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Ionic liquid mediated synthesis and molecular docking study of novel aromatic embedded Schiff bases as potent cholinesterase inhibitors

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ABSTRACT

Novel aromatic embedded Schiff bases have been synthesized in ionic liquid [bmim]Br and evaluated *in vitro* for their acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes inhibitory activities. Among the newly synthesized compounds, **5f**, **5h** and **7j** displayed higher AChE enzyme inhibitory activities than standard drug, galanthamine, with IC_{50} values of 1.88, 2.05 and 2.03 μM, respectively. Interestingly, all the compounds except for compound **5c** displayed higher BChE inhibitories than standard with IC_{50} values ranging from 3.49 to 19.86 μM. Molecular docking analysis for **5f** and **7j** possessing the most potent AChE and BChE inhibitory activities, disclosed their binding interaction templates to the active site of AChE and BChE enzymes, respectively.

1. Introduction

Alzheimer's disease (AD) is a an irreversible neurodegenerative disorder, which affected more than 37 million people around the world based on the World Health Organization (WHO) report [1]. Etiology of AD is limited to deposition of extracellular β-amyloid plaques and formation of intracellular neurofibrillary tangles that both play principal roles in the pathophysiology of this disease [2–6].

Besides, AD is clinically characterized by progressive cognitive impairments, memory loss, learning disabilities and diverse range of neuropsychiatric symptoms [7]. Cholinergic hypothesis suggests that loss of cholinergic neurons in the forebrain, cortex and hippocampus of AD patients' brain ensue decline in acetylcholine (ACh) neurotransmitter level and dysfunctions in cholinergic neurotransmission system. Thus, increasing acetylcholine levels to restore the substantial impairment of memory and cognitive dysfunctions in AD patients are of prime importance [8].

Current clinically approved treatments for AD are limited to cholinesterase inhibitors (ChEI's), acting by inhibiting cholinesterase enzymes from hydrolyzing acetylcholine and N-methyl D-aspartate receptor antagonists (e.g. memantine), which activates glutaminergic pathway [9–11]. Despite the tremendous efforts in search of disease modifying agents working *via* β-amyloid or tau pathways, none are clinically available due to their adverse effects. Therefore, the search for new cholinesterase enzymes inhibitors is still ongoing worldwide.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are two cholinesterase enzymes, which are responsible for degradation and regulation of acetylcholine in human body, however they are different in kinetics and substrate selectivity [12]. The active site of human AChE enzyme is located at the bottom of a 20 Å long, narrow gorge including five important regions to accommodate and hydrolyze the acetylcholine, including: catalytic triad [13], oxyanion hole [14], choline binding site [15], acyl binding pocket [16] and peripheral anionic site [17]. Acetylcholine or inhibitors guidance inside this channel is facilitated by hydrophobic interactions with aromatic amino acid residues lining the gorge wall such as phenylalanine (Phe), tryptophan (Trp) and tyrosine (Tyr) [18]. The overall structure of human BChE (*h*BChE) is similar to human AChE (*h*AChE), however in *h*BChE, aromatic residues are mostly replaced by hydrophobic ones such as leucine (Leu) and valine (Val), which makes BChE suitable for accommodation of bulky substrates and inhibitors [19].

In the context of green chemistry, the reactions assisted by unrivaled features of ionic solvents i.e. remarkable catalytic

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behavior, excellent chemical/thermal stability and good solvating ability, displayed significant merits of reduced reaction time and improved yields [20,21].

Schiff bases exhibit a wide range of biological activities such as antifungal [22,23], anti-bacterial [24–26], antiviral [27,28] as well as potent cholinesterase/A β-aggregation inhibitory properties [29,30].

