Synthesis, biological evaluation and docking studies of 2,3-dihydroquinazolin-4(*1H*)-one derivatives as inhibitors of cholinesterases

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Abstract:

In search of potent inhibitors of cholinesterases, we have synthesized and evaluate a number of 2,3-dihydroquinazolin-4(1H)-one derivatives. The synthetic approach provided an efficient synthesis of the target molecules with excellent yield. All the tested compounds showed activity against both the enzymes in micromolar range. In many case, the inhibition of both enzymes are higher than or comparable to the standard drug galatamine. With the selectivity index of 2.3 for AChE, compound **5f** can be considered as a potential lead compound with a feature of dual AChE/BChE inhibition with IC_{50} = 1.6±0.10 μM (AChE) and 3.7±0.18 μM (BChE). Binding modes of the synthesized compounds were explored by using GOLD (Genetic Optimization for Ligand Docking) suit v5.4.1. The computed binding modes of these compounds in the active site of AChE and BChE provide an insight into the mechanism of inhibition of these two enzymes.

Key Words: 2,3-dihydroquinazolin-4(1H)-one, Dual inhibitors, Cholinesterases, Alzheimer's disease

1. Introduction:

Alzheimer's disease (AD) is one of the most common Neurodegenerative Disorders (NDs) and is considered as the reason for almost 60% of the dementia cases among the persons over 65 years of age [1-2]. AD is a multifactorial disorder that is associated with dementia and memory impairment. Various pathological hypotheses have been put forth to explain the onset and progression of this disease. These include decreased levels of Acetylcholine (ACh) or enhanced activities of acetylcholinesterase (AChEs), amyloid-β (Aβ) deposits, tau (τ)-protein aggregation and oxidative stress. These all hypotheses are based on the biochemical phenomenon found to be happening in the brains of AD patients. Cholinesterases is found to be the only effective therapeutic approach for AD up till now [3-5].

For the symptomatic treatment of Alzheimer's disease, drug discovery researchers have developed multiple cholinesterase inhibitors (ChEI) including newer ChEIs (Phenserine, Tolserine, Esolerine and Tesofensine), naturally derived ChEIs (Nelumbo nucifera, Huperzine A, Huperzine B, Himatanthus lancifolius and Galangin, Cardanol Derivatives), hybrids (5-(N-Methyl-N-propargyl-aminomethyl) Quinolin-8-yl Dimethyl Carbamate and 5-(N-Methyl-N-propargylaminomethyl) Quinolin-8-yl Ethylmethyl Carbamate, Donepezil-AP2238, Donepezil-Tacrine Hybrids and Tacrine-8-hydroxyquinoline Hybrids), and synthetic analogues (N-Alkyl-7-methoxytacrine Hydrochlorides, Phenyl-5,6-dimethoxy-1-oxo-2,3-dihydro-1H-2-indenylmethanone Analogues, and Ladostigil) and many more which are under preclinical and clinical trials [3, 6-7].

Quinazoline, a class of fused pyrimidine consists of very useful nitrogen containing heterocyclic moieties which show various biological activities like anticonvulsant and hypnotic, anticancer, antimicrobial and antihistaminic, diuretic, antimalarial, antihypertensive, antagonism of ghrelin receptor, anti-inflammatory, analgesic and COX-2 inhibitory activities as well as antifungal

activities [8-17]. On the basis of substitution pattern on central ring of quinazoline, it can be divided into 2-substituted-4(3H)-quinazolinones, 3-substituted-4(3H)-quinazolinones, 2,3-disubstituted-4(3H)-quinazolinones, 4-substituted-quinazolines and 2,4-disubstituted-4(3H)-quinazolinones categories [18]. Of these synthesis of 2-substituted quinazolinones have attracted the chemists because of their wide range of medicinal and biological properties such as anti-inflammatory, antihypertensive, antibacterial activity, anticancer and antitumor [19-22]. Li et al reported the AChE/BChE inhibition of a series of novel 2-(2-indolyl-)-4(3H)-quinazolines derivates in nanomolar range [23]. Uraz et al reported 4(3*H*)-quinazolinone derivatives as good AChE inhibitors [24]. Similarly, highly potent spacer-modified bivalent quinazolinimines were reported by Chen et al as inhibitors of AChE/BChE [25].

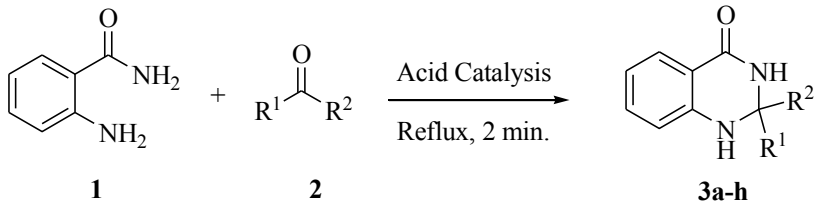
Recently, we have reported rational design and synthesis of dihydropyrimidine based dual binding site acetylcholinesterase inhibitors [26]. The objective of this study is to discover a new scaffold as inhibitor of cholinesterases. Herein, we reported the synthesis and *in vitro* bioactivity of 2,3-dihydroquinazolin-4(1H)-one derivatives as inhibitors of cholinesterases.

2. Results and discussion

2.1. Chemistry

Various chemists have attempted synthesis of a variety of quinazoline derivatives with focus on synthesis of 2-monosubstituted ones. Very few examples for the synthesis of 2,2-disubstituted quinazoline moieties have been recorded in literature due to their low yields, drastic reaction conditions and prolonged reaction times. However, in many cases use of catalytic methodologies as alternatives to condensation methods have provided many benefits to the scaffold synthesis. Catalytic carbonylation, domino reaction, hydrogen transfer process, use of metals such as iridium,

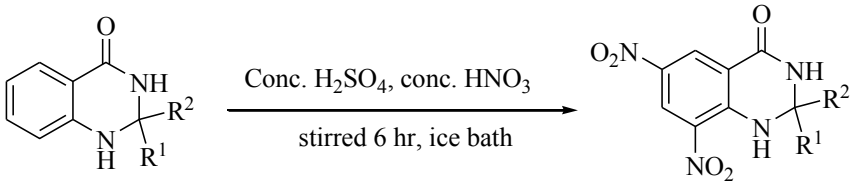
rhodium and palladium also provide mild reaction conditions and high yields [27-29]. We tried comparatively an inexpensive acid catalysis method for the synthesis of quinazolines. Starting from 2-amino benzamide and ketone, we were able to synthesize 2,3-dihydroquinazolin-4(1H)-one core under acid catalysis (1ml of HNO_3/HCl : 3/1) with excellent yield (**Scheme 1**).



