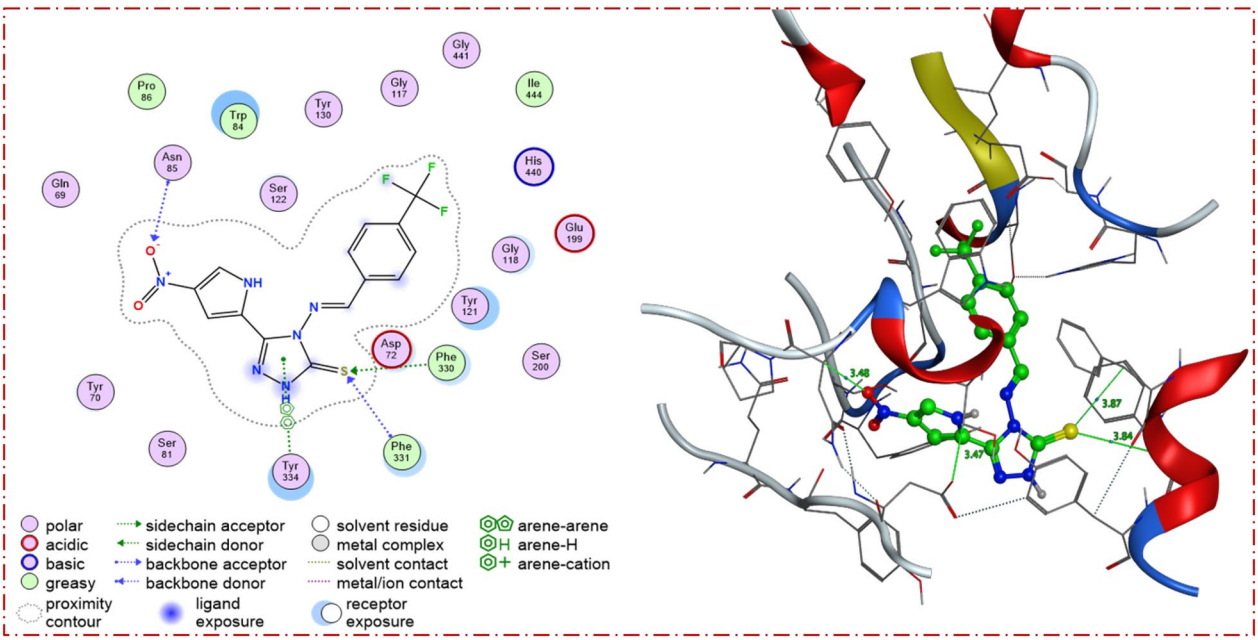


Fig. 14 Analog 7 binding affinity profile against BChE enzyme

Biological inhibitory activity of analog 6 was validated via *in-silico* molecular docking study and it was found that the analog strongly inhibits AChE enzymes through different interactions. A strong arene-H interaction was found between the amino acid TRP-84 and the phenyl ring bearing three –OH groups. Arene-cation interaction was also formed between the amino acid PRO-86 and oxygen atom of nitro group. The amino acid TYR-70 was also found to interact with the pyrrole ring. Other amino acids on active site of the AChE enzyme were found to interact via different Van der Waal's interactions. The docking score of analog 6 against AChE was −10.34. All these interactions and docking score reveal that binding affinity of analog 6 with target enzyme. 2D and 3D

