Synthesis, X-Rays Analysis, Docking Study and Cholinesterase Inhibition Activity of 2,3-dihydroquinazolin-4(*1H*)-one Derivatives

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ABSTRACT: *In search of potent cholinesterase inhibitors, we have carried out the synthesis and biologically evaluation of various benzaldehyde based 2,3-dihydroquinazolin-4(1H)-one derivatives. In vitro assay results revealed that all the synthesized compounds showed activity against both enzymes (AChE and BChE) and in few cases, the inhibition activity was even higher than or comparable to the standard drug galantamine. Overall, compounds having chloro or methoxy group attached to the para position of benzaldehyde resulted in potent cholinesterase inhibitors. Within the series, Bromo derivatives 4a-i were more active than their un-substituted counterparts. Amongst all, compound 4c (6,8-dibromo-2-(3-bromo-4-chloro-phenyl)-2,3-dihydro-1H-quinazolin-4-one) with selectivity index of 3.7 for AChE, displayed IC_{50} values of 3.7±1.05 μM (AChE) and 13.7±0.64 μM (BChE) and can be considered as potential lead compound with a feature of dual cholinesterase (AChE/BChE) inhibition. Insight into the mechanism of inhibition of the synthesized compounds was provided by computed binding modes in the active site of AChE and BChE. Docking study on both isomers of the quinazoline also supported in vitro assay results. Preliminary in silico studies by using online admetSAR server showed that all compounds possessed good pharmacokinetic profile except nitro and methoxy substituted derivatives which were predicted to exhibit AMES toxicity. The synthesized compounds can be used as a structural foundation for the preparation of new potent cholinesterase inhibitors.*

KEYWORDS: *Cholinesterases; 2,3-dihydroquinazolin-4(1H)-one; Dual inhibitors; Alzheimer's disease.*

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INTRODUCTION

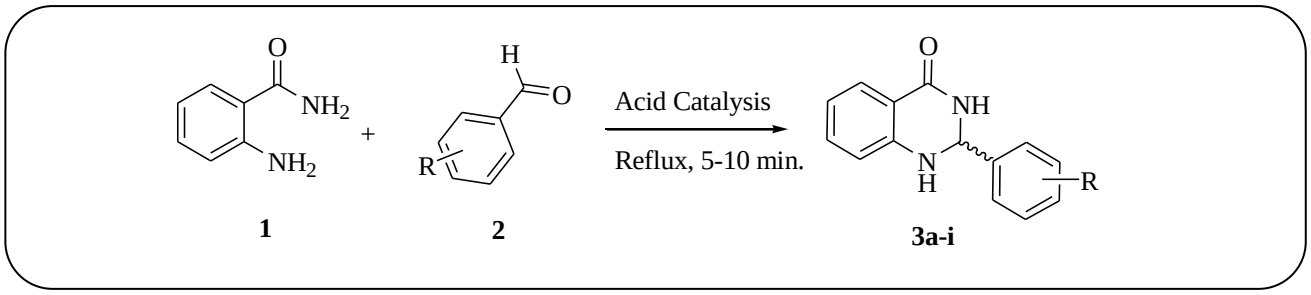
Heterocyclic compounds are building blocks of the majority of available therapeutic products that mimic natural products with physiological and biological activities [1]. Importance of heterocyclic compounds is heightened due to existence of their structural subunits in a large number of biological molecules such as vitamins, DNA, RNA, hormones, antibiotics and pigments and other natural compounds [2-4]. Literature survey of commercially available combinatorial libraries revealed that available bicyclic heterocyclic compounds are mainly limited to nitrogen containing moieties. These molecular motifs are considered to be "privileged structures" after the term coined by *Evans et al.* [5]. Privileged structures have versatile binding properties, so this approach has been widely applied in traditional medicinal chemistry as well as for the design of focused libraries [6]. Although, established synthetic methodologies are commonly used throughout the process of a drug discovery program, however, modern drug development design in comparison to classical approach- "*Let's make a change on an existing compound or synthesize a new structure and see what happens*" has rapidly grown as an approach to solve a drug design problem [7].

A number of natural and synthetic heterocyclic compounds especially nitrogen containing organic compounds possess fascinating biological properties and play key role in various biological processes. Many scientists have synthesized and studied different heterocyclic moieties for their versatile properties [8-11]. Various synthetic nitrogen heterocyclic moieties are well known to display a wide range of biological activities like antibacterial, antiviral, anti-inflammatory, antifungal, antioxidants, analgesics, anticancer, sedatives, anticonvulsants and hypnotics etc. Due to this reason, more than 75% structural assemblies of top 200 branded drugs available in the market are made up of heterocyclic fragments [12].

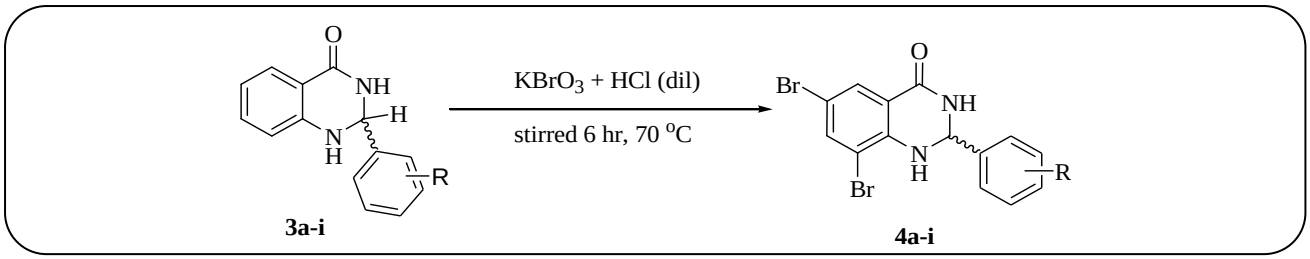
The cholinesterases including both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) catalyze the hydrolysis of the acetylcholine and regulate various physiological functions like blood coagulation, neurotransmission and digestion. As cholinesterase plays a critical role in maintaining and controlling different types of important physiological functions in the body, therefore several diseases and disorders such as thrombosis, pancreatitis and Alzheimer's Disease (AD) are associated with their activity. Cholinesterase inhibitors have clinical use in the management of myasthenia gravis, senile dementia, ataxia, AD and Parkinson's disease, along with several other disorders such as type 2 diabetes and chronic pain [13-17]. Cholinesterase (AChE and BChE) enzymes inhibition will elevate ACh level which can interact with neuronal receptor and ultimately reduces symptoms of AD and other neurotransmission disorders [18]. Four cholinesterase inhibitors (rivastigmine, tacrine, donepezil and galantamine) have been licensed as commercial drugs for symptomatic treatment of AD. Moreover, different classes of cholinesterase inhibitors such as tacrine, rivastigmine, donepezil, xanthostigmine, galantamine, *para*-aminobenzoic acid, coumarin, pyrrolo-isoxazole analogues and flavonoids (galangin, kaempferol, quercetin, myricetin, fisetin, apigenin, luteolin, rutin and other flavonoid derivatives having substituents like benzylpiperidine, amino alkyl/pyrrolidine/piperidine and carbamate moiety) have been developed as cholinesterase inhibitors [19]. Although cholinesterase inhibitors show a wide range of chemical diversity, but different heterocyclic compound and their derivatives are reported to exhibit potent inhibitory activity as dual cholinesterase inhibitors. Various simple and hybrid aromatic heterocyclic and *N*-heterocycles moieties with better interactions and enhanced anticholinesterase potency have been developed including imidazolidines, oxazolidines and benzoxazoles analogues [20-22]