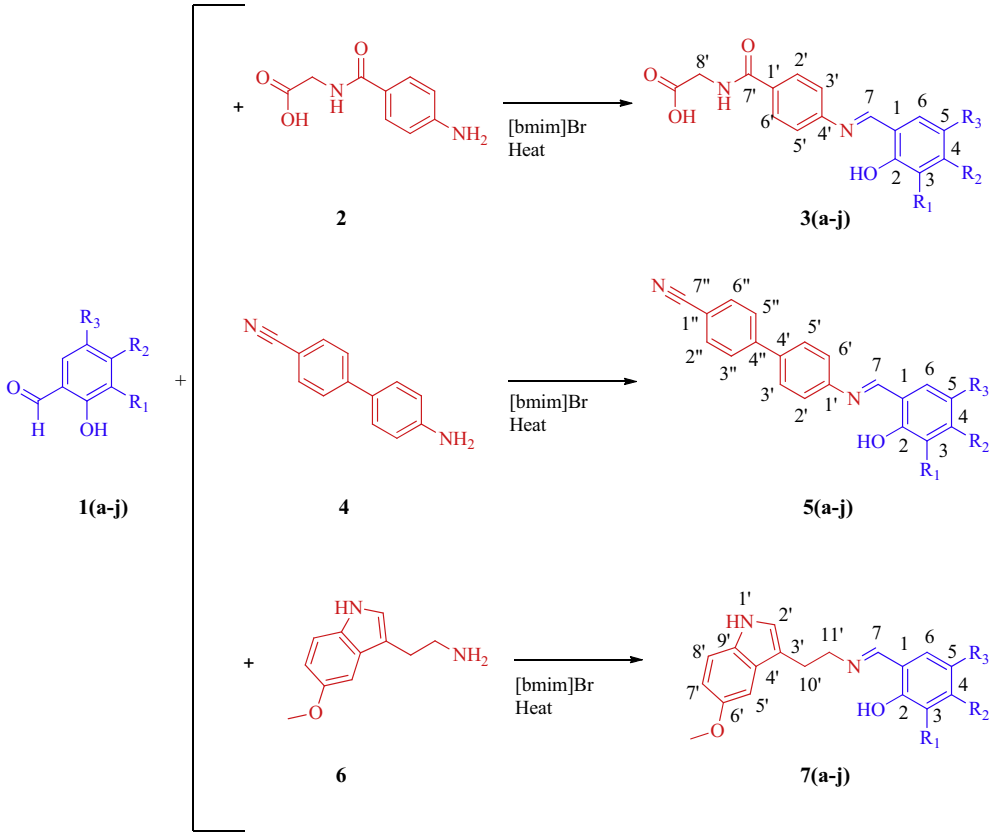
Inspired by the biological significance of Schiff base derivatives, in the present study we have reported an ionic liquid mediated synthesis of a library of Schiff base derivatives and their cholinesterase inhibitory activities. Subsequently, molecular docking analysis performed to disclose the plausible binding interaction mechanism of the most active derivatives to the active site of AChE and BChE receptors.

2. Results and discussions

A library of novel Schiff base derivatives **3(a–j)**, **5(a–j)** and **7(a–j)** were prepared by condensation of substituted benzaldehydes **1**(**a–j**) with *p*-aminohippuric acid (**2**), 4-(4′-aminophenyl)benzonitrile (**4**) and 5-methoxy tryptamine (**6**) in ionic liquid *viz.* 1-butyl-3-methylimidazolium bromide ([bmim]Br) (Scheme 1).

In order to find an optimal and handy protocol to synthesize Schiff bases **3**, **5** and **7**, equimolar mixture of substituted benzaldehydes and primary amines **2**, **4** and **6** were heated in presence of first; conventional organic solvents (e.g. ethanol) and second; novel, ionic solvents *viz.* [bmim]Br. Those reactions performed in ionic solvent, significantly showed merits of its unrivaled catalytic properties by furnishing to highly pure Schiff bases in a very short reaction time (>15 min), incomparable to the less pure products isolated from ethanol in much longer period of time (4–5 h). Therefore, to benefit ionic solvents' unique properties, all the condensation reactions were subsequently performed in [bmim]Br (Scheme 1).

Structural elucidation of the Schiff bases **3–7** was accomplished using 1D and 2D NMR spectroscopy techniques as well as elemental analysis. As a model, compound **3j** displayed a doublet at 6.08 ppm in ^{1}H NMR spectra belonging to H-3. This proton showed a H,H-COSY correlation to H-5 at 6.34 ppm. On the same basis, H-5 led to elucidation of H-6 as a doublet of doublets at 7.35 ppm owing to its H,H-COSY correlation to H-5. The coupling pairs of H-3′ and H-5′ as well as H-2′ and H-6′ showed up to two doublets at 7.40 and 7.93 ppm in ^{1}H NMR, respectively. Through HMBC, the singlet at 8.76 ppm was assignable to H-7, due to its correlation to



a: R_1=OCH_3, R_2=H, R_3=H
b: R_1=H, R_2=OCH_3, R_3=H
c: R_1=OH, R_2=H, R_3=H
d: R_1=H, R_2=OH, R_3=H
e: R_1=H, R_2=H, R_3=Br
f: R_1=OCH_2CH, R_2=H, R_3=H
g: R_1=OCH_3, R_2=H, R_3=$CH_2CH{=}CH_2$
h: R_1=NO_2, R_2=H, R_3=H
i: R_1=H, R_2=H, R_3=NO_2
j: R_1=H, R_2=$N(CH_2CH_3)_2$, R_3=H

Scheme 1. Synthesis of **3(a–j)**, **5(a–j)** and **7(a–j)**.