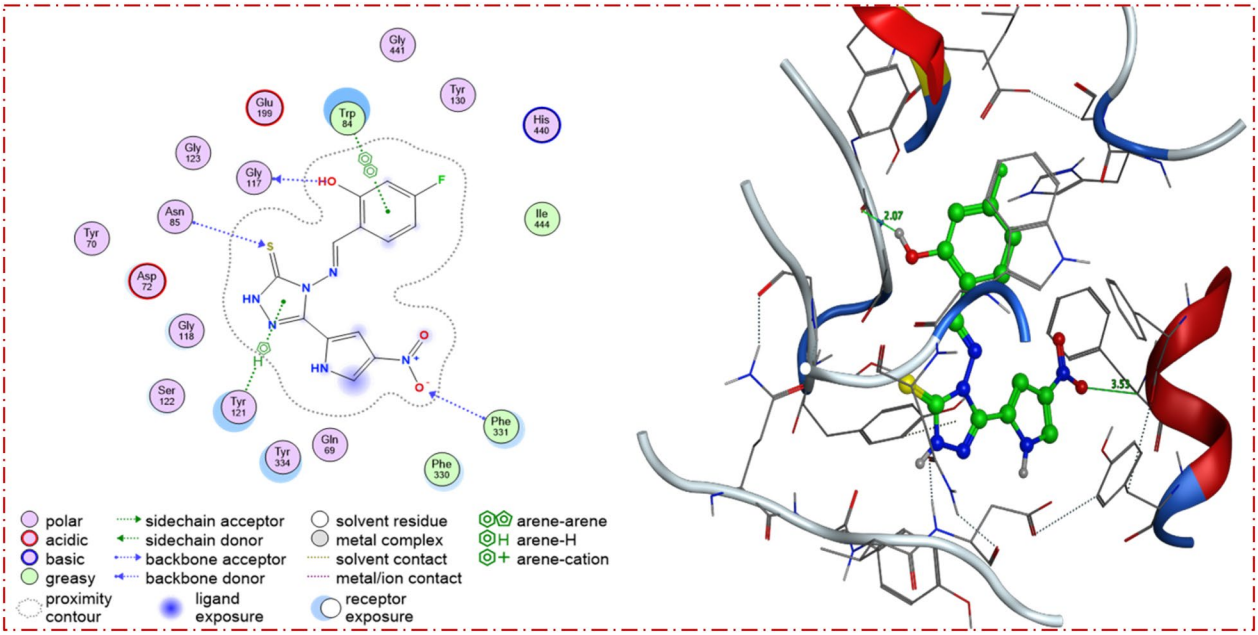


Fig. 15 Analog 11 binding affinity profile against AChE enzyme

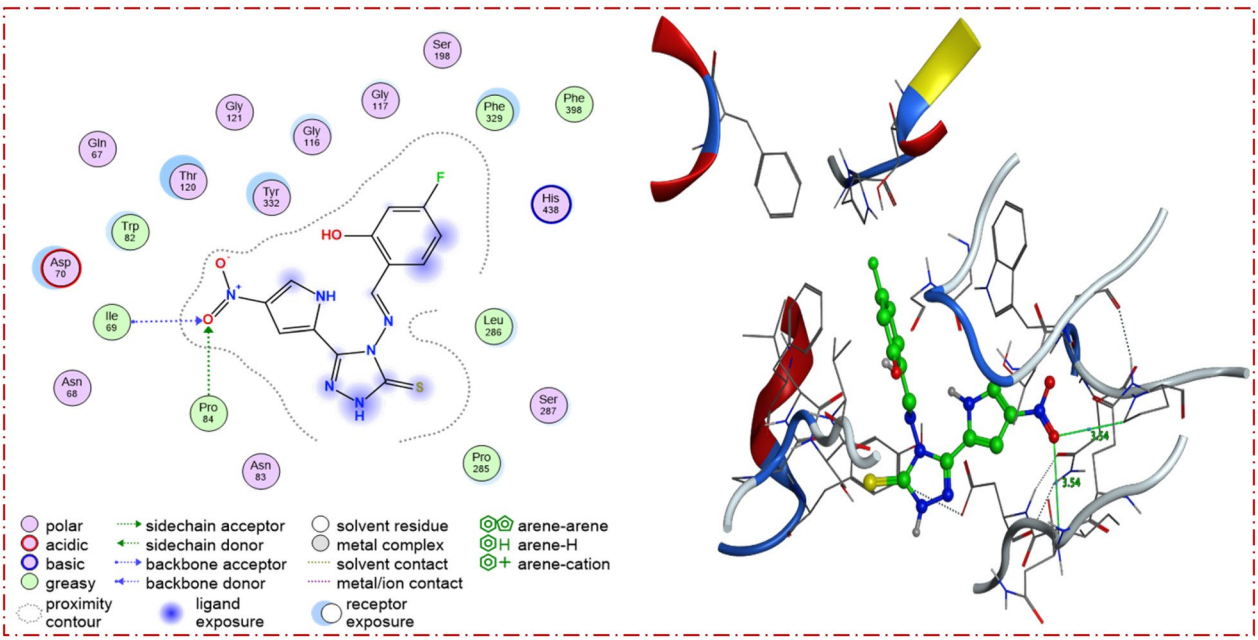


Fig. 16 Analog 11 binding affinity profile against BChE enzyme

(cartoon) interactions of analog 6 in AChE complex are shown in Fig. 11.