Quinazoline, a class of fused pyrimidine heterocyclic compounds show wide range of biological activities such as antimicrobial, anticonvulsant, hypnotic, anticancer, antihistaminic, anti-inflammatory, diuretic, antimalarial, antihypertensive, antifungal, antagonism of ghrelin receptor, analgesic and COX-2 inhibitory activities [23-26]. Few researchers have also reported biological potential of quinazoline compounds as inhibitors of AChE and BChE [27-28].

Recently, we have reported synthesis and biological activity of 2,3-dihydroquinazolin-4(*1H*)-one derivatives as dual binding site cholinesterase inhibitor [29-30]. In our present project, we have attempted to further optimize the structure of 2,3-dihydro-quinazolin-4(*1H*)-one core



Scheme 1: Synthesis of 2-substituted-2,3-dihydroquinazolin-4(1H)-ones (3a-i).



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

by replacing ketones with aromatic aldehydes having balanced hydrophilic and hydrophobic sites. The objective of present study is to develop 2,3-dihydroquinazolin-4(1*H*)-one derivatives as potent cholinesterase (AChE/BChE) inhibitors.

EXPERIMENTAL SECTION

All chemicals like 2-amino benzamide, aromatic aldehydes and solvents were of analytical grade and used asreceived. Acetylthiocholine iodide (code 101303874) was procured from Sigma-Aldrich UK and AChE (Electric eel type-VI-S, code 1001596210) was supplied by Sigma-Aldrich GmbH USA. Butyrylthiocholine Iodide (code 101334643) and BChE (Equine serum Lyophilized, code 101292670) were arranged from Sigma-Aldrich Switzerland and Sigma-Aldrich GmbH USA respectively. DTNB (code 101261619) from Sigma-Aldrich Germany and Galantamine hydrobromide Lycoris Sp. (code G1660) from Sigma-Aldrich France were used. ^{1}H NMR spectra were recorded on Bruker DRX 400 MHz NMR spectrometers and chemical shifts were reported in comparison to $SiMe_4$. Perkin-Elmer 241 polarimeter was used to determine optical rotations at the sodium D-line. The X-ray diffraction analysis of crystals was carried out on a Bruker Smart APEX II diffractometer.

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

To a stirred solution of 2-amino benzamide (100 mmol) in DCM, aldehyde (100 mmol) was added and stirring was continued for further 05 min. To this stirred mixture, conc. HNO_3/ HCl (1 mL) was added and reaction mixture was refluxed for 05-10 min. After completion of the reaction as checked by TLC, product was concentrated by rotary evaporator. After cooling, water (20mL) was added to the mixture and precipitates so formed were filtered, washed with excess water and dried in oven. Recrystallization of the product with ethyl acetate afforded crystals of 2-disubstituted-2,3-dihydroquinazolin-4(1*H*)-one which were characterized by spectroscopic and X-Rays crystallographic techniques.

Synthesis of 2-phenyl-2,3-dihydro-1H-quinazolin-4-one (3a)

Compound 3a was synthesized by following general procedure from 2-amino benzamide and benzaldehyde as white crystalline solid (89.5% yield), m.p. 228-230 °C, ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.80 (dd, ^{3}J = 8 Hz, ^{4}J = 2 Hz, 1H, Ar*H*), 7.32-7.28 (m, 1H, Ar*H*), 7.21-7.17 (m, 2H, Ar*H*), 7.12-7.07 (m, 3H, ArH), 6.91 (s, 1H, N*H*), 6.77 (t, ^{3}J = 8 Hz, 1H, Ar*H*), 6.59 (d, ^{3}J = 8 Hz, 1H, Ar*H*), 6.07 (m, 1H, Ar*H*), 5.58 (s, 1H, N*H*) ppm. ^{13}C

NMR (100 MHz, $CDCl_3$, 25 °C): δ = 166.0, 145.1, 144.2, 132.9, 128.7, 128.1, 127.2, 126.5, 118.4, 113.8, 68.7.

Synthesis of 2-(2-chloro-phenyl)-2,3-dihydro-1H-quinazolin-4-one (3b)

Synthesis of compound 3b was done by general procedure using a mixture of 2-amino benzamide and 2-chloro benzaldehyde as white crystalline solid (88.2% yield). m.p. 322-324 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.79 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.32-7.27 (m, 1H, Ar*H*), 7.20 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.06-7.00 (m, 3H, ArH), 6.91 (s, 1H, N*H*), 6.78 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.60 (d, ^3J = 8 Hz, 1H, Ar*H*), 6.05 (m, 1H, Ar*H*), 5.58 (s, 1H, N*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.0, 145.0, 144.3, 133.0, 132.6, 129.4, 128.3, 128.0, 118.4, 117.8, 113.8, 60.4.

Synthesis of 2-(4-chloro-phenyl)-2,3-dihydro-1H-quinazolin-4-one (3c)

Synthesis of 3c was carried out by general procedure using a mixture of 2-amino benzamide and 4-chloro benzaldehyde as white crystalline solid (90.0% yield). m.p. 328-330 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.79 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.32-7.27 (m, 1H, Ar*H*), 7.20 (d, ^3J = 8 Hz, 2H, Ar*H*), 7.08 (d, ^3J = 7 Hz, 2H, Ar*H*), 6.91 (s, 1H, N*H*), 6.79 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.60 (d, ^3J = 8 Hz, 1H, Ar*H*), 6.05 (m, 1H, Ar*H*), 5.58 (s, 1H, N*H*) ppm.

Synthesis of 2-(2-nitro-phenyl)-2,3-dihydro-1H-quinazolin-4-one (3d)

Compound 3d was obtained from the reaction of 2-amino benzamide and 2-nitro benzaldehyde by following the general procedure as light yellow crystalline solid (86.7% yield); m.p. 299-301 °C, ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ =8.17 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.82 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.59-7.55 (m, 1H, Ar*H*), 7.35-7.29 (m, 3H, Ar*H*), 6.89 (s, 1H, N*H*), 6.76 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.63 (d, ^3J = 8 Hz, 1H, Ar*H*),6.03 (m, 1H, Ar*H*), 5.82 (s, 1H, N*H*), ppm.

