



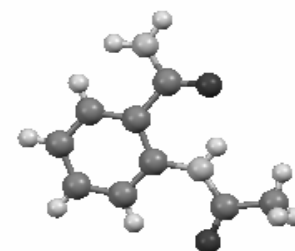
SYNTHESIS, *IN SILICO* STUDY AND CHOLINESTERASES INHIBITION ACTIVITY OF 2-SUBSTITUTED 2,3-DIHYDROQUINAZOLIN-4(*1H*)-ONE DERIVATIVES

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We have synthesized and evaluated a number of 2,3-dihydroquinazolin-4(*1H*)-one derivatives as inhibitors of cholinesterases. *In vitro* assay results revealed that all synthesized compounds are active against both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes and few compounds having longer alkyl chain at C-2 position showed better inhibition activity than standard drug galantamine. Moreover, bromo derivatives **6a-d** were more active than nitro **5a-d** and their un-substituted counterparts **4a-d**. Amongst all, compound **6d**, with IC₅₀ values of **4.8±1.01** μM (AChE) and **11.1±1.15** μM (BChE) can be considered as good cholinesterase (AChE/BChE), inhibitor with greater selectivity towards BChE. *In silico* calculations revealed that all compounds have good pharmacokinetic profile along with having high probabilities for penetration across blood brain barrier (BBB), human intestinal absorption (HIA), non-AMES toxicity and non-carcinogenicity except nitro substituted derivatives which were predicted to show AMES toxicity. The synthesized compounds may lead toward development of new potent cholinesterase inhibitors.



INTRODUCTION

Majority of organic heterocyclic compounds are well known to possess fascinating biological properties and play critical role in various biological and physiological processes as their structural subunits exist in a large number of biological molecules such as DNA, RNA, vitamins, hormones, antibiotics and pigments, etc.¹⁻³ Major portion of available therapeutic products are made up of various heterocyclic compounds that mimic natural products with physiological and biological activities.⁴ A number of biological activities including antifungal, antibacterial, antiviral, antioxidants, anti-inflammatory, analgesics, anticancer, sedatives, anticonvulsants and hypnotics are associated with many heterocyclic compounds especially synthetic nitrogen moieties. Due to biological potential of heterocyclic

compounds, they are building blocks of more than 75% of top 200 branded drug candidates available in the market.⁵

Quinazoline, a class of fused benzopyrimidine consists of very useful nitrogen containing bioactive heterocyclic moiety which exhibit promising biological activities including anti-inflammatory, antimicrobial, anticancer, anticonvulsant, hypnotic, antihistaminic, diuretic, antimalarial, antifungal, antihypertensive, antagonism of ghrelin receptor, analgesic and COX-2 inhibitory activities.⁶⁻¹⁰ Owing to biological significance of quinazoline compounds, it has been found that about 150 naturally occurring alkaloids like echinozolinone, glycosminine, rutaecarpine and deoxyvascinone, etc. belonging to animals, microorganisms and plants kingdoms are structural subunits of this scaffold.¹¹