1 **2** **3a-h**

Scheme 1: Synthesis of 2,3-dihydroquinazolin-4(1H)-ones (**3a-h**)

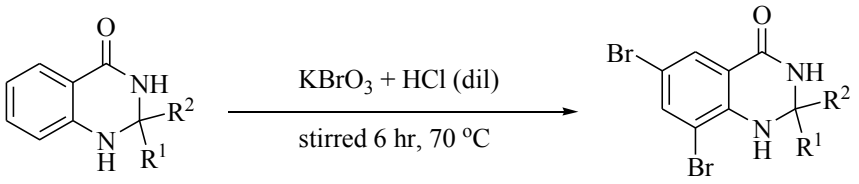
The synthesis of target compounds **(4a-h)** was commenced with reaction of unsubstituted phenyl ring of 2,3-dihydroquinazolin-4(1H)-one core (**3a-h**) with conc. HNO_3 in the presence of H_2SO_4 (**Scheme 2**).



3a-h **4a-h**

Scheme 2: Synthesis of dinitro derivatives of 2,3-dihydroquinazolin-4(1H)-ones (**4a-h**)

Dibrominated 2,3-dihydroquinazolin-4(1H)-ones (**5a-h**) were also synthesized by the reaction of **3a-h** with potassium bromate (KBrO3) in acidic medium (**Scheme 3)**.



3a-h **5a-h**

Scheme 3: Synthesis of dibromo derivatives of 2,3-dihydroquinazolin-4(1*H*)-ones (**5a-h**)

In the ^{1}H NMR spectra of 2,3-dihydroquinazolin-4(1*H*)-ones (**3a-h**), unsubstituted phenyl ring of quinazoline was deduced from their four signals of one proton each, chemical shift values and their coupling constants. One ^{1}H-signal as dd (J_{ortho} = 8 Hz and J_{meta} = 2Hz), one triplet (J_{ortho} = 8 Hz), one multiplet and one doublet (J_{ortho} = 8 Hz) was assigned to protons H-5, H-6, H-7 and H-8 respectively. ^{1}H-NMR signals with splitting at 1.70-1.74 ppm (doublet, J = 6 Hz) and 1.38-1.78 (multiplet) were assigned to –CH_2 protons of isobutyl / ethyl / propyl substituents at C-2 respectively. Intrinsic splitting pattern at about 1.85-1.94 (m) and 0.86-0.93 (t, J_{ortho} = 7 Hz) corresponds to –CH and -CH_3 of ethyl / propyl / isobutyl group respectively. Two broad singlets at 5.76-5.87 and 6.81-6.92 ppm were interpreted for the –NH protons at position-1 and 3 of the quinazoline ring respectively. ^{1}H-NMR of the series **4a-h** and **5a-h** were deduced with almost same splitting pattern. However, two highly deshielded signals in the range of 9.21-9.23 and 9.22-9.06 ppm (d, J = 8 Hz) appeared due to the –NO_2 groups (**4a-h**) whereas due to Br substitution (**5a-h**) signals appeared at 7.81-7.89 and 7.38-7.68 (d, J = 8 Hz) for H-5 and H-7 respectively. In ^{13}C-NMR, a characteristic most downfield signal at 1630-164.6 ppm was interpreted for C-4 carbonyl group. A relatively upfield signal was C-9 at 143.9-147.2 ppm. While C-10 signal was observed at 116.0-119.6 ppm. The quinazoline C-2 was detected at 66.8-72.6 ppm.

2.2. XRD

The structures of the synthesized compounds were also confirmed by single crystal X-ray diffraction studies. Crystallographic structures and data of some represented compounds are presented in Supplementary information **(Figure S1-S3** and in Table **S-1** and **S-2**).

2.3. *In vitro* pharmacology

2.3.1 AChE and BChE inhibition assay

The inhibitory potency (IC_{50} values) of the synthesized compounds against AChE (*Electrophorus electricus*) and BChE inhibition (equine serum) was evaluated according to the method [26, 30]. The *in vitro* results and selectivity index for AChE and BChE inhibition are summarized in **Table 1**.

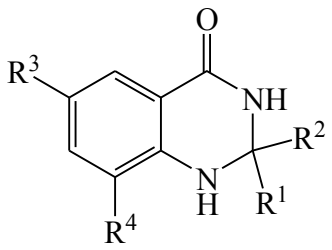
All the synthesized quinazolines showed good to moderate degree of inhibition and the IC_{50} value of some of compounds are in low micromolar range toward both enzymes. In general, a clear trend appears that the derivatives with 4-choloropheny group at C-2 position of quinazoline ring (**3f**, **4f** and **5f**) possess high AChE/BChE inhibitory activity in their respective series of analogues. Similarly, compounds with di-isobutyl group (**3h**, **4h** and **5h**) also exhibited good inhibition toward both enzymes. Compounds **3a-h** with unsubstituted phenyl ring are less potent than their dinitro (**4a-h**) and dibromo (**5a-h**) counterparts.

Compounds **4f, 4h, 5b, 5f** and **5h** were more potent AChE inhibitors than the standard drug Galantamine with IC_{50} values in the range of 1.6 μM to 3.9 μM. Amongst the dinitro series (**4a-h**), compound **4f** emerged as potent compound with IC_{50} value of 3.8±0.14 μM. Compound **4h** also exhibited good AChE inhibition (IC_{50} = 3.9±0.17 μM) comparable to that of **4f**. Dibromo derivatives (**5a-h**) emerged as inhibitor as good as their dinitro counterparts. Introduction of 4-

chlorophenyl group at C-2 position led to potent compound **5f** (IC_{50} = 1.6±0.10 μM). Methyl and ethyl substituents at C-2 position did not improve the AChE inhibition. However, replacing these substituents by isobutyl /diisobutyl group (**5g** and **5h**) resulted in moderate to good activity.