Synthesis of 2-(4-nitro-phenyl)-2,3-dihydro-1H-quinazolin-4-one (3e)

Synthesis of compound 3e was achieved from a mixture of 2-amino benzamide and 4-nitro benzaldehyde by general procedure as light yellow crystalline solid (86.8% yield), m.p. 304-305 °C, ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ =8.11 (d, ^3J = 8 Hz, 2H, Ar*H*), 7.82 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.36 (d, ^3J = 7 Hz, 2H, Ar*H*), 7.31-7.27 (m, 1H, Ar*H*), 6.89 (s, 1H, N*H*), 6.76 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.62 (d, ^3J = 8 Hz, 1H, Ar*H*), 6.04 (m, 1H, Ar*H*), 5.82 (s, 1H, N*H*), ppm.

Synthesis of 2-(3-methoxy-phenyl)-2,3-dihydro-1H-quinazolin-4-one (3f)

Synthesis of compound 3f was carried out by general procedure using a mixture of 2-amino benzamide and 3-methoxy benzaldehyde as white crystalline solid (78.2% yield). m.p. 308-309 °C, ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.81 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.33-7.27 (m, 1H, Ar*H*), 7.06 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.92 (s, 1H, N*H*), 6.83 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.65-6.58 (m, 4H, Ar*H*), 6.04 (m, 1H, Ar*H*), 5.85 (s, 1H, N*H*), 3.81 (s, 3H, CH_3) ppm.

Synthesis of 2-(4-methoxy-phenyl)-2,3-dihydro-1H-quinazolin-4-one (3g)

Compound 3g was synthesized by general procedure using a mixture of 2-amino benzamide and 4-methoxy benzaldehyde as white crystalline solid (76.8% yield). m.p. 319-320 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.81 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.33-7.27 (m, 1H, Ar*H*), 6.99 (d, ^3J = 8 Hz, 2H, Ar*H*), 6.92 (s, 1H, N*H*), 6.83 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.65-6.58 (m, 3H, Ar*H*), 6.04 (m, 1H, Ar*H*), 5.85 (s, 1H, N*H*), 3.79 (s, 3H, CH_3) ppm.

Synthesis of 2-(3-hydroxy-phenyl)-2,3-dihydro-1H-quinazolin-4-one (3h)

Reaction of 2-amino benzamide and 3-hydroxy benzaldehyde was carried out to synthesize compound 3h by adopting general procedure as white crystalline solid (81.5% yield). m.p. 366-364 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.83 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.31-7.26 (m, 1H, Ar*H*), 7.03 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.92 (s, 1H, N*H*), 6.83 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.64 (d, ^3J = 7 Hz, 2H, Ar*H*), 6.56-6.52 (m, 2H, Ar*H*), 6.04 (m, 1H, Ar*H*), 5.85 (s, 1H, N*H*), 5.11 (s, 1H, OH) ppm.

Synthesis of 2-(4-hydroxy-phenyl)-2,3-dihydro-1H-quinazolin-4-one (3i)

Synthesis of compound 3i was done from the reaction of 2-amino benzamide and 4-hydroxy benzaldehyde by using general procedure as white crystalline solid (82.7% yield).

m.p. 370-372 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.81 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, Ar*H*), 7.31-7.27 (m, 1H, Ar*H*), 6.94 (d, ^3J = 8 Hz, 2H, Ar*H*), 6.89 (s, 1H, N*H*), 6.83 (t, ^3J = 8 Hz, 1H, Ar*H*), 6.64-6.59 (m, 3H, Ar*H*), 6.04 (m, 1H, Ar*H*), 5.85 (s, 1H, N*H*), 5.10 (s, 1H, OH) ppm.

General procedure for the synthesis of 6,8-dibromo-2-disubstituted-2,3-dihydroquinazolin-4(1H)-one (4a-j)

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

Synthesis of 6,8-dibromo-2-(3-bromo-phenyl)-2,3-dihydro-1H-quinazolin-4-one (4a)

Compound 4a was synthesized by following general procedure 4.2 from 3a as brownish solid (69.5% yield), ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.89 (s, 1H, Ar*H*), 7.65 (s, 1H, Ar*H*), 7.26-7.21 (m, 2H, Ar*H*), 7.06-7.01 (m, 2H, ArH), 6.90 (s, 1H, N*H*), 6.07 (m, 1H, Ar*H*), 5.58 (s, 1H, N*H*) ppm.

Synthesis of 6,8-dibromo-2-(3-bromo-2-chloro-phenyl)-2,3-dihydro-1H-quinazolin-4-one (4b)

Synthesis of compound 4b was done by general procedure from 3b as brownish crystalline solid (68.1% yield); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.89 (s, 1H, Ar*H*), 7.65 (s, 1H, Ar*H*), 7.24 (d, ^3J = 8 Hz, 1H, Ar*H*), 7.01-6.65 (m, 2H, ArH), 6.90 (s, 1H, N*H*), 6.06 (m, 1H, Ar*H*), 5.54 (s, 1H, N*H*) ppm.

Synthesis of 6,8-dibromo-2-(3-bromo-4-chloro-phenyl)-2,3-dihydro-1H-quinazolin-4-one (4c)

Synthesis of 4c was carried out by general procedure from 3c as brownish solid (69.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.89 (s, 1H, Ar*H*), 7.65 (s, 1H, Ar*H*), 7.21 (s, 1H, Ar*H*), 7.05 (d, ^3J = 8 Hz, 1H, ArH), 6.96 (d, ^3J = 7 Hz, 1H, ArH), 6.90 (s, 1H, N*H*), 6.06 (m, 1H, Ar*H*), 5.54 (s, 1H, N*H*) ppm.

4c(I) $[\alpha]_D^{25}$ = −56.84 (*c* 1.04, $CHCl_3$) and 4c(II) $[\alpha]_D^{25}$ = +56.72 (*c* 1.02, CHCl3). Spectroscopy data of both enantiomers was exactly identical to the racemic mixture.

Synthesis of 6,8-dibromo-2-(3-bromo-2-nitro-phenyl)-2,3-dihydro-1H-quinazolin-4-one (4d)

Synthesis of compound 4d was carried out from 3d by following the general procedure as light brownish solid (66.7% yield); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.88 (s, 1H, Ar*H*), 7.66 (s, 1H, Ar*H*), 7.58 (d, ^3J = 8 Hz, 1H, Ar*H*), 7.46 (t, ^3J = 8 Hz, 1H, Ar*H*), 7.30 (d, ^3J = 7 Hz, 1H, Ar*H*), 6.91 (s, 1H, N*H*), 6.06 (m, 1H, Ar*H*), 5.54 (s, 1H, N*H*) ppm.