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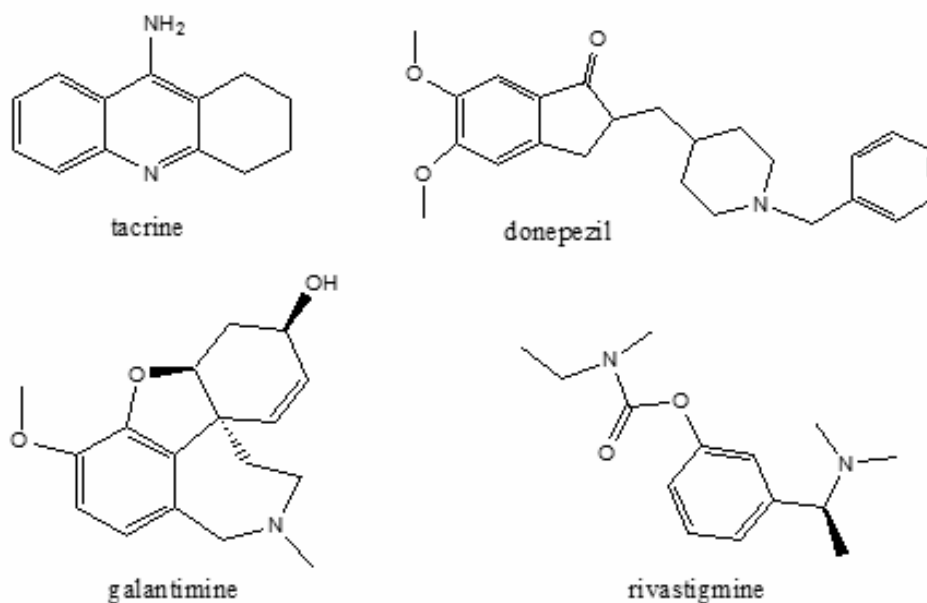


Fig. 1 – Chemical structure of approved drugs used for treatment of AD.

Quinazolines importance as traditional cure remedies is known since ancient times. For example, trypanthrin is known to be used as a remedy for fungal infections in Japanese traditional herbal medicines. Deoxyvascinone has also been widely employed for symptomatic treatment of cold, cough, bronchitis and asthma in indigenous medicines.¹² Dichroafebrifuga, a renowned medicinal herb, has been used for the possible treatment of malaria for decades in China. Whereas, halofuginone, a halogenated derivative of febrifugine, exhibited cocidiostat activity.¹³

The cholinesterases (AChE and BChE) are known to catalyze the hydrolysis of acetylcholine (ACh) and are involved in regulation of various physiological functions such as digestion, blood coagulation and neurotransmission. Therefore, several diseases and disorders like pancreatitis, thrombosis and Alzheimer's disease (AD) are linked to over activity of these enzymes.¹⁴⁻¹⁶

Inhibition of these enzymes will increase ACh level which will reduce symptoms of AD and other neurotransmission disorders.¹⁷ Various natural and synthetic cholinesterase inhibitors including approved drugs donepezil, tacrine, rivastigmine, galantamine (**Figure 1**) and other moieties like *para*-aminobenzoic acid, xanthostigmine, flavonoid, coumarin and pyrrolo-isoxazole analogues have been studied against AD and other disorders.¹⁸ Besides clinical use of cholinesterase inhibitors in senile dementia, myasthenia gravis, ataxia, Parkinson's disease and AD, they have also been employed for treatment of several other conditions like chronic pain and type 2 diabetes.¹⁹⁻²²

A wide range of compounds are effective cholinesterase inhibitors, but different heterocyclic compounds and their derivatives are most potent AChE and BChE inhibitors. Various simple and hybrid aromatic heterocyclic compounds including oxazolidines, imidazolidines and benzoxazoles analogues with better interactions and enhanced anti-cholinesterase activity have been developed for the purpose.²³⁻²⁶ Other than these, few researchers have reported anti-cholinesterase potential of quinazoline compounds.^{27,28}

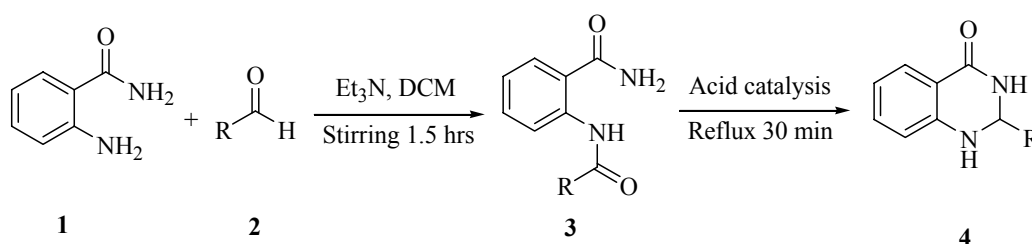
Recently, our research group have reported potential of various 2-aminobenzamide derivatives and 2,2-disubstituted-2,3-dihydroquinazolin-4(*1H*)-ones as dual binding site cholinesterase inhibitors.^{29,30} In our current research project, we have tried to extend chain length at C-2 position of 2,3-dihydroquinazolin-4(*1H*)-one core. The objective of present study was to optimize structure of 2,3-dihydroquinazolin-4(*1H*)-one for developing potent cholinesterases (AChE/BChE) inhibitors.