H-6. CH_2-8′ appeared as a doublet in proton NMR at 3.94 ppm, having H,H-COSY correlation with NH at 8.83 ppm. Using HMQC, C-3, C-5 and C-6 elucidated at 96.7, 104.1 and 134.4 ppm in ^{13}C NMR. C-2′, C-6′ and C-3′, C-5′ showed up to two signals at 120.5 and 130.3. In addition, C-7 appeared at 163.5 and C=O groups emerged at 165.8 and 171.3 ppm (Fig. 1). The chemical shifts of Schiff bases in series **3**(**a–j**), **5**(**a–j**) and **7**(**a–j**) were precisely elucidated using the same approach. The analytical data including CHN, ^1H and ^{13}C chemicals shifts as well as IR data for compounds **3j**, **5b** and **7c** are reported in this context as a sample. The complete analytical data are reported in Supplementary data. In addition, structure and stereochemistry of Schiff bases **3e** and **7j** were confirmed by the single crystal X-ray crystallographic analysis and depicted in Figs. 2 and 3.

The mechanism for the formation of Schiff bases **3**–**7** plausibly is triggered by the addition of amine moiety of primary amines **2**, **4** and **6** to the carbonyl moiety of substituted benzaldehydes **1** to generate carbinolamine intermediate as shown in Scheme 2. Primary amines and substituted benzaldehyde are strongly stabilized by hydrogen bonding interaction to ionic solvent. These bonding interactions plausibly generate a favorable low energy pass to form carbinolamine intermediate in a very short reaction time and high product yields. It is worth to note that ionic solvent counter ion (Br-) is responsible for initiating dehydration procedure in carbinolamine and generating final products.

All newly synthesized compounds were evaluated for their cholinesterase inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes and the findings are summarized in Table 1. To explain, the compounds in series **3** showed good to moderate inhibitory activity against AChE, therein, compound **3g** with methoxy at R_1 and allyl at R_3 positions of