To evaluate the selectivity profile, IC_{50} values of the synthesized compounds toward BChE were also determined. As seen from **Table 1**, with exception of compounds **3f and 3g**, all the other compounds of subset **3a-h** exhibited moderate to very little BChE inhibition. However, the compounds of this series have reduced the selectivity for AChE inhibition. Di-isobutyl derivative **3g** has inhibitory selectivity for BChE (SI=0.5). Compounds **3a, 3c-e** exhibited very little BChE inhibitory activity. Instead, good BChE inhibition was found within the dinitro subset (**4a-h**) where 7 out of 8 derivatives were more active than galatamine having IC_{50} values in the range of 5.3±0.21 μM to 14.2±0.53 μM. Compound **4h** with BChE IC_{50} value of 5.3±0.21 μM has good inhibitory activity (SI=1.3). Dibromo subset (**5a-h**) is displayed less BChE inhibition than corresponding dinitro subset. With the selectivity index of 2.3 for AChE, compound **5f** can be considered as a potential lead compound as dual AChE / BChE inhibitor with IC_{50} = 1.6±0.10 μM (AChE) and 3.7±0.18 μM (BChE).

Table 1: *In vitro* AChE and BChE inhibitory activity of the synthesized compounds



Compound	R^1	R^2	R^3	R^4	IC_{50} (µM ±SEM)		Selectivity Index [a]
					eeAChE	eqBChE	
3a	CH_3	CH_3	H	H	80.3±1.8	97.5±2.40	1.2
3b	CH_3	C_6H_5	H	H	24.5±0.70	20.3±0.52	0.8
3c	CH_3	CH_2CH_3	H	H	29.4±0.60	58.3±1.74	1.9
3d	CH_2CH_3	CH_2CH_3	H	H	54.2±1.63	57.9±1.62	**1.0**
3e	CH_3	$CH_2CH_2CH_3$	H	H	31.1±1.08	33.4±0.66	1.0
3f	CH_3	4-Cl. C_6H_4	H	H	9.9±0.10	11.2±0.28	1.1
3g	CH_3	$CH_2CH(CH_3)_2$	H	H	31.0±0.82	17.9±0.57	0.5
3h	$CH_2CH(CH_3)_2$	$CH_2CH(CH_3)_2$	H	H	10.4±0.23	23.4±0.63	2.2
4a	CH_3	CH_3	NO_2	NO_2	24.4±0.62	21.0±0.70	0.8
4b	CH_3	C_6H_5	NO_2	NO_2	**6.2±0.13**	**9.9±0.44**	1.6
4c	CH_3	CH_2CH_3	NO_2	NO_2	20.7±0.31	**13.9±0.48**	0.6
4d	CH_2CH_3	CH_2CH_3	NO_2	NO_2	19.3±0.57	**14.2±0.53**	0.7
4e	CH_3	$CH_2CH_2CH_3$	NO_2	NO_2	12.1±0.42	**13.3±0.33**	1.1
4f	CH_3	4-Cl. C_6H_4	NO_2	NO_2	**3.8±0.14**	**10.3±0.30**	2.7
4g	CH_3	$CH_2CH(CH_3)_2$	NO_2	NO_2	4.6±0.19	**12.2±0.42**	2.6
4h	$CH_2CH(CH_3)_2$	$CH_2CH(CH_3)_2$	NO_2	NO_2	**3.9±0.17**	**5.3±0.21**	1.3
5a	CH_3	CH_3	Br	Br	25.0±0.90	86.2±3.44	3.4
5b	CH_3	C_6H_5	Br	Br	**3.5±0.12**	**6.4±0.21**	1.8
5c	CH_3	CH_2CH_3	Br	Br	29.4±0.90	22.1±0.88	0.7
5d	CH_2CH_3	CH_2CH_3	Br	Br	17.9±0.82	16.2±0.40	0.9
5e	CH_3	$CH_2CH_2CH_3$	Br	Br	5.4±0.16	23.4±0.58	4.3
5f	CH_3	4-Cl. C_6H_4	Br	Br	**1.6±0.10**	**3.7±0.18**	2.3
5g	CH_3	$CH_2CH(CH_3)_2$	Br	Br	11.2±0.22	18.4±0.64	1.6
5h	$CH_2CH(CH_3)_2$	$CH_2CH(CH_3)_2$	Br	Br	**2.5±0.10**	14.2±0.34	5.7
Galatamine					**4.0±0.10**	**15.0±0.67**	**3.7**

[a] Values are expressed as mean (standard error of the mean of at least three experiments.

[b] Selectivity Index = IC_{50} ratio (BChE/AChE).

2.4. Molecular modelling

2.4.1. Molecular modeling studies on AChE and BChE inhibition

For designing the drug and to understand the molecular mechanism of AD, the three dimensional (3D) structure of AChE is essential. X-ray structures of AChE from different species such as *Electrophorus electricus* (eel), *Torpedo californica* (*Tc*AChE), *Drosophila melanogaster*, human (*h*AChE) and mouse have been reported in the literature [31-32]. The structure of *Tc*AChE provide insights into the mechanism of enzyme catalysis. However, recent studies have shown that the binding site of *h*AChE is different from *Tc*AChE. Therefore, we studied the binding interactions of our synthesized compounds with both the enzymes by using GOLD (Genetic Optimization for Ligand Docking) suit v5.4.1 [33]. The X-ray crystallographic structure of *Tc*AChE (PDB Code 1EVE) and *h*AChE (PDB Code 4EY7) in complex with donepezil were used as enzyme structures. In general, visual inspection of the docked compound-enzyme complexes revealed following observations: All the docked compounds were stabilized in the cavity by forming different types of interactions. With few exceptions, the best-scored binding poses of compounds suggest that the benzene ring of quinazoline is caged into the choline binding site and forms π-π stacking interactions with Trp84 (Trp86 in *h*AChE). Phenyl ring at 2-position of the quinazoline ring oriented towards the peripheral anionic site (PAS) comprising Asp72, Trp279 and Tyr334 (*Tc*AChE numbering). Alkyl chains (methyl, ethyl and isobutyl) at 2-position of the quinazoline ring forms π-σ interactions with Tyr121, Phe290, Phe330, Phe331 and.Tyr334. It was also observed that bromine atoms of compounds **5a-h** establish significant van der Waals interactions with Leu127, Tyr130 and Phe330. 6,8-dinitro group at quinazoline ring (**4a-h**) is involved in the hydrogen bonding interactions. Ion-dipole interactions were also observed between NH of quinazoline and the deprotonated aspartic acid (Asp72).