RESULTS AND DISCUSSION

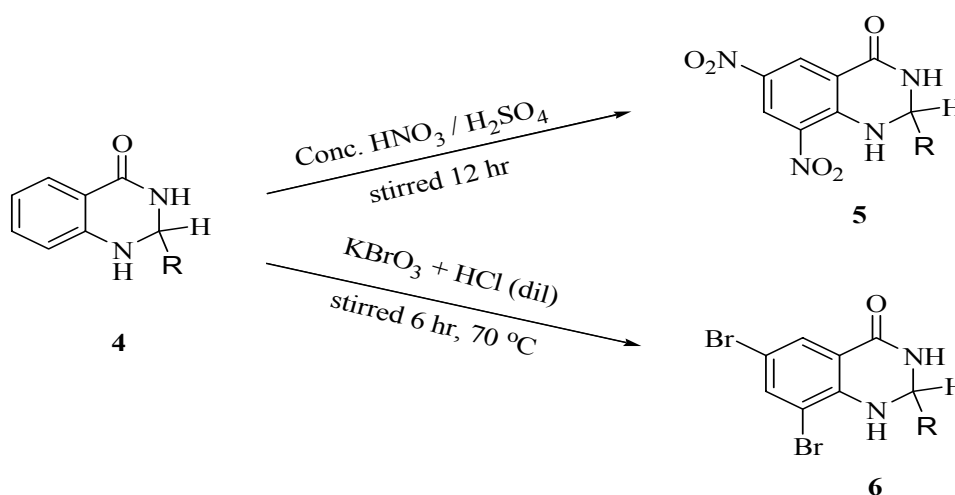
Different synthetic methodologies like Niementowski reaction, multicomponent reaction, reactions via benzoxazinone intermediates, Domino reaction and condensation of 2-aminobenzamide with various aldehydes and ketones in the presence of metallic and organic catalysts have been reported for the construction of quinazolinone moiety.³¹⁻³⁵ We tried a relatively different protocol for the synthesis of 2-substituted-2,3-dihydro-

quinazolin-4(1*H*)-one. Anthranilamide was reacted with acetyl chloride in dichloromethane (DCM) under basic conditions by using triethyl amine (Et_3N) to form 2-acetyl-amino-benzamide which further cyclized into 2-methyl-2,3-dihydroquinazolin-4(1*H*)-one by following Mannich reaction (**Scheme 1**). Detail of substituents is given in **Table 2**.

As our previous study revealed that nitro and bromo derivatives of 2,2-disubstituted-2,3-dihydroquinazolin-4(1*H*)-one were having better cholinesterase activity than their un-substituted counterparts.²⁹



Scheme 1 – Synthesis of 2-methyl-2,3-dihydroquinazolin-4(1*H*)-ones (4a-d).



Scheme 2 – Synthesis of dinitro 5a-d and dibromo 6a-d derivatives.