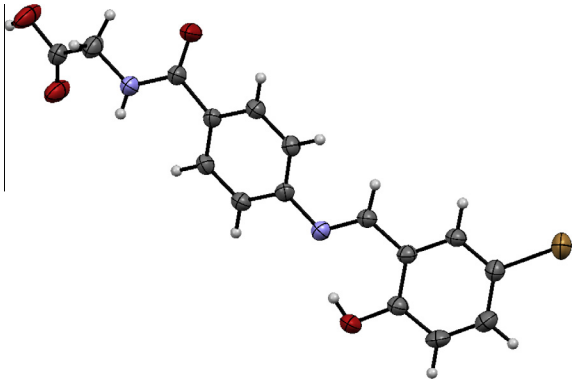


Fig. 2. X-ray crystallographic diagram for compound **3e.**

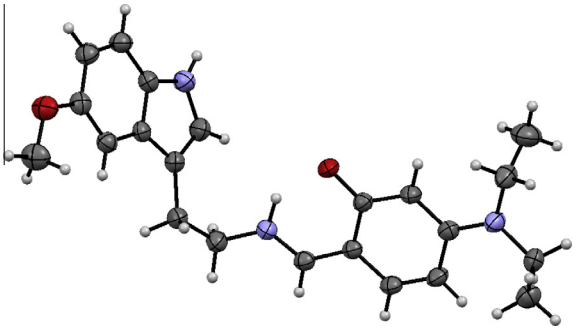


Fig. 3. X-ray crystallographic diagram for compound **7j.**

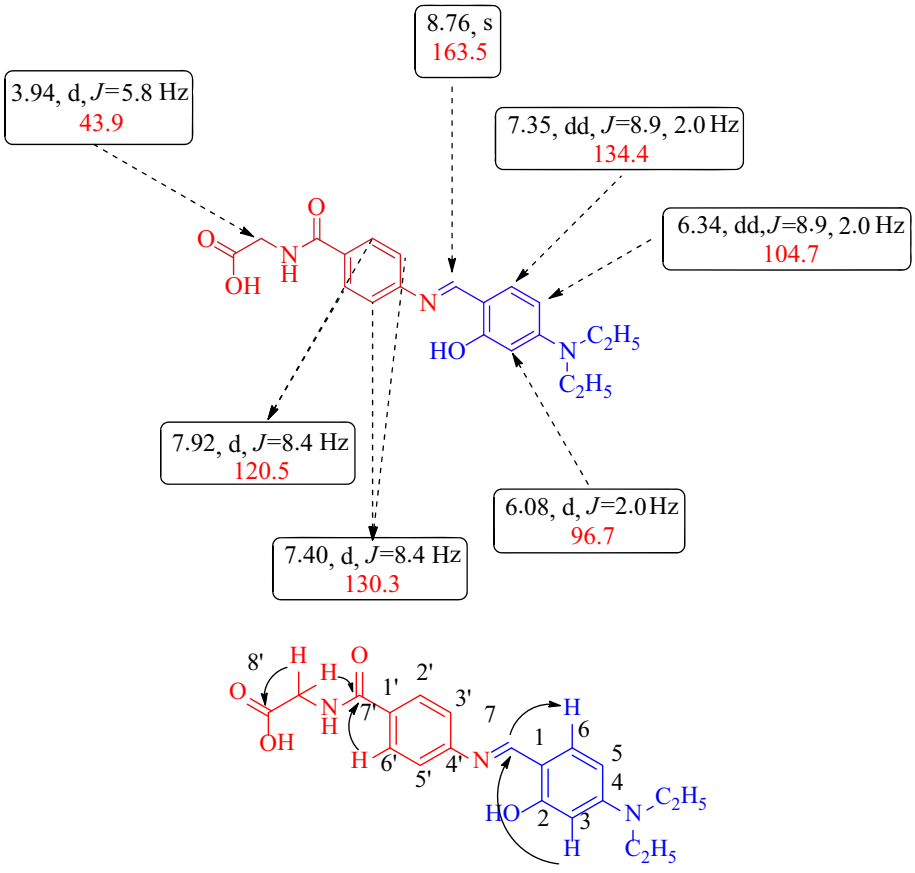
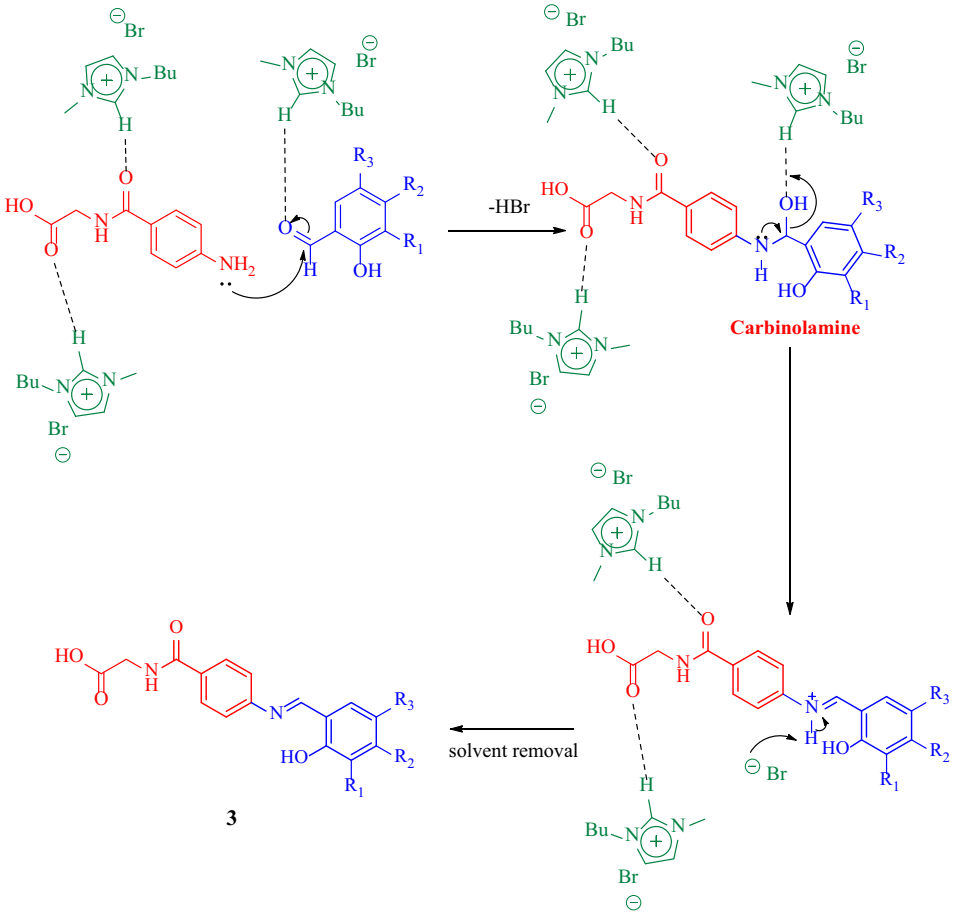