Superposition of the docked poses of the most active compound **5f** into the binding pockets of *Tc*AChE and *h*AChE is shown in **Figure 1a**. The binding mode analysis of **5f**-*Tc*AChE and **5f**-*h*AChE complex reveals mostly similar binding mode as shown in **Figure 1a**. Depiction of the top-scored docking pose of the most active compound **5f** in the binding site of *Tc*AChE and *h*AChE is presented in **Figure 1a-b**. Dibromo phenyl ring is oriented toward Trp84 (Trp86 in *h*AChE) and engaged in π-π stacking interactions. In *Tc*AChE, two T-shaped orthogonal π-π stacking interactions were observed between chlorophenyl at C-2 and Phe331 and another between same substituent and Tyr334 (**Figure 1b**). Bromine atoms interacts with the π-system of Trp84, Tyr130, Phe330, and His440, While, in *h*AChE-**5f** complex, chlorophenyl ring is properly packed in the narrow gorge and forms some π-π stacking and π-σ interactions with Tyr337 and Tyr341 respectively. Although, the binding pattern of donepezil in *h*AChE is different from *Tc*AChE [32], however, our small molecules bind in same conformation as in *Tc*AChE (**Figure 1a)**. This was further confirmed by their Gold fitness score values. Gold fitness score for **5f** is 72.3148 (*Tc*AChE) and 72.8581 (*h*AChE).

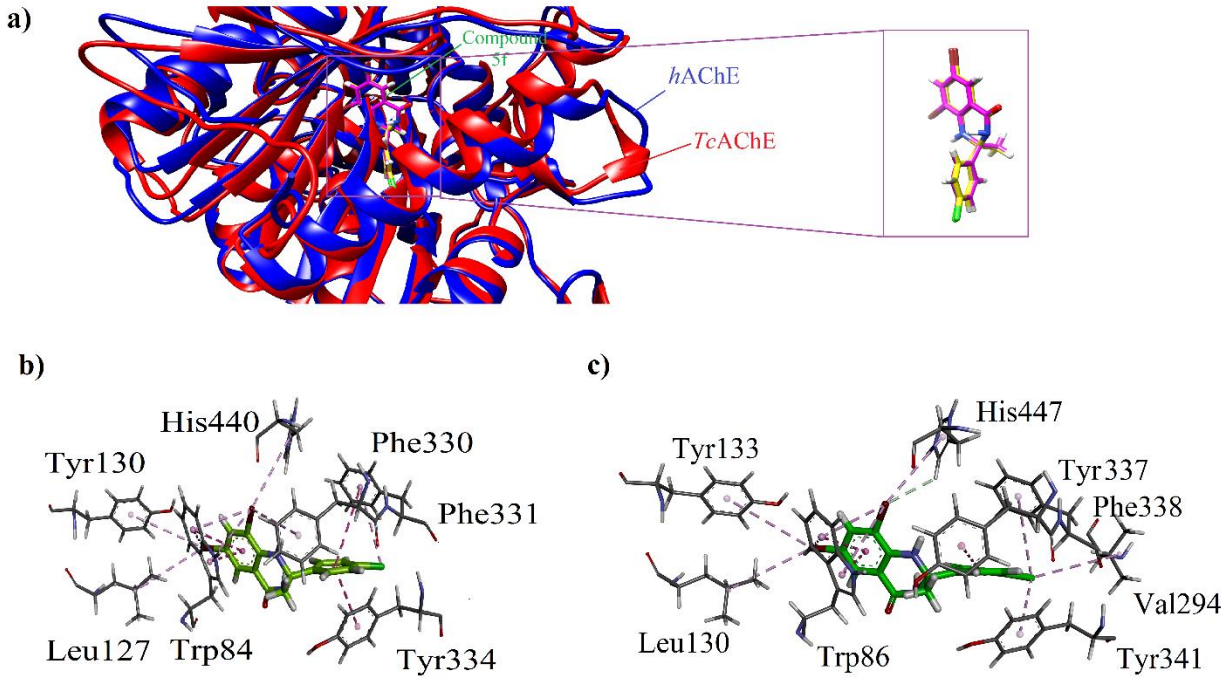


Figure 1: (**a**) Superimposed ribbon diagram of the top-scoring docking pose for **5f**-*Tc*AChE (red ribbons) and **5f**-*h*AChE complex (blue ribbon); (**b**) Close-up depiction of the docking pose of compound **5f** in the binding site of 1EVE; (**c**) Close-up depiction of the docking pose of compound **5f** in the binding site of 4EY7. The key residues are represented as stick model.

The binding mode of compound **4b** (IC_{50} = 6.2 μM) in the binding pocket of *Tc*AChE was also studied. Compound **4b** is embedded in the deep and narrow catalytic gorge. As depicted in **Figure 2a**, oxygen atom of nitro group interact with the imidazole of His440 at a distance of 2.92 Å. Side chain of Asp72, one of the acidic residue present in the active site gorge, forms an ionic interaction with NH-quinazoline (ion-dipole interaction) at the distance of 2.12 Å. Compound **4b** forms a π-π stacking interactions with the PAS residue Tyr334 at a distance of 4.46 Å. The backbone α-amine of Gly118, one of the glycine residue of oxyanion hole, forms a hydrogen bond with oxygen atom of nitro group. The Gold fitness score compounded for the best-scoring binding pose (**Figure 2a**) for **2f** is 69.6228. Compound **3a** is the least active compound with IC_{50} value of 80.3±1.8 μM. Compound **3a** oriented itself at a distance of 4.36 Å and interacts with Trp84 *via* π-π stacking

interactions (**Figure 2b**). This only interaction is considered to be the reason of the very low inhibition of **3a**.