Synthesis of 6,8-dibromo-2-(3-bromo-4-nitro-phenyl)-2,3-dihydro-1H-quinazolin-4-one (4e)

Synthesis of compound 4e was achieved from 3e by general procedure as light brown solid (68.8% yield), ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.01 (d, ^3J = 8 Hz, 1H, Ar*H*), 7.89 (s, 1H, Ar*H*), 7.65 (s, 1H, Ar*H*), 7.53 (s, 1H, Ar*H*), 7.27 (d, ^3J = 7 Hz, 1H, Ar*H*), 6.90 (s, 1H, N*H*), 6.06 (m, 1H, Ar*H*), 5.54 (s, 1H, N*H*) ppm.

X-rays crystallographic study

Crystallization of the synthesized compounds was done by slow evaporation in a solution ethyl acetate/DCM/methanol (2:1:1) and suitable crystals of the individual compounds were analysed at Bruker KAPPA Apex II diffractometer having graphite-monochromatized Mo Ka radiation, λMo = 0.710 73 Å at 100 K. Data reduction and structure refinement was achieved by using SAINT and SHELXL-2013 program package respectively. Material for publication was prepared by using PLATON software [42-43]. Finalized crystal structure data was deposited with Cambridge Crystallographic Data Centre (CCDC) which can be obtained free of charges.

Determination of AChE and BChE inhibitory activity

Anti-cholinesterase activity of the synthesized compounds was determined by using Ellman's methodology [38] by dissolving compounds in phosphate buffer (0.1 M) having pH 8.0. (KH_2PO_4/K_2HPO_4). Appropriate amount of Ellman's reagent (DTNB), 0.03 U/mL of enzymes (AChE and BChE) and test compounds were mixed and this reaction mixture was pre-incubated at 30 °C for 10 min, followed by adding 1mM ATCI or BTCI and incubated again for 15 min. µQuant microplate spectrophotometer (MQX200, BioTek USA) was used to monitor the enzymatic hydrolysis at 412 nm. The IC_{50}

values were determined by plotting the sample solution concentrations against the inhibition. Galantamine was used as reference drug and all reactions were carried out in triplicate.

Resolution of quinazoline racemic mixture

To a cooled solution of 4c racemic mixture in THF at -78˚C, slow addition of NaHMDS (1.1 mol equiv.) in hexane (1.0 M) was carried out. Solution was stirred at -78˚C for 15 min and then resolution agent (N-phthalyl-L-alanine chloride) was added. Resulting mixture was stirred for further 1 h before treating with saturated NH_4Cl solution and water. Extraction with CH_2Cl_2 and purification by chromatography (hexane/AcOEt) resulted the corresponding diastereomer. Evaporation and further addition of Bu_4NOH solution in excess at 0˚C to the appropriate diastereomer in THF under stirring conditions for 12 h yielded corresponding enantiomer. Finally, the mixture was concentrated and further purified by employing column chromatography (hex/AcOEt 50:50) to obtain the desired product.

Docking studies

Docking experiments were performed *via* Molecular Operating Environment (MOE) docking program version 2016.08. Crystal structures of of *Tc*AChE, *h*AChE and BChE with PDB codes 1EVE, 4EY7 and 1P0I respectively were selected for these studies. All the water molecules were removed from the protein structure. The 3D protonation of the downloaded enzymes was done for all the atoms in the implicit solvated environment (GB/VI) at temperature 300K, pH=7 and Salt concentration=0.1. After 3D protonation step, energy optimization was carried out using default force field [gradient: 0.05, Force Field: MMFF94X]. Partial charges of all atoms were calculated by using current force field method before docking.The resulting model was subjected to systematic conformational search at default parameters with RMS gradient of 0.01 kcal/mol using Site Finder. For 1EVE and 4EY7, the active site of the prepared enzyme was defined as the residues within 10 Å of the reference ligand (donepezil). However, for 1P0I (BChE), the enzyme was searched for its active site and dummy atoms were created using alpha spheres as centroids. The important amino acid residues from the list reported in the literature. A key tryptophan residue in AChE, Trp84 (TcAChE numbering), is conserved in BChE (Trp82). Other key residues are: Asp70, Gly116, Gly117, Tyr128, Glu197, Ser198, Phe329, His438.The backbone and residues were kept fixed and the energy minimization was performed. The lowest energy minimized pose was used for further analysis. Ligand-interaction module of MOE was used to calculate the 2D ligand-enzyme interactions. The view of the docking results and analysis of their surface with graphical representations were done using MOE and discovery studio visualizer [44].

Determination of in silico pharmacokinetic properties

The ADMET structure-activity relationship server, known as admetSAR, is a comprehensive knowledge and tool used to predict Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of various drug candidates and other chemicals [45]. As experimental evaluation of ADMET profiles is costly and time consuming. Therefore, *in silico* pharmacokinetic study plays an important role for development of a synthesized compound toward an effective drug [46]. Online *admet*SAR server was used to carry out *in silico* predictions of all synthesized compounds including BBB, HIA, AT and carcinogenicity. Structures of all synthesized compounds were drawn in ChemDrawsoftware and SMILES files were used to predict ADMET properties in comparison to standard drug donepezil.

RESULTS AND DISCUSSION

Synthesis and chemistry

Many researchers have made use of traditional condensation methods and catalytic methodologies like catalytic carbonylation, domino reaction, hydrogen transfer process, use of metals such as iridium, rhodium and palladium and green catalysts for the construction of quinazoline derivatives [31-34]. However, drastic reaction conditions, low yield, prolonged reaction time and use of sophisticated research methodologies were the main concerns. We tried relatively an inexpensive acid catalysis method for the synthesis of 2-substituted-2,3-dihydroquinazolin-4(1*H*)-one derivatives from the reaction of anthranilamide and aromatic aldehydes under acid catalysis (1ml of HNO_3/HCl : 3/1) with excellent yield (Scheme 1). Detail of substituents is presented in Table 2.

As our previous study revealed that bromo derivatives of 2,2-disubstituted-2,3-dihydroquinazolin-4(1*H*)-one were more active towards cholinesterase inhibition

as compared to their un-substituted analogues [28]. Therefore, bromination of the synthesized compounds was carried out to synthesize bromo derivatives **(**4a-i) by the reaction of **3a-i** with potassium bromate in acidic medium (Scheme 2).