Similarly, the inhibitory potential of analog 6 against BChE was also confirmed through molecular docking. The key interactions included arene-H, arene-cation and arene-arene with different amino acids. Van der Waals interactions were also formed between amino acids on active site of BChE which contributed to the strong binding affinity of analog 6. The docking score of analog 6 against BChE was −9.02. 2D and 3D (cartoon) interactions of analog 6 in BChE complex are shown in Fig. 12.

The inhibitory potential of analog 7 against acetylcholinesterase (AChE) was confirmed through in silico molecular docking. Key interactions included arene-H

between triazole ring and the amino acids TRP-84 and GLY-123. Furthermore, arene-cation interaction was found between HIS-440 and nitro group oxygen. Additional Van der Waals interactions with active site amino acids contributed to the strong binding affinity of analog 7. The docking score of analog 7 against AChE was −12.71. 2D and 3D (cartoon) interactions of analog 7 in AChE complex are shown in Fig. 13.

Molecular docking simulations revealed that analog 7 exhibits potent inhibitory activity against BChE, mediated by specific arene-H, arene-cation, and arene-arene interactions with key amino acids. Arene-arene interaction was developed between the amino acid TYR-334 and triazole ring. Strong interactions of S atom substituted on triazole ring were visualized with amino acid PHE-330 and PHE-331. Moreover, nitro group on pyrrole ring also interacts with the amino acid ASN-85. Extensive Van der Waals interactions and a docking score of −11.08 confirmed high binding affinity, underscoring analog 7's potential as a BChE inhibitor. 2D and 3D (cartoon) interactions of analog 7 in BChE complex are shown in Fig. 14.

Inhibitory activity of analog 11 was also visualized under molecular docking study. Strong inhibition against AChE enzymes was visualized through different interactions. Arene-arene interaction was found between the amino acid TRP-84 and the phenyl ring bearing –OH and F groups. This OH group was also found to interact with the amino acid GLY-117. Arene-cation interaction was formed between the amino acid PHE-331 and oxygen atom of nitro group. The amino acid TYR-121 was also found to interact via arene-H binding with the triazole ring. Strong binding interaction was also formed between S atom on triazole and the amino acid ASN-85. Other amino acids were also found to interact via different Van der Waal's interactions. The docking score of analog 11 against AChE was –9.51. All these interactions and docking score reveal that binding affinity of analog 11 with target enzyme. 2D and 3D (cartoon) interactions of analog 6 in AChE complex are shown in Fig. 15.

In silico molecular docking studies validated analog 11's inhibitory activity against butyrylcholinesterase (BChE). Arene-H, arene-cation, and arene-arene interactions with specific amino acids uplift the biological profile of the analog. The favorable docking score of −9.17 indicated strong binding affinity of analog against BChE. 2D and 3D (cartoon) interactions of analog 11 in BChE complex are shown in Fig. 16.

ADMET analysis

Safety protocols of active molecules were predicted by ADMET parameters, which were performed by using SwissADME tool. This analysis mostly revealed the pharmacokinetics, physiochemical, lipophilic, water solubility and other medicinal capabilities of active scaffolds. Active compounds underwent investigation under certain ADMET guidelines. These were mostly based on the molecular weight, number of heavy atoms as well as hydrogen bond donor and acceptor atoms, skin permeability, solubility, GI absorption etc., parameters. Results showed that these analogues have therapeutically safe characteristics as they didn't showed any critical violations of log Kp, Lipinski, PAINS, Brenk, Ghose, and Muegge parameters (illustrated in Table 2 and Figs. 17, 18, 19 They have favorable drug like properties and they can be used as safe drug candidates. Moreover, we presented an innovative, intuitive methodology for concurrently predicting two critical ADME parameters: human intestinal absorption (HIA) and blood–brain barrier (BBB) penetration, facilitating early-stage drug development shown in Fig. 20. This Fig. 20 displays a spatial mapping of ADME properties, with white areas indicating high gastrointestinal absorption and yellow regions (yolk) signifying high brain permeability.