Table 1

XRD analysis data of compound 3a

Compound	3a		
Chemical Formula	C9 H10 N2 O2		
M (g mol ⁻¹)	178.07		
Temperature (K)	296(2)		
Crystal system	Monoclinic		
Space group	c 2/c		
Cell volume	2057.2(4)		
a(Å)	14.3643(15)	A	90.0
b(Å)	18.942(2)	B	91.428(4)
c(Å)	7.5633(9)	Γ	90.0
C1	0.66904(15)	0.32782(14)	0.3624(3)
C2	0.74063(18)	0.37547(16)	0.3298(4)
H2	0.7943	0.3598	0.2757

Table 1 (continued)

C3	0.7327(2)	0.44485(16)	0.3763(5)
H3	0.7809	0.4759	0.3525
C4	0.6538(2)	0.46973(17)	0.4588(5)
H4	0.6485	0.5171	0.4891
C5	0.5841(2)	0.42303(16)	0.4943(4)
H5	0.5316	0.4392	0.5514
C6	0.58912(16)	0.35241(14)	0.4481(3)
C7	0.73938(17)	0.22063(15)	0.2283(3)
C8	0.7168(2)	0.14561(16)	0.1887(5)
H9A	0.7071	0.1398	0.0635
H9B	0.6613	0.1324	0.2485
H9C	0.7675	0.1161	0.2284
N1	0.67292(14)	0.25636(12)	0.3140(3)
H1	0.6256	0.2315	0.3435
C2F	0.51076(16)	0.30406(17)	0.4929(3)
O1	0.81351(13)	0.24755(11)	0.1850(3)
N2	0.44062(13)	0.33647(11)	0.5644(3)
H3G	0.3884	0.3103	0.5992
H3F	0.4471	0.3842	0.5978
O3	0.51067(13)	0.24090(12)	0.4668(3)

3a)

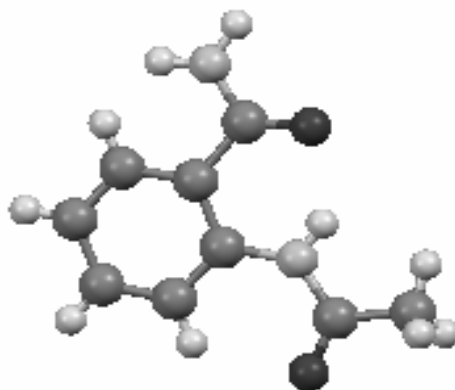


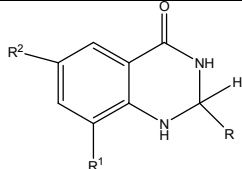
Fig. 2 – ORTEP diagram of compounds 3a.

Un-substituted phenyl ring of synthesized quinazoline compounds **4a-d** was deduced from chemical shift values, coupling constants and their four ^1H signals of one proton each as dd ($J_{\text{ortho}} = 8 \text{ Hz}$ and $J_{\text{meta}} = 2 \text{ Hz}$), triplet ($J_{\text{ortho}} = 8 \text{ Hz}$), multiplet and doublet ($J_{\text{ortho}} = 8 \text{ Hz}$) in ^1H NMR spectra. 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. Extension in chain at C–2 position has deduced appearance of signals for –CH₂ and –CH₃. ^1H -NMR of the series **5a-d** and **6a-d** was deduced with almost the same splitting pattern. However, two highly deshielded signals for H–5 and H–7 appeared in the range of 9.21–9.23 and 9.22–9.06 ppm due to the –NO₂ groups and due to Br substitution these signals appeared at 7.81–7.89 and 7.38–7.68 respectively.

IN VITRO PHARMACOLOGY

Synthesized compounds **4a-d**, **5a-d** and **6a-d** were evaluated for anti-cholinesterase activity (AChE and BChE) according to the reported method. The *in vitro* assay results as inhibitory potency (IC₅₀ values) and selectivity index (SI = BChE/AChE) are summarized in **Table 2**. All synthesized compounds showed moderate degree of inhibition and none of the compounds was more potent AChE inhibitor than standard drug glantamine, however two compounds **4c** and **4d** showed BChE inhibition better than glantamine. In general, derivatives having long chain length at C–2 position of quinazoline ring exhibited better AChE/BChE inhibitory activity as compared to other analogues of their respective series. Among the series, dinitro compounds **5a-d** were better cholinesterase inhibitors than their un-substituted counterparts. But dibromo derivatives **6a-d** emerged as most potent cholinesterase inhibitors as compared to nitro and un-substituted analogues.