It has been observed that synthesized compounds possess a chiral center at C-2 position and product exist as racemic mixture. Each enantiomer might have quite different pharmacological properties. Therefore, it is necessary to resolve the racemic mixtures to investigate the biological properties of each enantiomer separately. Different researchers have used diversified techniques for resolution of racemic mixture. However, use of column chromatography by using commercially available Chiral Stationary Phases (CSP) like cellulose and amylose derivatives, especially phenylcarbamate derivatives have been widely employed for the separation of chiral biologically active substances [35-36]. We have employed chiral column chromatography methodology by using hexane/ AcOEt solvent for the separation of racemic mixture of our hit compound **4c** into (-) and (+) enantiomers and studied their biological activity. The resolution of the two enantiomers was achieved by forming diastereomers with *N*-phthalyl-L-alanine chloride (S)-12 used as resoluting agent in presence of NaHMDS at -78˚C [37].

NMR and X-ray Crystallography Characterization

In ^{1}H NMR spectra of **3a-i**, un-substituted phenyl ring of quinazoline was deduced from chemical shift values, coupling constants and their four ^{1}H signals of one proton each as dd (J_{ortho} = 8 Hz and J_{meta} = 2Hz), triplet (J_{ortho} = 8 Hz), multiplet and doublet (J_{ortho} = 8 Hz) for protons H-5, H-6, H-7 and H-8 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-i** was deduced with almost same splitting pattern. However, due to Br substitution signals for H-5 and H-7 appeared at 7.81-7.89 and 7.38-7.68 (d, *J* = 8 Hz) respectively. The structures of the synthesized compounds were also confirmed by single beam X-ray diffraction studies. Suitable crystals were grown in ethyl acetate and DCM mixture (2:1) and XRD data was obtained to confirm structure of the synthesized compounds as shown in Fig. 1.

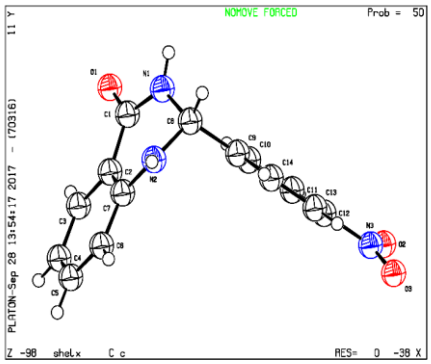
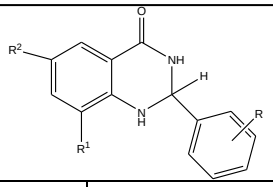


Fig. 1: XRD structure of compound 3e (CCDC No. 1577081).

In vitro pharmacology: AChE and BChE inhibition assay

Synthesized quinazoline derivatives were evaluated for cholinesterase (AChE and BChE) inhibition activity according to reported method [38]. The *in vitro* results of the synthesized compounds as inhibitory potency (IC_{50} values) are summarized in Table 1. All compounds showed good to moderate degree of inhibition and the IC_{50} values of some of compounds are in low micromolar range toward both enzymes. In general, all derivatives having para chloro, methoxy and hydroxy phenyl group at C-2 position of quinazoline ring (3c,3g, 3i, 4c, 4g and 4i) have shown better AChE/BChE inhibitory activity as compared to other compounds. Amongst un-substituted series, compound 3g with 4-OCH_3 phenyl group at C-2 position whereas dibromo derivative 4c with 4-chlorophenyl group attached to C-2 position of the pyrimidine ring has shown highest inhibition activity towards both of the enzymes in their respective series of analogues. Overall, dibromo derivatives 4a-i emerged as more potent dual cholinesterase inhibitors than their un-substituted counterparts (3a-i). Compounds 3g, 4c, 4g and 4i are better BChE inhibitors as compared to standard drug galantamine. Introduction of 4-chlorophenyl group at C-2 position led to the preparation of most potent compound 4c with IC_{50} value of 3.7±1.05 µM for AChE and 13.7±0.64 µM for BChE**.** Hence, compound 4c with the selectivity index of 3.7 for AChE, can be considered as a potential lead compound as dual AChE / BChE inhibitor. Furthermore, resolution of racemic mixture of lead compound 4c and *in vitro* assay results of the separated enantiomers showed that (+)-enantiomer 4c(II)

Table 1: In vitro cholinesterase inhibitory activity of the synthesized compounds.

Compound						
	R	R^1	R^2	IC_{50} (µM ±SEM)[a]		SI[b]
				eeAChE	eqBChE	
3a	H	H	H	118.3±1.8	137.4±2.40	1.2
3b	2-Cl	H	H	38.5±0.70	49.1±0.52	1.3
3c	4-Cl	H	H	15.4±0.60	23.7±1.74	1.8
3d	2-NO_2	H	H	24.4±1.63	51.4±1.62	2.1
3e	4-NO_2	H	H	21.1±1.08	33.4±0.66	1.6
3f	3-OCH_3	H	H	19.5±0.10	21.2±0.28	1.1
3g	4-OCH_3	H	H	7.8±0.82	[c]13.6±0.57	1.8
3h	3-OH	H	H	13.4±0.23	26.4±0.63	2.2
3i	4-OH	H	H	12.7±0.62	25.0±0.70	1.9
4a	H	Br	Br	78.3±2.4	92.4±1.22	1.2
4b	2-Cl	Br	Br	23.3±0.71	40.1±0.15	1.7
4c	4-Cl	Br	Br	3.7±1.05	13.7±0.64	3.7
4d	2-NO_2	Br	Br	17.3±0.83	21.4±1.37	1.2
4e	4-NO_2	Br	Br	8.4±1.0	14.1±1.34	1.7
4f	3-OCH_3	Br	Br	11.9±1.10	19.2±1.28	1.6
4g	4-OCH_3	Br	Br	6.1±0.61	11.6±1.17	1.9
4h	3-OH	Br	Br	12.4±0.23	24.4±0.63	1.9
4i	4-OH	Br	Br	7.4±1.02	9.9±1.20	1.3
4c(I)	4-Cl	Br	Br	3.9±0.75	16.3±1.34	4.1
4c(II)	4-Cl	Br	Br	3.3±1.10	9.7±0.76	2.9
Galantamine				4.0 ± 0.10	15.0 ± 0.67	3.7
Donpezil				0.03 ± 0.01	5.4 ± 0.27	180

with IC_{50} value of 3.3±1.10 µM (AChE) and 9.7±0.76 (BChE) have displayed better anti-cholinesterase activity as compared to (-)-enantiomer 4c(I**)** having IC_{50} value of 3.9±0.75 µM for AChE and 16.3±1.34 for BChE.