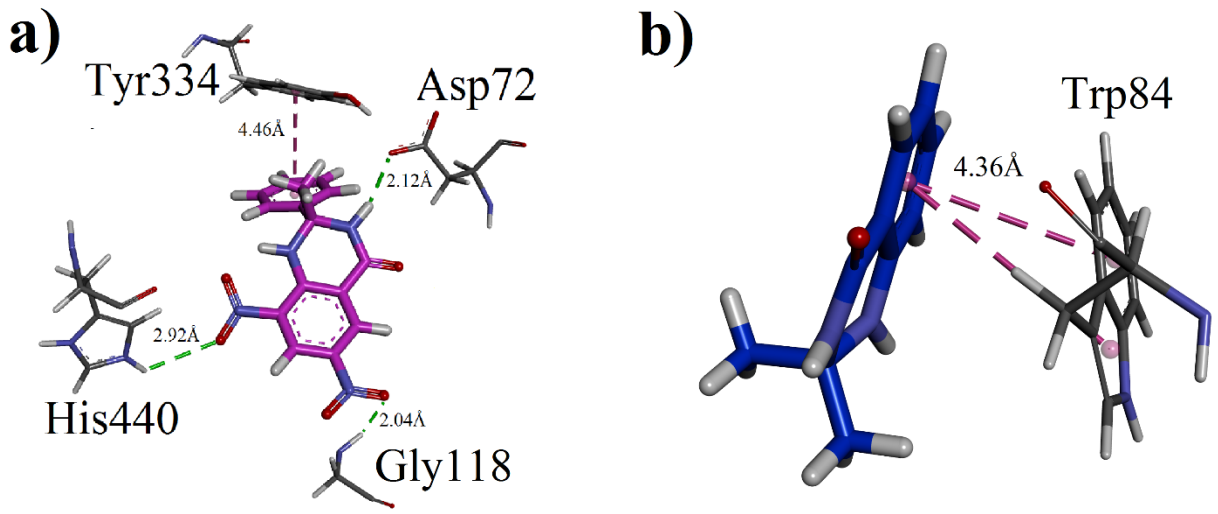


Figure 2: Hydrogen bond and π-π stacking interactions in the binding pocket of *Tc*AChE (1EVE) and the distance (in Angstrom) of compounds; a) **4b** and b) **3a**

For gaining insight into the mechanism of BChE inhibition, the X-ray crystallographic structure of human BChE (PDB Code 1P0I) was used as enzyme structure. The most potent BChE inhibitor **5f** displayed the most was docked into the active site of *Hu*BChE (1P0I). The computed Gold fitness score of the top-scoring docking pose is 69.8391. Compound **5f** displayed multiple binding pattern within the 5 Å distance. In the larger gorge it was engaged in π-π stacking interactions with Trp82. The most potent ion-dipole interaction was established between NH of the ring and deprotonated Glu197 at a distance of 1.58 Å. Carbonyl oxygen of the quinazoline ring established two hydrogen bond interactions with the backbone α-NH groups of Gly116 (1.88 Å) and Gly117 (2.91 Å). Dibromophenyl ring occupies hydrophobic pocket and formed π-π stacking interactions with Phe329 at a distance of 4.59 Å.

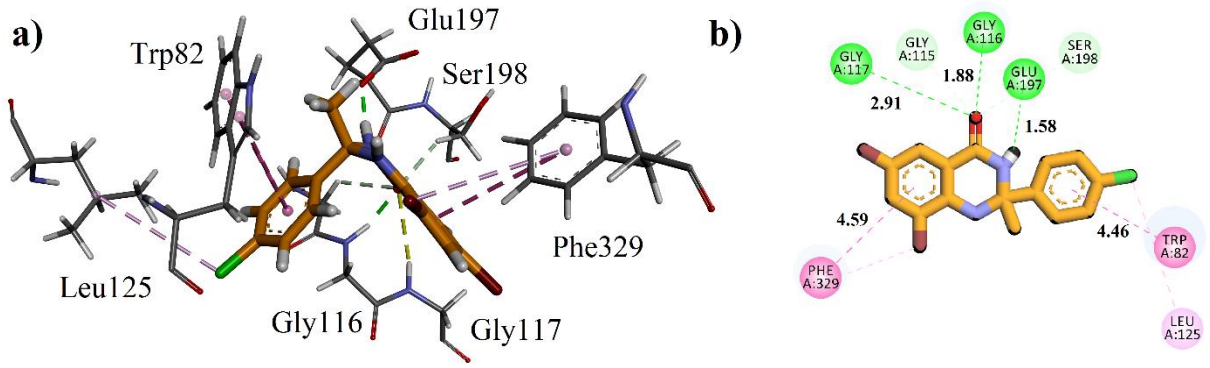


Figure 3: Hydrogen bond and π-π stacking interactions of **5f** in the binding pocket of human BChE (PDB Code 1P0I **a)** 3D interactions and **b)** 2D interactions.

3. Conclusion

In the light of above findings, it can be concluded that we have synthesized and tested quinazoline derivatives for AChE and BChE inhibitory potency. All the derivatives showed activity against both the enzymes. All the tested compounds showed activity against both the enzymes in micromolar range. In many case, the inhibition of both enzymes are higher than or comparable to the standard drug galatamine. The findings of this study suggest that appropriate structural modification of quinazoline scaffold may result in potential AChE or BChE inhibitors.

4. Materials and Methods

Analytical grade chemicals obtained from commercial suppliers were used as received. Melting points were measured using a SMP30 Stuart Scientific melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 400 MHz NMR spectrometers. ^{1}H and ^{13}C chemical shifts are reported Vs $SiMe_4$ and were determined by reference to the residual ^{1}H and ^{13}C solvent peaks. The X-ray diff raction data of crystals was collected on a Bruker Smart APEX II diff ractometer. Data reduction was carried out using the SAINT program [34]. The structure solution and refinements were performed with the SHELXL-2013 program package [35].

4.1. General procedure for the synthesis of 2,2-disubstituted-2,3-dihydroquinazolin-4(1*H*)-one (3a-h)

Stirred solution of 2-amino benzamide (100 mmol) and ketone (200 mmol) in presence of conc. HNO_3/ HCl (1 mL) as catalyst was refluxed for 05 minute and stirring was continued for further 30 minutes. After completion of the reaction, product was concentrated by rotary evaporator and after cooling, water (20ml) was added to the mixture. Precipitates formed were filtered, washed with water and dried in oven. Recrystallization of the product with ethyl acetate afforded crystals of 2,2-disubstituted-2,3-dihydroquinazolin-4(1*H*)-one which were characterized by X-Rays crystallographic and NMR techniques.

4.1.1. Synthesis of 2,2- dimethyl-2,3-dihydroquinazolin-4(1*H*)-one (3a).

3a was synthesized by general procedure using a mixture of 2-amino benzamide (13.6 g, 100mmol) and acetone (14.5 mL, 200 mmol) as white crystalline solid (99.5% yield), m.p. 182-183 °C, CCDC No. 1442341. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.83 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.29-7.25 (m, 1H, Ar*H*), 6.89 (s, 1H, N*H*), 6.78 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.59 (d, ^3J = 8 Hz, 1H, Ar*H*), 5.78 (s, 1H, N*H*), 1.37 (s, 6H, 2xCH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 163.0, 147.0, 133.2, 127.2, 116.4, 114.2, 113.8, 66.8, 29.0.