Experimental

Materials

Highly pure reactants and essential reagents needed for the efficient synthesis of derivatives (1–16) were purchased from the sigma Aldrich. To interpret and elucidate their structure, NMR machine (Bruker AM) was used to perform ^{1}HNMR (600 MHz) and ^{13}CNMR (125 MHz). Different splitting patterns such as doublet, triplet, doublet of doublet, multiplet etc. were observed in NMR spectra. Coupling constant for these splitting patterns were measured in Hertz (Hz). Buchi M-560 was used for measuring the boiling point of these synthesized derivatives. Reaction completion was confirmed by silica plates purchased from Merck, Germany. These plates were coated with silica gel containing aluminum and spots on these TLC plates were observed by means of UV light. This UV lamp has 254 and 365 nm range of wavelength.

Synthetic methodology for pyrrole derived triazole based Schiff base scaffolds synthesis

Pyrrole derived triazole based Schiff base hybrid scaffolds were synthesized by facile synthetic pathway comprised of two steps reaction.

First step: pyrrole derived triazole bearing amine and thione group moiety synthesis

In this procedure, first 1 equivalent amount of pyrrole bearing acid moiety (I) was allowed to react with 1 equivalent hydrazine carbothiohydrazide in 10 mL ethanol and reaction was catalyzed by 3 drops of triethylamine

Table 2 Safety profile of lead candidate 7, which illustrate its physiochemical, water solubility, lipophilicity, pharmacokinetics and drug likeness properties

Compound 7	
Physiochemical Properties	
Formula	$C_{14}H_9F_3N_6O_2S$
Molecular weight	382.32 g/mol
Number of heavy atoms	26
Number of aromatic heavy atoms	16
Fraction Csp^3	0.07
Number of rotatable bonds	5
Number of H-bond acceptors	7
Number of H-bond donors	2
Molar Refractivity	90.46
TPSA	139.67 A^2
Water Solubility	
Log S (ESOL)	-4.29
Solubility	1.97e-02 mg/ml; 5.14e-05 mol/l
Class	Moderately soluble
Log S (Ali)	-5.70
Solubility	7.61e-04 mg/ml; 1.99e-06 mol/l
Class	Moderately soluble
Log S (SILICOS-IT)	-4.69
Solubility	7.82e-03 mg/ml; 2.05e-05 mol/l
Class	Moderately soluble
Lipophilicity	
Log $P_{o/w}$ (ILOGP)	2.40
Log $P_{o/w}$ (XLOGP3)	3.10
Log $P_{o/w}$ (WLOGP)	4.90
Log $P_{o/w}$ (MLOGP)	1.45
Log $P_{o/w}$ (SILICOS-IT)	2.84
Consensus Log $P_{o/w}$	2.94
Pharmacokinetics	
GI absorption	Low
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-6.43 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	No; 1 violation: TPSA>131.6
Muegge	Yes
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	4 alerts: imine-1, nitro-group, oxygen-nitrogen-single bond, thiocarbonyl group
Leadlikeness	No; 1 violation: MW>350
Synthetic accessibility	3.22