Fig. 1. Selected HMBCs and ^1H and ^{13}C chemical shifts of **3j.**



Scheme 2. Plausible mechanism for the formation of **3.**

aromatic rings, showed the highest activity in this series with remarkable IC_{50} value of 2.29 μM. In addition, compounds **3f** with ethoxy at R_1 and **3h** with N-ethyl at R_2 positions also showed significant activities with IC_{50} values of lower than 3 μM. Remaining compounds in this series, had moderate activities ranging from 9.93 to 25.78 μM. It can be justified that presence of electron donating moieties such as ethoxy and methoxy at R_1 position as well as N-ethyl at R_2 position of aromatic ring had great impact on the AChE inhibitory activities observed in this series. For BChE, except for compounds **3d**, **3e** and **3i**, which showed moderate activities of more than 10 μM, remaining compounds showed good inhibitory potential for BChE. Therein, compound **3j** with N-ethyl at R_2 position, showed the highest activity with IC_{50} value of 5.51 μM. Compounds **3a**, **3b**, **3c**, **3f**, **3g** and **3h** had IC_{50} values of 8.86, 9.51, 9.97, 9.17, 6.55 and 8.11 μM, respectively.

The Schiff series **5**, showed strong inhibitory activity toward AChE having compound **5f** with ethoxy and **5h** with NO_2 at R_2 position, which showed significant IC_{50} values of lower than 2.05 μM. In addition, compounds **5a** with R_1 = methoxy, **5c** with R_1 = OH, **5e** with R_3 = Br, **5g** with R_1 = methoxy, R_3 = allyl and **5j** with R_2 = N-ethyl also displayed remarkable IC_{50} values of lower than 10 μM. Except for **5j**, it seems that presence of either electron donating or electron withdrawing substituents at R_1 and R_3 position of aromatic ring, had major impact on the activities observed in this series. On the other hand, for BChE, only four compounds, namely **5b** with R_2 = methoxy, **5e** with R_3 = Br, **5h** with $R_1 = NO_2$ and **5j** with R_2 = N-ethyl displayed good activities with IC_{50} values of lower than 10 μM. The six remaining Schiff bases in this series showed moderate inhibitory activities with IC_{50} values ranging from 11.84 to 28.29 μM.

The compounds in series **7**(**a–j**), relatively displayed good inhibitory activity toward AChE, including compounds **7j** with R_2 = N-ethyl and **7h** with $R_1 = NO_2$ having remarkable IC_{50} values of 2.03 and 2.74 μM, respectively. Compound **7c** with R_1 = OH, **7e** with R_3 = Br, **7f** with R_1 = ethoxy and **7g** with R_1 = methoxy, R_3 = allyl also showed AChE IC_{50} values of lower than 10 μM. In this series, compounds bearing substation at R_2 position of aromatic ring displayed the lowest AChE inhibition. For BChE, compound **7j** showed the highest inhibition with IC_{50} value of 3.49 μM, followed by **7d**, **7e**, **7g** and **7h** having IC_{50} values of 9.46, 7.95, 6.62 and 6.65 μM, respectively. Other Schiff base derivatives in this series displayed moderate activities ranging from 11.74 to 19.86 μM.

Comparing series **3**, **5** and **7**, triple core Schiff bases in series **5** relatively showed better AChE inhibitory activities than their dual core analogues in series **3** and **7**. Presumably, presence of more aromatic cores in the chemical structure of these inhibitors, relatively improved their insertion and accommodation inside the AChE gorge through successive hydrophobic interactions to the amino acid residues having aromatic side chain.