4.1.2. Synthesis of 2-methyl-2-phenyl-2,3-dihydroquinazolin-4(1*H*)-one(3b)

3b was synthesized by general procedure using a mixture of 2-amino benzamide (13.6 g, 100 mmol) and acetophenone (23.5 mL, 200 mmol) as white crystalline solid (98.2% yield). m.p. 222-224 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.84 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.54 (d, ^3J = 8 Hz, 2H,Ar*H*), 7.34-7.26 (m, 4H, Ar*H*), 6.81(t, ^3J = 8 Hz, 2H, N*H*, Ar*H*), 6.68 (d, ^3J = 8 Hz, 1H, Ar*H*), 5.76 (s, 1H, N*H*), 1.89 (s, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C), δ=164.5, 145.6, 144.7, 134.2, 128.6, 128.6, 128.5, 128.3, 125.3, 119.2, 114.9, 71.0, 29.9.

4.1.3. Synthesis of 2-ethyl-2-methyl-2,3-dihydroquinazolin-4(1*H*)-one (3c)

3c was synthesized by general procedure using a mixture of 2-amino benzamide (13.6 g, 100 mmol) and ethyl methyl ketone (18 mL, 200 mmol) as white crystalline solid (99.5% yield). m.p. 179-180 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.82 (dd, ^{3}J = 8 Hz, ^{4}J = 2 Hz, 1H, Ar*H*), 7.29-7.24 (m, 1H, Ar*H*), 6.90 (s, 1H, N*H*), 6.80 (t, ^{3}J = 8 Hz, 1H, Ar*H*), 6.60 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 5.76 (s, 1H, N*H*), 1.68-1.58 (m, 2H, CH_2), 1.32 (s, 3H, CH_3), 0.86 (t, ^{3}J = 7 Hz, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 163.1, 147.2, 133.1, 127.1, 116.0, 114.0, 113.5, 69.2, 33.9, 27.4, 8.2.

4.1.4. Synthesis of 2,2-diethyl-2,3-dihydroquinazolin-4(1*H*)-one (3d)

3d was synthesized by general procedure using a mixture of 2-amino benzamide (13.6 g, 100 mmol) and diethyl ketone (21.5 mL, 200 mmol) as white crystalline solid (99.5% yield); m.p. 197-200 °C, ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.84 (dd, ^{3}J = 8 Hz, ^{4}J = 2 Hz, 1H, Ar*H*), 7.29-7.26 (m, 1H, Ar*H*), 6.89 (s, 1H, N*H*), 6.77 (t, ^{3}J = 8 Hz, 1H, Ar*H*), 6.59 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 5.82 (s, 1H, N*H*), 1.78-1.73 (m, 4H, 2×CH_2), 0.97 (t, ^{3}J = 7 Hz, 6H, 2×CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C), δ=164.4, 146.2, 134.0, 128.3, 118.2, 114.1, 114.1, 72.6, 32.9, 7.9.

4.1.5. Synthesis of 2-methyl-2-propyl-2,3-dihydroquinazolin-4(1*H*)-one (3e)

3e was synthesized by general procedure using a mixture of 2-amino benzamide (13.6 g, 100 mmol) and methyl propyl ketone (21.5 mL, 200 mmol) as white crystalline solid (99.5% yield), m.p. 192-195 °C, ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.86 (dd, ^{3}J = 8 Hz, ^{4}J = 2 Hz, 1H, Ar*H*), 7.29-7.25 (m, 1H, Ar*H*), 6.84 (s, 1H, N*H*), 6.80 (t, ^{3}J = 8 Hz, 1H, Ar*H*), 6.59 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 5.87 (s, 1H, N*H*), 1.76-1.72 (m, 2H, CH_2), 1.50 (s, 3H, CH_3), 1.47-1.38 (m, 2H, CH_2), 0.93 (t, ^{3}J = 7 Hz, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C), δ=164.3, 145.9, 134, 128.3, 118.6, 114.5, 114.4, 69.9, 44.5, 28.1, 17.2, 14.1.

4.1.6. Synthesis of 2-(4-chlorophenyl)-2-methyl-2,3-dihydroquinazolin-4(1*H*)-one (3f)

3f was synthesized by general procedure using a mixture of 2-amino benzamide (6.8 g, 50 mmol) and 4-chloro acetophenone (13.0 mL, 100 mmol) as white crystalline solid (99.5% yield). m.p. 209-211 °C, ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.84 (dd, ^{3}J = 8 Hz, ^{4}J = 2 Hz, 1H, Ar*H*), 7.47 (d, ^{3}J = 8 Hz, 2H, Ar*H*), 7.33-7.27 (m, 3H, Ar*H*), 6.92 (s, 1H, N*H*), 6.83 (t, ^{3}J = 8 Hz, 1H, Ar*H*), 6.67 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 5.85 (s, 1H, N*H*), 1.86 (s, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C), δ=164.0, 145.3, 143.5, 134.3, 128.8, 128.7, 128.5, 126.8, 119.6, 115.1, 70.6, 30.0.

4.1.7. Synthesis of 2-methyl-2-(2-methylpropyl)-2,3-dihydroquinazolin-4(1*H*)-one (3g)

3g was synthesized by general procedure using a mixture of 2-amino benzamide (13.6 g, 100 mmol) and methyl isobutyl ketone (25 mL, 200 mmol) as white crystalline solid (99.2% yield). m.p. 170-172 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.86 (dd, ^{3}J = 8 Hz, J = 2 Hz, 1H, Ar*H*), 7.32-7.27 (m, 1H, Ar*H*), 6.92 (s, 1H, N*H*), 6.81 (t, ^{3}J = 8 Hz, 1H, Ar*H*), 6.62 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 5.81 (s, 1H, N*H*), 1.94-1.88 (m, 1H, CH), 1.70 (d, ^{3}J = 6 Hz, 2H, CH_2), 1.54 (s, 3H, CH_3), 0.97 (t, ^{3}J = 7 Hz, 6H, 2×CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C), δ=164.4, 145.9, 133.9, 128.3, 118.4, 114.5, 114.4, 70.2, 50.3, 28.8, 24.5, 24.3, 24.1.

4.1.8. Synthesis of 2,2-bis(2-methylpropyl)-2,3-dihydroquinazolin-4(1*H*)-one (3h)

3h was synthesized by general procedure using a mixture of 2-amino benzamide (6.8 g, 50 mmol) and diisobutyl ketone (17.5 mL, 100 mmol) as white crystalline solid (99.5% yield). m.p. 170-172 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.83 (dd, ^{3}J = 8 Hz, ^{4}J = 2 Hz, 1H, Ar*H*), 7.33-7.28 (m, 1H, Ar*H*), 6.89 (s, 1H, N*H*), 6.78 (t, ^{3}J = 8 Hz, 1H, Ar*H*), 6.60 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 5.78 (s, 1H, N*H*), 1.92-1.85 (m, 2H, 2×CH), 1.74 (d, ^{3}J = 6 Hz, 2H, CH_2), 1.71 (d, ^{3}J = 6 Hz, 2H, CH_2), 0.96 (d, ^{3}J = 7 Hz, 6H, 2×CH_3), 0.94 (d, ^{3}J = 7 Hz, 6H, 2×CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C), δ=164.6, 143.9, 133.9, 128.3, 117.8, 114.5, 115.4, 68.9, 52.3, 24.6, 24.3, 21.4.