Table 2
In vitro cholinesterase inhibitory activity of the synthesized compounds

Compound				IC ₅₀ (μM ±SEM)		SI ^a
	R	R ¹	R ²	ecAChE	eqBChE	
				4a	CH ₃	
4b	C ₂ H ₅	H	H	108.5±1.70	149.4±1.02	1.4
4c	C ₃ H ₇	H	H	85.4±0.98	123.0±0.74	1.4
4d	C ₄ H ₉	H	H	54.1±0.73	71.4±1.0	1.3
5a	CH ₃	NO ₂	NO ₂	91.1±1.0	103.4±1.66	1.1
5b	C ₂ H ₅	NO ₂	NO ₂	79.5±1.10	98.2±1.28	1.2
5c	C ₃ H ₇	NO ₂	NO ₂	37.8±1.02	63.1±1.27	1.7
5d	C ₄ H ₉	NO ₂	NO ₂	11.4±1.23	26.4±1.63	2.3
6a	CH ₃	Br	Br	18.7±0.92	39.0±1.70	2.1
6b	C ₂ H ₅	Br	Br	8.9±0.13	18.7±1.44	2.1
6c	C ₃ H ₇	Br	Br	7.3±1.4	12.4±1.22	1.6
6d	C ₄ H ₉	Br	Br	4.8±1.01	11.1±1.15	2.3
Galantamine				4.0 ± 0.10	15.0 ± 0.67	3.7

^aValues are expressed as mean (standard error of the mean of at least three experiments. ^bSelectivity Index (SI) = IC₅₀ ratio (BChE/AChE).

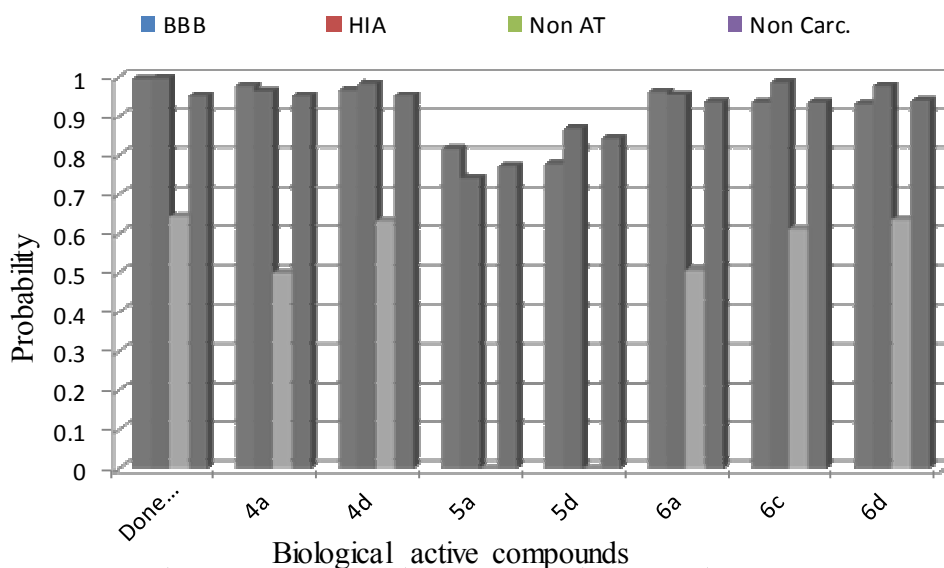


Fig. 3 – Comparison of *in silico* calculated probabilities of biological active compounds with donepezil.