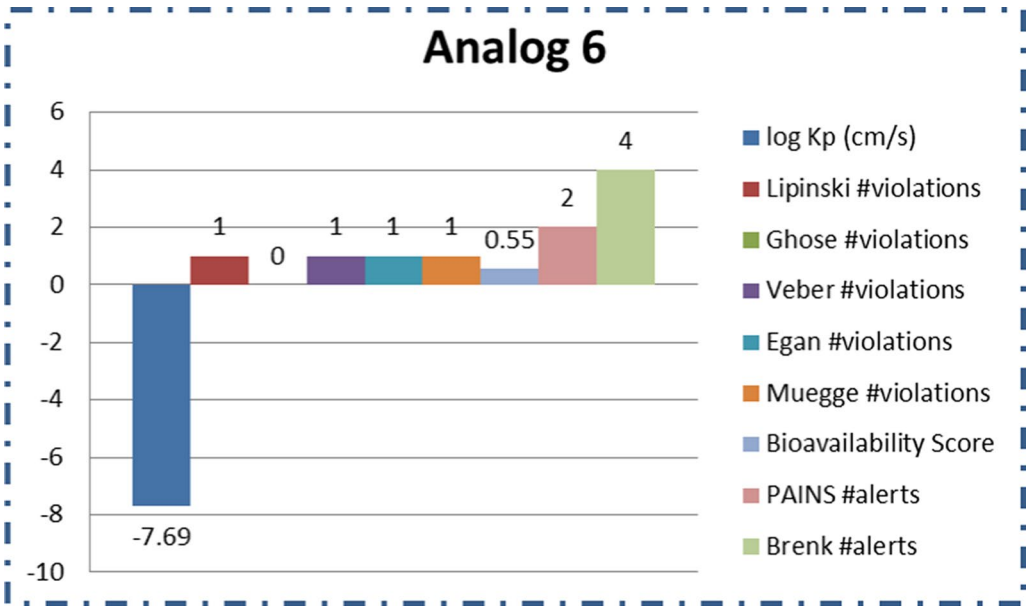


Fig. 17 Safety profile of analog 6 explored via ADMET

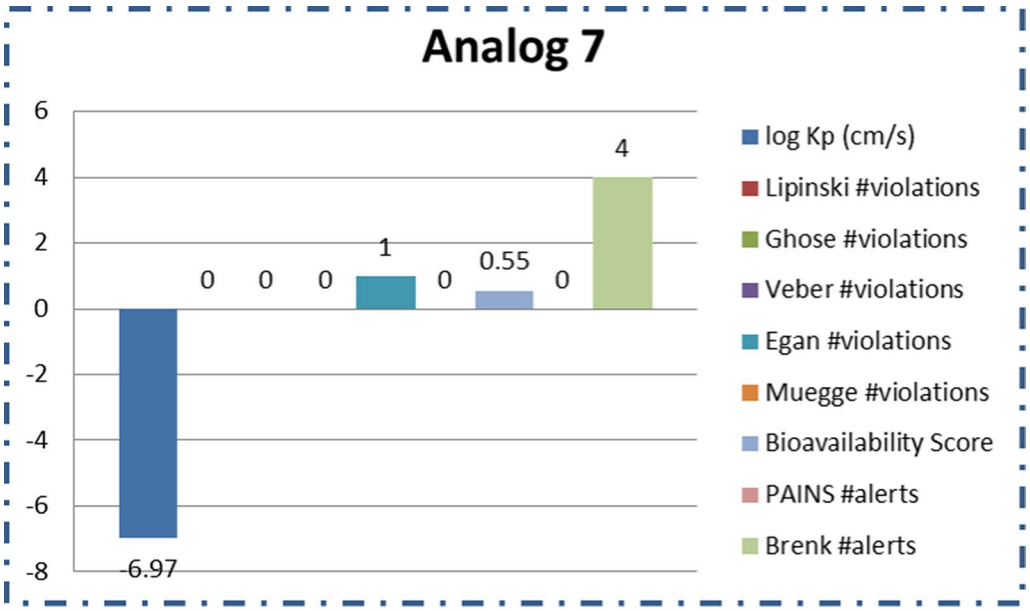


Fig. 18 Safety profile of analog 7 explored via ADMET

catalyst under refluxed for 18 h to obtain pyrrole derived triazole bearing amine and thione group moiety.

Second step: pyrrole derived triazole based Schiff base analogues synthesis

After confirmation of first step reaction completion by TLC, 1 equivalent of intermediate II was then treated with benzaldehyde having versatile substitution (each 1 equivalent) in 10 mL of ethanol solvent and 2 mL of acetic acid (catalyst) under refluxed for 5 h to afford varied substituted pyrrole derived triazole based Schiff base analogues (1–16).