4.2. General Procedure for the synthesis of 6,8-dinitro-2,2-disubstituted-2,3-dihydroquinazolin-4(1*H*)-one (4a-h)

Above synthesized 2,3-disubstituted quinazoline (**3a-h**) (0.01 mole) was added to conc. H_2SO_4 (10mL) and stirred to dissolve in an ice bath. To this solution conc. HNO_3 (0.5mL) was added drop wise and stirring was continued for further 6 hours. Mixture was poured on crushed ice and left overnight. Precipitates formed were filtered and washed with plenty of water, dried in oven and recrystallized from ethanol to afford yellowish crystals of 6,8-dinitro-2,2-disubstituted-2,3-dihydroquinazolin-4(1*H*)-one which were further characterized by NMR and XRD techniques.

4.2.1. Synthesis of 6,8-dinitro-2,2-dimethyl-2,3-dihydroquinazolin-4(1H)-one (4a).

Yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.22 (d, ^3J = 8 Hz, 1H, Ar*H*), 9.02 (d, ^3J = 8 Hz, 1H, Ar*H*), 6.90 (s, 1H, N*H*), 5.80 (s, 1H, N*H*), 1.38 (s, 6H, 2×CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$,25 °C), δ=161.2, 144.8, 130.6, 128.3, 128.5, 125.5, 121.7, 67.4, 31.6.

4.2.2. Synthesis of* 2-methyl-6,8-dinitro-2-(3-nitro-phenyl)-2,3-dihydro-1H-quinazolin-4-one *(4b)

Yellow crystalline solid.CCDC 1509031.^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.23 (d, ^3J = 8 Hz, 1H, Ar*H*), 9.06 (d, ^3J = 8 Hz, 1H, Ar*H*), 8.22 (d, ^3J = 8 Hz, 2H, Ar*H*), 7.81 (d, ^3J = 8 Hz, 2H, Ar*H*), 6.90 (s, 1H, N*H*), 5.83 (s, 1H, N*H*), 1.85 (s, 3H, CH_3) ppm.

4.2.3. Synthesis of 6,8-dinitro-2-ethyl, 2-methyl-2,3-dihydroquinazolin-4(1H)-one (4c)

Yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.21 (d, ^3J = 8 Hz, 1H, Ar*H*), 9.02 (d, ^3J = 8 Hz, 1H, Ar*H*), 6.91 (s, 1H, N*H*), 5.78 (s, 1H, N*H*), 1.99-1.93 (m, 2H, CH_2), 1.24 (s, 3H, CH_3), 1.04 (t, ^3J = 7 Hz, 3H, CH_3) ppm.

4.2.4. Synthesis of 6,8-dinitro-2,2-diethyl-2,3-dihydroquinazolin-4(1H)-one (4d)

Yellow crystalline solid.^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.21 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 9.02 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 6.92 (s, 1H, N*H*), 5.67 (s, 1H, N*H*), 1.95-1.88 (m, 4H, 2×CH_2), 1.05 (t, ^{3}J = 7 Hz, 6H, 2×CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C), δ=160.2, 145.8, 130.6, 130.3, 128.5, 127.3, 122.0, 74.6, 32.2, 7.6.

4.2.5. Synthesis of 6,8-dinitro-2-methyl-2-propyl-2,3-dihydroquinazolin-4(1H)-one (4e)

Yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.22 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 9.03 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 6.91 (s, 1H, N*H*), 5.84 (s, 1H, N*H*), 1.75-1.72 (m, 2H, CH_2), 1.51 (s, 3H, CH_3), 1.47-1.38 (m, 2H, CH_2), 0.93 (t, ^{3}J = 7 Hz, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C), δ=161.2, 145.6, 131.6, 130.8, 128.7, 124.9, 121.3, 71.4, 44.8, 28.4, 16.9, 14.5.

4.2.7. Synthesis of 6,8-dinitro-2-methyl-2-(2-methylpropyl)-2,3-dihydroquinazolin-4(1H)-one (4g)

Yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.21 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 9.04 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 6.88 (s, 1H, N*H*), 5.86 (s, 1H, N*H*), 1.95-1.88 (m, 1H, CH), 1.85 (d, ^{3}J = 6 Hz, 2H, CH_2), 1.72 (s, 3H, CH_3), 1.02 (d, ^{3}J = 6 Hz, 6H, 2×CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C), δ=160.1, 145.0, 136.7, 130.9, 130.4, 127.3, 116.9, 71.5, 51.5, 30.7, 24.2, 24.0.

4.2.8. Synthesis of 6,8-dinitro-2,2-bis(2-methylpropyl)-2,3-dihydroquinazolin-4(1H)-one (4h)

Yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.21 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 9.05 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 6.92 (s, 1H, *NH*), 5.86 (s, 1H, *NH*), 1.91-1.85 (m, 2H, 2×CH), 1.74 (d, ^{3}J = 6 Hz, 2H, CH_2), 1.71 (d, ^{3}J = 6 Hz, 2H, CH_2), 0.96 (d, ^{3}J = 6 Hz, 6H, 2×CH_3), 0.94 (d, ^{3}J = 6 Hz, 6H, 2×CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C), δ=160.4, 145.2, 136.2, 131.4, 130.7, 126.6, 117.2, 69.3, 52.8, 24.8, 24.6, 20.8.

4.3. Synthesis of 6,8-dibromo-2,2-disubstituted-2,3-dihydroquinazolin-4(1*H*)-one (5a-h)

To a stirred solution of 2,2-disubstituted quinazoline (10 mmol) and $KBrO_3$ (10 mmol) in acetonitrile (10ml) was added dil. HCl (10 mL, 1N) drop wise and stirring was continued for 6 hrs at 70 ^oC. Concentration of the mixture was done at rotary evaporator and washing was done with water. Recrystallization of the product with ethanol resulted required product.