Molecular modeling studies on AChE and BChE inhibition

The structure of AChE is helpful in providing insights into the mechanism of enzyme catalysis. Therefore, different researchers have utilized X-ray structures of AChE from different species including human (*h*AChE), *Torpedo californica* (*Tc*AChE), *Electrophorus electricus* (eel), *Drosophila melanogaster* and mouse as reported in literature [39-40]. We have studied the binding interactions of our synthesized compounds with *Tc*AChE by using Molecular Operating Environment 2016 (MOE) by utilizing X-ray crystallographic structure of *Tc*AChE (PDB Code 1EVE) in complex with donepezil as enzyme structure [41]. As a first

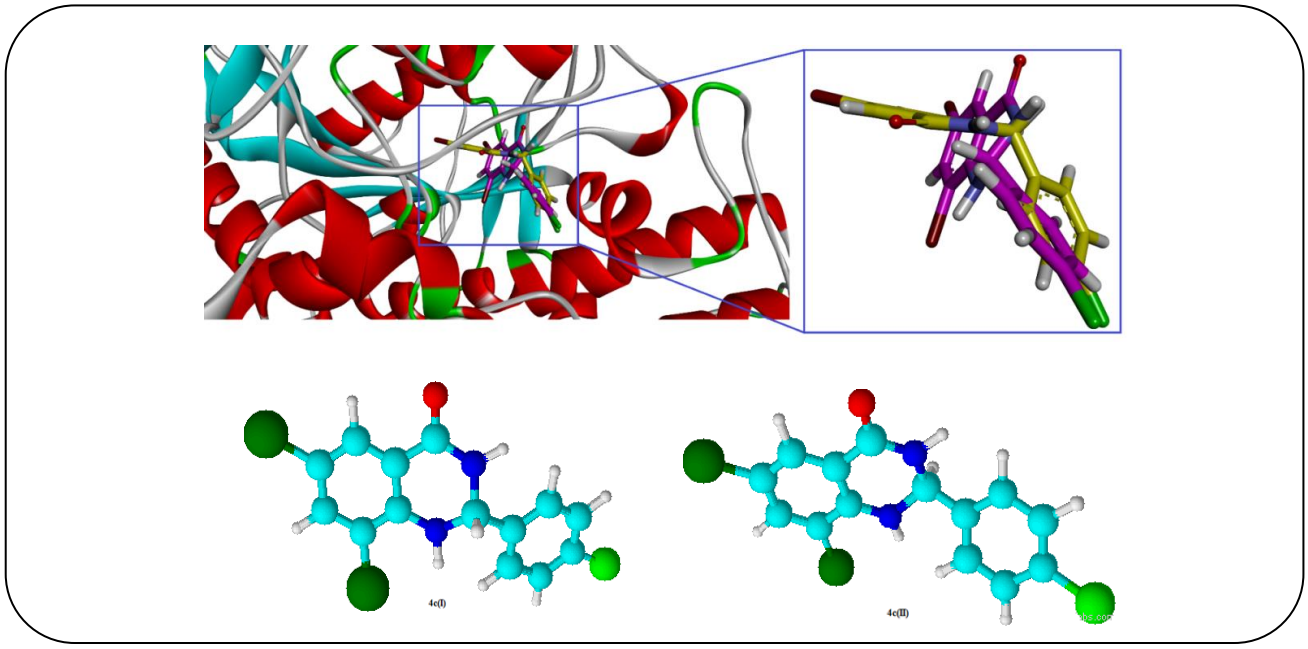


Fig 2: Superimposed diagram of the top-ranked docking pose of (R)-isomer (pink) and (S)-isomer (yellow) of compound 4c into the binding site of 1EVE.

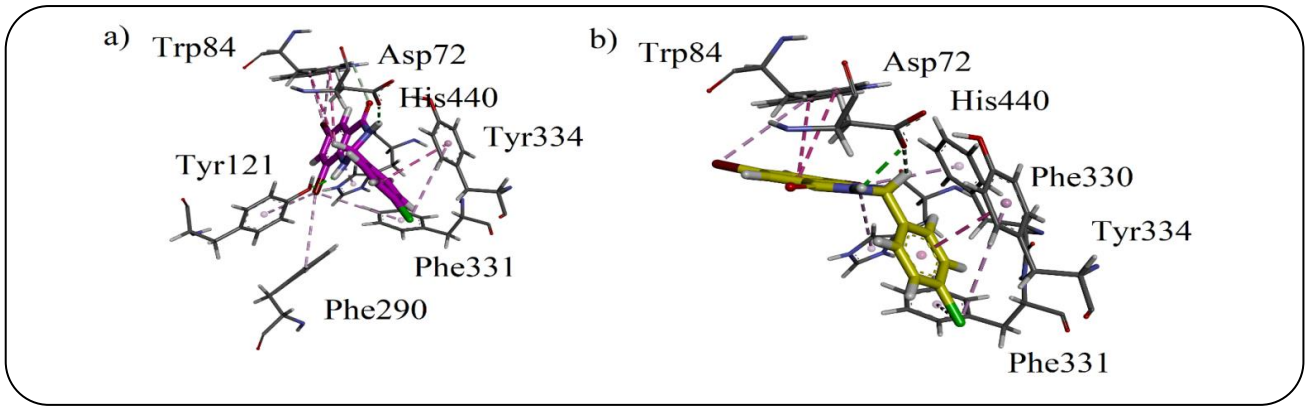


Fig 3: Close-up view of binding orientation of quinazoline isomers in the active site 1EVE. (a) (R)-isomer 4c(I); (b) (S)-isomer 4c(II).

step, the ability of the docking algorithm was validated to reproduce the co-crystallized pose of donepezil in the 1EVE pocket. The validation was carried out using RMSD method. The co-crystalized ligand was re-docked and the root mean square deviation (RMSD) between co-crystallized and re-docked conformation was determined. The RMSD value of <2.0 Å is considered as accurate in predicting binding orientation of ligand. It has been observed that docked quinazoline isomers were stabilized in the cavity of enzyme through different types of π-π stacking, π-alkyl and hydrogen bonding interactions. Binding pose of two isomers is shown in Fig. 2.

Binding mode analysis of the docked poses of the (*R*)-enantiomer 4c(I) having (IC_{50} = 3.9±0.75 µM) into the binding pocket of 1EVE revealed that it establishes π-alkyl interactions with catalytic site (CAS) residues Trp84 and Tyr121, catalytic triad residue His440, acyl pocket residue Phe290. While, -NH of the quinazoline ring forms a hydrogen bond interaction with Asp72. Chlorophenyl ring oriented itself towards CAS and forms π-π stacking interactions with Tyr334. While, dibromophenyl ring establishes π-π stacking interactions with Trp84 (Fig. 3a). (*S*)-Enantiomer 4c(II) having (IC_{50} = 3.3±1.10 µM) forms strong π-π

Table 2: Binding affinities and docking scores of the docked isomers of quinazoline.