Overall, dibromo compounds **6c** and **6d** with IC₅₀ values of 7.3 ± 1.4 and 4.8 ± 1.01 μM respectively can be considered good AChE inhibitor but their BChE inhibition potential with IC₅₀ values of 12.4 ± 1.22 and 11.1 ± 1.15 μM was even better than standard drug glantamine. Reaction of 2-aminobenzamide with butyryl chloride resulted quinazolinone **4d** which on bromination prepared compound **6d**, and this compound with IC₅₀ values of 11.1 ± 1.15 μM can be reported as most potent BChE inhibitor.

PRELIMINARY *IN SILICO* PHARMACOKINETIC

In silico calculations of the synthesized compounds regarding penetration across blood brain barrier (BBB), human intestinal absorption (HIA), AMES toxicity (AT) and carcinogenicity were carried out by using online ADMET SAR server. Comparison of predicted properties with standard drug donepezil revealed that all compounds are predicted to cross the BBB, absorb

in human intestine along with having non-carcinogenicity and non-AMES toxicity except nitro derivatives **5a–d** which are calculated to be AMES toxic. Calculated probabilities of biological active compounds were compared with standard drug donepezil which shows that few compounds have good probabilities for BBB, HIA, Non AT and Non-carcinogenicity as shown in **Figure 3**.

MATERIALS AND METHODS

All chemicals used for synthesis were of analytical grade and used as received from commercial suppliers. Acetylthiocholine iodide was procured from Sigma-Aldrich UK, code 101303874, AChE (Electric eel type-VI-S) from Sigma-Aldrich GmbH USA, code 1001596210, Butyrylthiocholine Iodide (code 101334643) from Sigma-Aldrich Switzerland, BChE (Equine serum Lyophilized, code 101292670) from Sigma-Aldrich GmbH USA, DTNB (code 101261619) from Sigma-Aldrich Germany and Galantamine hydrobromide Lycoris Sp. (code G1660) from Sigma-Aldrich France. Bruker DRX 400 MHz NMR spectrometers was used for recording ¹H NMR spectra and chemical shifts of individual protons were reported in comparison to SiMe₄. The X-ray diffraction analysis of crystals was carried out on a Bruker Smart APEX II diffractometer.

GENERAL PROCEDURE FOR THE SYNTHESIS OF 2-SUBSTITUTED-2,3-DIHYDROQUINAZOLIN-4(1H)-ONE (**4a–d**)

To a stirred solution of 2-amino benzamide (100 mmol) in DCM, acid chloride (100 mmol) was added dropwise under nitrogen environment and stirring was continued for 1.5 hrs. After completion of the reaction, the product was concentrated by rotary evaporator and washed with ice cold water. Precipitates so formed were filtered and dried in microwave oven to obtain **3a–d**. Compound **3** (100 mmol) was dissolved in acetone and refluxed for 30 min in the presence of HCl / HNO₃ as acid catalyst. After completion of reaction, product was again concentrated by rotary evaporator and recrystallization was done with ethyl acetate / DCM to obtain crystals of 2-substituted-2,3-dihydroquinazolin-4(1H)-ones which were further characterized by spectroscopic and X-Rays crystallographic techniques.

SYNTHESIS OF 2-METHYL-2,3-DIHYDRO-1H-QUINAZOLIN-4-ONE (**4a**)

Compound **4a** was synthesized by following general procedure from 2-amino benzamide and acetyl chloride as light yellowish crystalline solid (88.5% yield), m.p. 165-166 °C, ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 7.80 (dd, ³J = 8 Hz, ⁴J = 2 Hz, 1H, ArH), 7.32-7.28 (m, 1H, ArH), 6.95 (s, 1H, NH), 6.77 (t, ³J = 8 Hz, 1H, ArH), 6.63 (d, ³J = 8 Hz, 1H, ArH), 4.85 (s, 1H, ArH), 1.51 (s, 3H, CH₃) ppm. ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 169.0, 143.1, 132.9, 128.3, 118.4, 117.5, 113.8, 62.7, 25.4.