4.3.1. Synthesis of 6,8-dbromo-2,2-dimethyl-2,3-dihydroquinazolin-4(1H)-one (5a).

Brownish crystalline solid. CCDC 1442364.^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.85 (d, ^3J = 8 Hz, 1H, Ar*H*), 7.55 (d, ^3J = 8 Hz, 1H, Ar*H*), 6.90 (s, 1H, *NH*), 5.86 (s, 1H, *NH*), 1.41 (s, 6H, 2×CH_3) ppm.

4.3.2. Synthesis of 6,8-dibromo-2-methyl-2-phenyl-2,3-dihydroquinazolin-4(1H)-one (5b)

Brownish crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.87 (d, ^3J = 8 Hz, 1H, Ar*H*), 7.63 (d, ^3J = 8 Hz, 1H, Ar*H*), 7.24 (d, ^3J = 8 Hz, 2H, Ar*H*), 7.14 (d, ^3J = 7 Hz, 2H, Ar*H*), 6.93 (s, 1H, N*H*), 5.86 (s, 1H, N*H*), 1.89 (s, 3H, CH_3) ppm

4.3.3. Synthesis of 6,8-dibromo-2-ethyl, 2-methyl-2,3-dihydroquinazolin-4(1H)-one (5c)

Brownish crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.83 (d, ^3J = 8 Hz, 1H, Ar*H*), 7.53 (d, ^3J = 8 Hz, 1H, Ar*H*), 6.89 (s, 1H, *NH*), 5.78 (s, 1H, *NH*), 1.84-1.83 (m, 2H, CH_2), 1.49 (s, 3H, CH_3), 1.25 (t, ^3J = 7 Hz, 3H, CH_3) ppm.

4.3.4. Synthesis of 6,8-dibromo-2,2-diethyl-2,3-dihydroquinazolin-4(1H)-one (5d)

Brownish crystalline solid.^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.81 (d, ^3J = 8 Hz, 1H, Ar*H*), 7.63 (d, ^3J = 8 Hz, 1H, Ar*H*), 6.91 (s, 1H, N*H*), 5.80 (s, 1H, N*H*), 1.79-1.74 (m, 4H, 2×CH_2), 0.97 (t, ^3J = 7 Hz, 6H, 2×CH_3) ppm.

4.3.5. Synthesis of 6,8-dibromo-2-methyl-2-propyl-2,3-dihydroquinazolin-4(1H)-one (5e)

Brownish crystalline solid.^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.86 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 7.38 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 6.94 (s, 1H, N*H*), 5.82 (s, 1H, N*H*), 1.75-1.69 (m, 2H, CH_2), 1.49 (s, 3H, CH_3), 1.46-1.40 (m, 2H, CH_2), 0.93 (t, ^{3}J = 7 Hz, 3H, CH_3) ppm.

4.3.6. Synthesis of 6,8-Dibromo-2-(3-bromo-4-chloro-phenyl)-2-methyl-2,3-dihydro-1H-quinazolin-4-one (5f)

Brownish crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.89 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 7.68 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 7.35 (s, 1H, Ar*H*), 7.19 (d, ^{3}J = 7 Hz, 1H, Ar*H*), 7.08 (d, ^{3}J = 7 Hz, 1H, Ar*H*), 6.89 (s, 1H, *NH*), 5.86 (s, 1H, N*H*), 1.89 (s, 3H, CH_3) ppm.

4.3.7. Synthesis of 6,8-dibromo-2-methyl-2-(2-methylpropyl)-2,3-dihydroquinazolin-4(1H)-one (5g)

Brownish crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.87 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 7.68 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 6.93 (s, 1H, N*H*), 5.85 (s, 1H, N*H*), 1.94-1.88 (m, 1H, CH), 1.85 (d, ^{3}J = 6 Hz, 2H, CH_2), 1.73 (s, 3H, CH_3), 1.04 (d, ^{3}J = 6 Hz, 6H, 2×CH_3) ppm.

4.3.8. ***Synthesis of 6,8-dibromo-2,2-bis(2-methylpropyl)-2,3-dihydroquinazolin-4(1H)-one (5h)***

Brownish crystalline solid.^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.88 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 7.67 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 6.91 (s, 1H, *NH*), 5.82 (s, 1H, *NH*), 1.92-1.84 (m, 2H, 2 × *CH*), 1.73 (d, ^{3}J = 7 Hz, 2H, CH_2), 1.71 (d, ^{3}J = 7 Hz, 2H, CH_2), 0.98 (d, ^{3}J = 6 Hz, 6H, 2×CH_3), 0.96 (d, ^{3}J = 6 Hz, 6H, 2×CH_3) ppm.

4.4. Determination of AChE and BChE inhibitory activity

AChE (Electric eel type-VI-S, Sigma-Aldrich GmbH USA, code 1001596210), BChE (Equine serum Lyophilized Sigma-Aldrich GmbH USA, code 101292670), Acetylthiocholine iodide

(Sigma-Aldrich UK, code 101303874), Butyrylthiocholine Iodide (Sigma-Aldrich Switzerland, code 101334643), DTNB (Sigma-Aldrich Germany, code 101261619), Galantamine hydrobromide Lycoris Sp. (Sigma-Aldrich France, code G1660). All the other chemicals used were of analytical grade.

Galantamine was used as reference drugs. The synthesized quinazolines were dissolved in 0.1 M phosphate buffer of pH 8.0. (KH_2PO_4/K_2HPO_4. The reaction mixture consisted of appropriate amount of, DTNB (Ellman's reagent), test compounds, of 0.03 U/ml of enzymes (AChE and BChE). The mixture was pre-incubated at 30 °C for 10 min and followed by adding 1mM ATCI or BTCI and incubated again for 15 min. The enzymatic hydrolysis was monitored at 412 nm using µQuant microplate spectrophotometer (MQX200, BioTek USA). All reactions were carried out in triplicate. The IC_{50} values were determined by plotting the inhibition against the sample solution concentrations.

4.5. Computational studies

Docking studies were carried out using GOLD (Genetic Optimization for Ligand Docking) suit v5.4.1. The X-ray crystallographic structure *Tc*AChE (PDB Code 1EVE) and *h*AChE (PDB Code 4EY7) and human BChE (PDB Code 1P0I) was used as enzyme structures. The default docking protocol was applied and the synthesized tin complexes were submitted to 10 GA runs using GOLD fitness score. Other docking parameters were set to the software's default values. The view of the docking results and analysis of their surface with graphical representations were done using Discovery Studio Visualizer [36] and Chimera 1.11.2rc [37].

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