Washing and drying

Minor impurities were removed from product by using n-hexane solvent (washing) and then solvent were evaporated at reduced pressure and fine products were

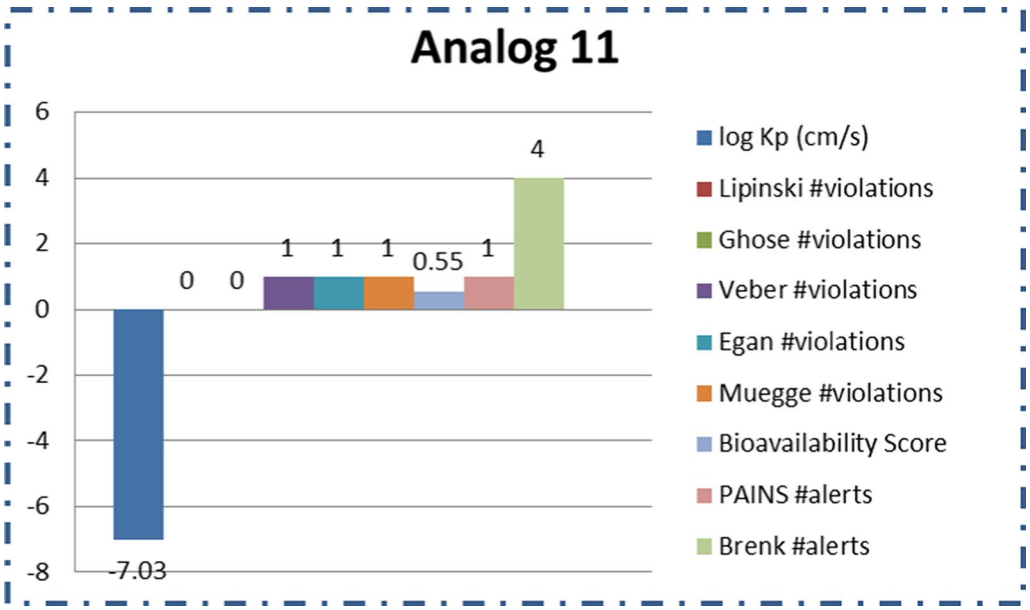


Fig. 19 Safety profile of analog 11 explored via ADMET

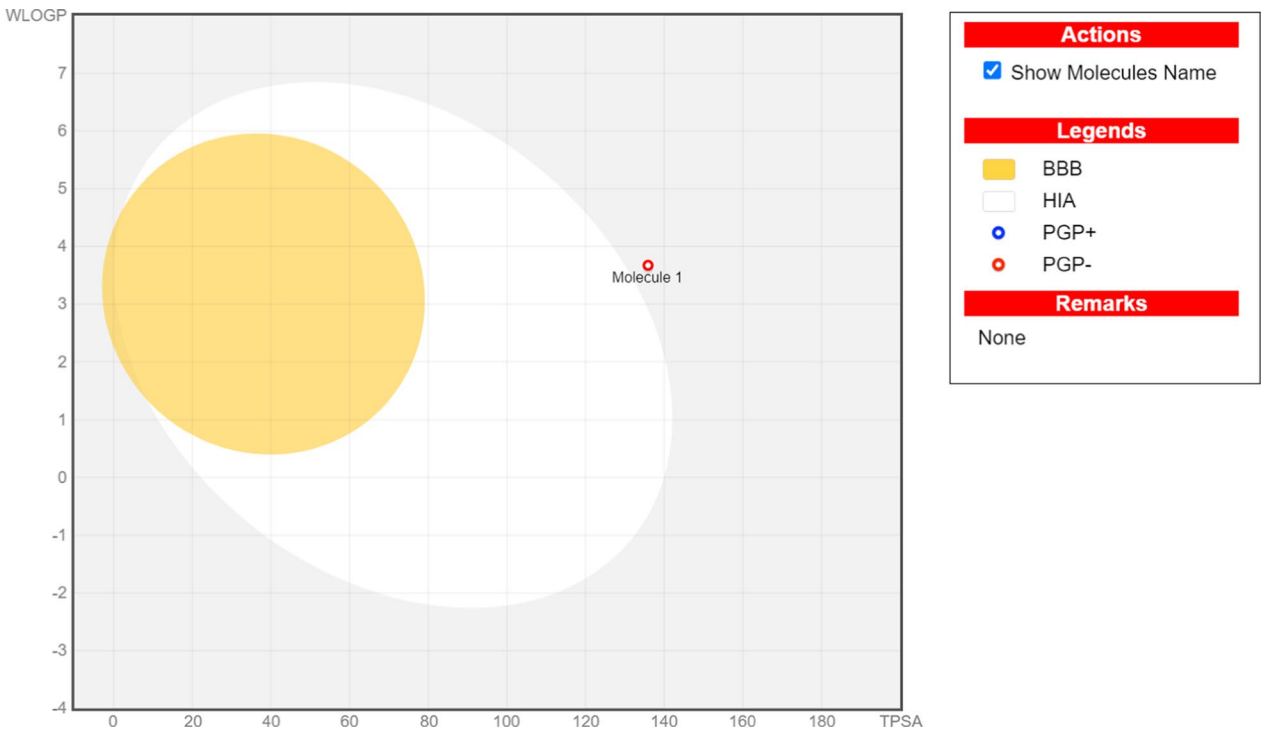


Fig. 20 Boiled-egg representing human intestinal absorption (HIA) and blood–brain barrier (BBB) penetration of potent analog **7**

collected. These synthesized products were characterized via ^{1}HNMR, ^{13}CNMR and HREI-MS.

Spectral analysis
Provided in supplementary information.

Assay protocol for AChE and BChE inhibition
Provided in supplementary information.

Assay protocol for molecular docking
Provided in supplementary information.

Conclusion

This study presents a facile synthetic route for highly effective anti-Alzheimer agents. These compounds were synthesized by treating pyrrole bearing acid moiety with hydrazine carbothiohydrazide, followed by reacting the obtained product with varied substituted benzaldehyde to obtained desired products pyrrole derived triazole based Schiff base scaffolds (1–16). Structural confirmation of all the compounds was carried out by NMR (^{1}HNMR and ^{13}CNMR) and HREI-MS. These compounds were further assesed for their anti-Alzheimer potential against both cholinesterase enzymes (AChE and BChE). The *in-vitro* results showed that these derivatives have strong biological profile and have inhibitory concentration ranging between (5.10 ± 0.40–27.10 ± 0.10 μM) for AChE and (5.60 ± 0.30–28.40 ± 0.30 μM) for BChE in comparison to reference drugs donepezil (10.20 ± 0.10 and 10.80 ± 0.20 μM) and allanzanthone (12.40 ± 0.10 and 13.10 ± 0.10 μM). Among all the members of novel series, analog 7 (IC_{50} = 5.10 ± 0.40, 5.60 ± 0.30 μM) bearing $-CF_3$ on *para*-position emerged as excellent inhibitor of both enzymes. The spellbinding inhibitory profile of analog 7 is due to the formation of strong interactions such as hydrogen bond with the amino acids present on active site of the