	TcAChE (1EVE)		hAChE (4EY7)		BChE (1P0I)	
	Binding affinity (kcal/mol)	Docking score	Binding affinity (kcal/mol)	Docking score	Binding affinity (kcal/mol)	Docking score
(R)-enantiomer 4c(I)	-6.4016	-10.6864	-7.0903	-11.1228	-6.3799	-10.2716
(S)-enantiomer 4c(II)	-6.4160	-11.4354	-7.0883	-11.1819	-6.3938	-10.7905

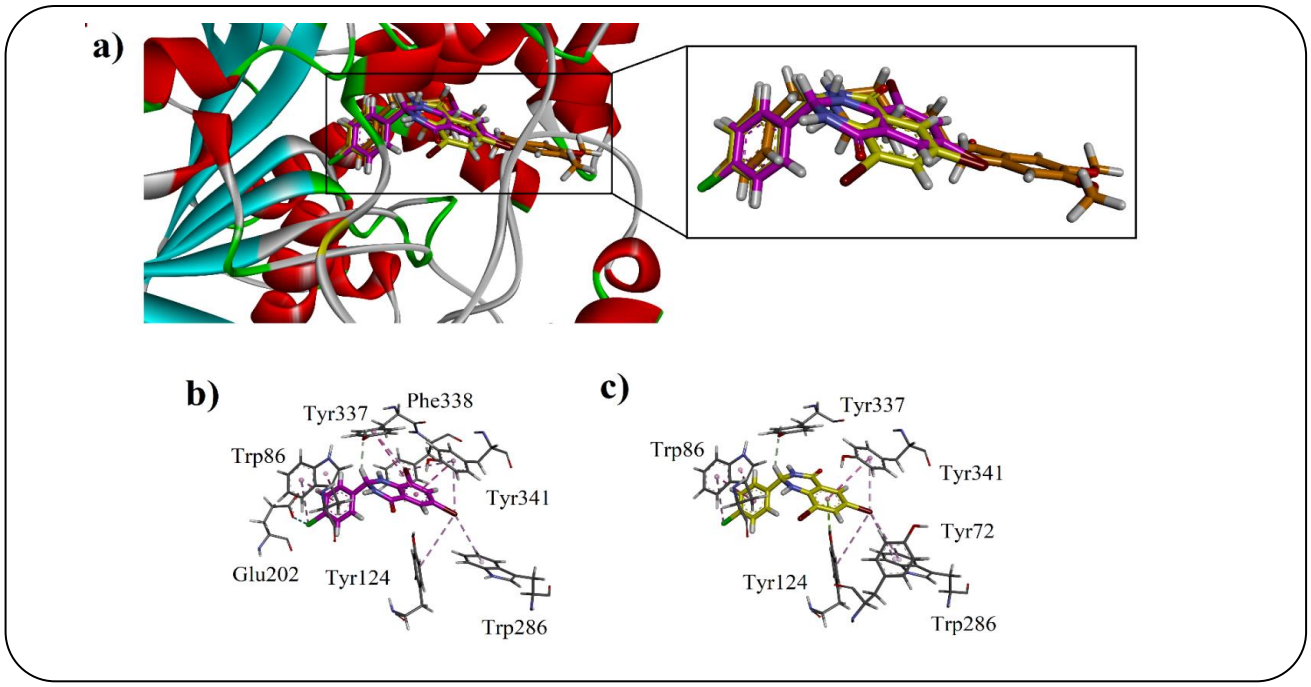


Fig. 4: a) Superimposed diagram of the top-ranked docking pose of (R)-isomer (pink), (S)-isomer (yellow) of compound 4c and co-crystallized donepezil (brown) into the binding site of human AChE (4EY7). (b-c) Close-up view of binding orientation of two isomers in the active site 1EVE. (b) (R)-isomer 4c(I); (c) (S)-isomer 4c(II).

stacking interactions with Trp84 and Tyr334. Interactions of π-alkyl type are found with amino acid residue of active site as in (R)-isomer. Asp72 forms hydrogen bond with –NH of quinazoline ring (Fig. 3b). However, the binding affinity for (R) and (S)-isomers is -6.4016 and -6.4106 kcal/mol respectively (Table 2). The docking scores for (R) and (S)-isomers is -10.6804 and -11.4354 respectively.

To understand binding of human AChE (*h*AChE), we docked both the isomers of 4c into the binding site of hAChE. Three-dimensional structure of hAChE in complex with donepezil was retrieved from PDB with code 4EY7. Binding affinity data and docking scores tabulated in Table 2 showed that these isomers bound more tightly in the binding site of *h*AChE(4EY7) than *Tc*AChE (1EVE). The best ranked superimposed binding pose of two isomers is shown in Fig. 4a. Phenyl rings of both isomers forms π-π stacking interactions with Trp86 (Trp84 in *Tc*AChE). While, Trp286 establishes π-alkyl interactions (Fig. 4b-c), the binding affinity for (R)- and (S)-isomers is -7.0903 and -7.0883 kcal/mol respectively (Table 2). The docking scores for (R)- and (S)-isomers is -11.1228 and -11.1819 respectively.

Docking studies were also carried out on X-ray structure of human BChE (PDB ID 1P0I). Binding pose of two isomers in the binding site of BChE is shown in Fig. 5. (R)-Isomer 4c(I) (IC_{50} =16.3±1.34 μM) establishes arene-H interactions with His 438 (catalytic triad residue) and hydrogen bond interactions with Glu197 (Fig. 6a).

The binding affinity and docking score for this isomer is -6.3799 kcal/mol and -10.2716 respectively. (S)-isomer

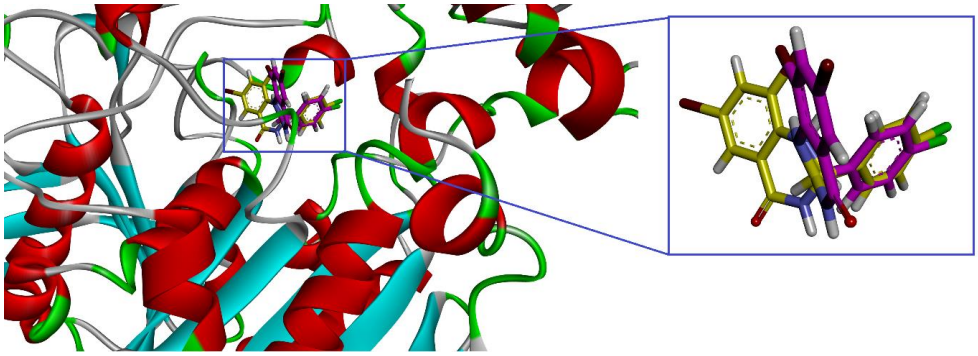


Fig. 5: Superimposed diagram of the top-ranked docking pose of (R)-isomer (pink) and (S)-isomer (yellow) of compound 4c into the binding site of 1P0I.