GENERAL PROCEDURE FOR THE SYNTHESIS OF 6,8-DINITRO-2-SUBSTITUTED-2,3-DIHYDROQUINAZOLIN-4(1H)-ONE (**5a–d**)

Above synthesized 2,3-disubstituted quinazoline (**4a–d**) (100 mmole) was added to conc. H₂SO₄ (10mL) and stirred to dissolve in an ice bath. To this solution conc. HNO₃ (0.5mL) was added dropwise 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-disubstituted-2,3-dihydroquinazolin-4(1H)-one which were further characterized by NMR and XRD techniques.

SYNTHESIS OF 2-METHYL-6,8-DINITRO-2,3-DIHYDRO-1H-QUINAZOLIN-4-ONE (**5a**)

Yellow crystalline solid. ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 9.21 (d, ³J = 8 Hz, 1H, ArH), 9.02 (d, ³J = 8 Hz, 1H, ArH), 6.90 (s, 1H, NH), 5.80 (s, 1H, NH), 4.97 (s, 1H, ArH), 1.51 (s, 3H, CH₃) ppm.

GENERAL PROCEDURE FOR THE SYNTHESIS OF 6,8-DIBROMO-2-SUBSTITUTED-2,3-DIHYDROQUINAZOLIN-4(1H)-ONE (**6a–d**)

To a stirred solution of 2-substituted quinazoline **4a–d** (10 mmol) and KBrO₃ (10 mmol) in acetonitrile (10 ml) was added dil. HCl (10mL, 1N) dropwise 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 in required product.

Synthesis of 6,8-Dibromo-2-methyl-2,3-dihydro-1*H*-quinazolin-4-one (**6a**)

Brownish solid (69.5% yield), ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 7.89 (s, 1H, ArH), 7.65 (s, 1H, ArH), 6.90 (s, 1H, NH), 5.58 (s, 1H, NH), 4.95 (s, 1H, ArH), 1.52 (s, 1H, CH₃) ppm.

X-RAYS CRYSTALLOGRAPHIC STUDY

Slow fusion of the synthesized compounds was done in a solution ethyl acetate/DCM/methanol (2:1:1) to obtain crystals of the synthesized compounds which were analyzed at Bruker KAPPA Apex II diffractometer with graphite-monochromatized Mo K α radiation, $\lambda_{\text{Mo}} = 0.71073 \text{ \AA}$ at 100 K. Data collection was carried out under monoclinic system with space groups C 2/c. SAINT and SHELXL-2013 program packages were employed for data reduction and structure refinement respectively.^{36,37}

DETERMINATION OF ACHE AND BChE INHIBITORY ACTIVITY

Ellman's methodology was used for determining anti-cholinesterase activity of the synthesized compounds with reference to galantamine as standard drug³⁸. Synthesized compounds were dissolved in 0.1 M phosphate buffer (KH₂PO₄/K₂HPO₄) of pH 8.0. Appropriate amount of Ellman's reagent (DTNB), 0.03 U/mL of enzymes (AChE and BChE) and test compounds were mixed and pre-incubated at 30 °C for 10 min, then 1mM ATCI or BTCI was added and reaction mixture was again incubated for 15 min. Enzymatic hydrolysis was monitored at 412 nm by using μ Quant microplate spectrophotometer (MQX200, BioTek USA) and IC₅₀ values were calculated by plotting sample solution concentrations against the inhibition. All the reactions were carried out in triplicate.

CONCLUSION

We have synthesized 2-substituted-2,3-dihydro-quinazolin-4(*IH*)-one and their nitro and bromo derivatives and studied their cholinesterase (AChE and BChE) inhibitory potential. *In vitro* results revealed that few compounds have shown good activity against both the enzymes. But only two compounds **6c** and **6d** with IC₅₀ values of

12.4±1.22 and **11.1±1.15** μM respectively can be considered potent selective BChE inhibitors. ADMET SAR calculations showed that except for nitro derivatives **5a-d**, all other compounds have good BBB, HIA, Non-AT and Non-carcinogenicity probabilities.

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