target enzymes. Other members of series including analog 6 having trihydroxy substituents and analog 11 having –OH and –F group also have better biological profile and considered as 2nd and 3rd most potent scaffolds of synthesized series. The biological profile of all the potent compounds was also validated via *in-silico* molecular docking study. This study provided an insight into the binding interactions of potent compounds with target enzymes. Moreover, the drug profile of all the potent compounds was also analyzed under ADMET analysis to explore pharmacokinetic properties of the potent compounds. All the *in-vitro* and *in-silico* studies conducted in the current research work were found coherent. This study provides substantial therapeutic agents highlighting promising clinical applications for the treatment of Alzheimer disease in future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13065-024-01340-x.

Additional file 1.

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Author contributions
Shoaib Khan has proposed the methodology, conceptualization and written the original draft of the manuscript. Tayyiaba Iqbal has conducted computational analysis using different software tools and performed visualization and validation. Muhammad Bilal Khan has conducted the investigation of the manuscript. Rafaqat Hussain contributed for the data curation. Yousaf Khan has performed formal analysis of the manuscript. Hany W. Darwish has the contribution of write and review. All authors reviewed the manuscript.

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Availability of data and materials
All the data generated during and analyzed during the current study is not publicly available and is highly confidential, but is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not Applicable.

Consent for publication
All data presented in this manuscript is original and does not require consent for publication.

Competing interests
The authors declare no competing interests.

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