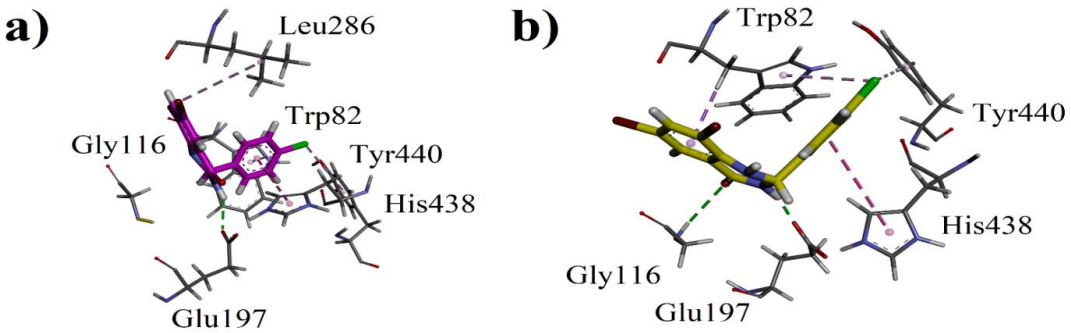


Fig. 6: Three-dimensional (2D) binding-poses in the active site human BChE. (a) (R)-isomer 4c(I); (b) (S)-isomer 4c(II).

4c(II) (IC_{50} =9.7±0.76 μM**)** is well stabilized into the active by forming arene-H interactions with anionic site residue Trp82 and with catalytic triad residue His438. A hydrogen bond interaction was also found between –NH of quinazoline ring and Glu197 (Fig. 6b). Gly116 forms a hydrogen bonding interaction with carbonyl oxygen of quinazolinone ring. The binding affinity and docking score for this isomer is -6.3938 kcal/mol and -10.7905 respectively (Table 2).

Preliminary in silico pharmacokinetic

In silico predictions of all synthesized compounds regarding penetration across Blood Brain Barrier (BBB), Human Intestinal Absorption (HIA), AMES Toxicity (AT) and carcinogenicity were compared with the standard drug donepezil. Results revealed that all un-substituted as well as bromo derivatives are predicted to be absorbed in human intestine, cross the BBB and have non-carcinogenic behavior. Probabilities of penetration across BBB, HIA and carcinogenicity ranged from 0.8765-0.9842, 0.9601-0.9945 and 0.7178-0.9384 respectively. Probability for non-AT varied from 0.5112 to 0.7164, however compounds 3d-g in un-substituted series 3a-i and derivatives 4d-f from brominated series 4a-i are predicted to be AMES toxic with high probability range from 0.5273 to 0.8445. All AMES toxic compounds possess nitro and methoxy substituents attached to the phenyl ring at C-2 position. Interestingly, most potent dual cholinesterase inhibitor compound 4c having dibromo substituents at benzene ring and chloro group attached to phenyl ring at C-2 is not predicted to show AMES toxicity**.** Calculated probabilities of biological active compounds were compared with standard drug donepezil which shows that except few compounds, all other have good probabilities for BBB, HIA, Non AT and Non-carcinogenicity as shown in Table 3.

Table 3: Comparison of in silico calculated probabilities.

Sample Code	BBB		HIA		AMES Toxicity		Carcinogens	
	Results	Probability	Results	Probability	Results	Probability	Results	Probability
Donepezil	BBB+	0.9953	HIA+	0.9966	Non AT	0.6441	Non-carcinogens	0.9528
3a	BBB+	0.9842	HIA+	0.9811	Non AT	0.602	Non-carcinogens	0.9384
3b	BBB+	0.9651	HIA+	0.9855	Non AT	0.6261	Non-carcinogens	0.8952
3c	BBB+	0.9651	HIA+	0.9855	Non AT	0.6261	Non-carcinogens	0.8952
3d	BBB+	0.917	HIA+	0.9677	AMES toxic	0.8445	Non-carcinogens	0.7533
3e	BBB+	0.917	HIA+	0.9677	AMES toxic	0.8445	Non-carcinogens	0.7533
3f	BBB+	0.9684	HIA+	0.9948	AMES toxic	0.6669	Non-carcinogens	0.9385
3g	BBB+	0.9684	HIA+	0.9948	AMES toxic	0.6669	Non-carcinogens	0.9385
3h	BBB+	0.9676	HIA+	0.9877	Non AT	0.6193	Non-carcinogens	0.9138
3i	BBB+	0.9676	HIA+	0.9877	Non AT	0.6193	Non-carcinogens	0.9138
4a	BBB+	0.974	HIA+	0.9758	Non AT	0.5981	Non-carcinogens	0.9204
4b	BBB+	0.9602	HIA+	0.99	Non AT	0.6254	Non-carcinogens	0.8938
4c	BBB+	0.9602	HIA+	0.99	Non AT	0.6254	Non-carcinogens	0.8938
4d	BBB+	0.8765	HIA+	0.9601	AMES toxic	0.7658	Non-carcinogens	0.7178
4e	BBB+	0.8765	HIA+	0.9601	AMES toxic	0.7658	Non-carcinogens	0.7178
4f	BBB+	0.9584	HIA+	0.9934	AMES toxic	0.5273	Non-carcinogens	0.9174
4g	BBB+	0.9399	HIA+	0.9944	Non AT	0.5112	Non-carcinogens	0.9111
4h	BBB+	0.9484	HIA+	0.9844	Non AT	0.6807	Non-carcinogens	0.8913
4i	BBB+	0.9259	HIA+	0.9868	Non AT	0.7167	Non-carcinogens	0.8824

CONCLUSIONS

We have synthesized and tested quinazoline derivatives for AChE and BChE inhibitory potency and found that all the derivatives have showed activity against both the enzymes in micromolar range. Few compounds have shown inhibition of both enzymes (AChE and BChE) higher than or comparable to the standard drug galatamine. Compound 4c (6,8-dibromo-2-(3-bromo-4-chloro-phenyl)-2,3-dihydro-1H-quinazolin-4-one) with the selectivity index of 3.7 for AChE, can be considered as a potential lead compound as dual AChE / BChE inhibitor with IC_{50} values of 3.7±1.05 µM and 13.7±0.64 µM for AChE and BChE respectively. Further resolution of racemic mixture showed that (+)-enantiomer 4c(II) with IC_{50} value of 3.3±1.10 µM (AChE) and 9.7±0.76 (BChE) is more potent cholinesterase inhibitor. Binding mode analysis of the docked poses of synthesized compounds with *Tc*AChE, *h*AChE and BChE also supported *in vitro* results. Docking study showed that compound 4c(II) establishes strong interactions with enzyme cavity thus showing better inhibition activity. The findings of this study suggest that appropriate structural modification of quinazoline scaffold may result potent AChE or BChE inhibitors.

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CONFLICT OF INTEREST

Authors of this research paper declare no conflicts of interest

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