In comparison to standard drug, compounds **5f**, **5h** and **7j** significantly showed higher AChE inhibitory activities than galanthamine with IC_{50} values of 1.88, 2.05 and 2.03 μM. For BChE, excluding compound **5c**, all newly synthesized Schiff bases showed higher inhibitory potentials than galanthamine.

The most active AChE and BChE inhibitors, **5f** and **7j**, were docked into the active site of AChE and BChE enzymes derived from crystal structure of *Torpedo californica* AChE and human BChE, respectively. Compound **5f** showed strong hydrogen bonding interaction with side chain residues at peripheral anionic site (Arg 289, 2.13 Å) and catalytic triad of the AChE enzyme (Tyr 130, 2.12 Å).

Table 1
AChE and BChE inhibitory activities of **3(a–j)**, **5(a–j)** and **7(a–j)**.

Entry	Compound	R_1	R_2	R_3	IC_{50}				Selectivity	
					AChE		BChE			
					μg/mL	μM	μg/mL	μM	AChE[a]	BChE[b]
1	3a	OCH_3	H	H	3.38 ± 0.17	10.24	3.10 ± 0.16	8.86	0.86	1.16
2	3b	H	OCH_3	H	8.51 ± 0.23	25.78	3.33 ± 0.17	9.51	0.37	2.71
3	3c	OH	H	H	3.12 ± 0.16	9.93	3.49 ± 0.19	9.97	1	1
4	3d	H	OH	H	5.77 ± 0.29	17.48	4.22 ± 0.21	12.06	0.69	1.45
5	3e	H	H	Br	4.10 ± 0.21	12.42	3.59 ± 0.18	10.26	0.83	1.21
6	3f	OC_2H_5	H	H	0.91 ± 0.11	2.41	3.21 ± 0.16	9.17	3.81	0.26
7	3g	OCH_3	H	allyl	0.84 ± 0.12	2.29	2.41 ± 0.12	6.55	2.85	0.35
8	3h	NO_2	H	H	3.50 ± 0.18	10.28	2.78 ± 0.14	8.11	0.79	1.27
9	3i	H	H	NO_2	4.28 ± 0.21	12.96	6.58 ± 0.27	18.8	1.45	0.69
10	3j	H	N–$(C_2H_5)_2$	H	0.88 ± 0.09	2.38	2.03 ± 0.10	5.51	2.3	0.43
11	5a	OCH_3	H	H	2.98 ± 0.15	8.51	4.88 ± 0.24	13.94	1.64	0.21
12	5b	H	OCH_3	H	3.90 ± 0.20	11.14	3.12 ± 0.16	8.91	0.8	0.44
13	5c	OH	H	H	2.11 ± 0.11	6.03	9.90 ± 0.28	28.29	4.69	0.07
14	5d	H	OH	H	4.50 ± 0.23	12.86	5.54 ± 0.17	15.83	1.23	0.28
15	5e	H	H	Br	1.67 ± 0.08	4.43	2.81 ± 0.14	8.03	1.81	0.21
16	5f	OC_2H_5	H	H	0.64 ± 0.10	1.88	4.51 ± 0.23	12.89	6.85	0.14
17	5g	OCH_3	H	Allyl	2.79 ± 0.14	7.97	4.36 ± 0.21	11.84	1.49	0.24
18	5h	NO_2	H	H	0.70 ± 0.11	2.05	1.83 ± 0.16	5.35	2.6	0.38
19	5i	H	H	NO_2	2.16 ± 0.14	12.86	4.21 ± 0.21	12.03	0.94	0.18
20	5j	H	N–$(C_2H_5)_2$	H	1.99 ± 0.15	5.42	2.59 ± 0.13	7.02	1.3	0.28
21	7a	OCH_3	H	H	6.95 ± 0.25	19.86	4.11 ± 0.21	11.74	0.59	1.69
22	7b	H	OCH_3	H	9.01 ± 0.21	25.74	6.95 ± 0.25	19.86	0.77	1.3
23	7c	OH	H	H	3.25 ± 0.16	9.29	6.02 ± 0.18	17.2	1.85	0.54
24	7d	H	OH	H	4.25 ± 0.21	12.14	2.93 ± 0.15	9.46	0.69	1.45
25	7e	H	H	Br	2.54 ± 0.13	6.83	2.97 ± 0.17	7.95	1.17	0.86
26	7f	OC_2H_5	H	H	2.84 ± 0.14	7.64	4.97 ± 0.21	14.2	1.75	0.57
27	7g	OCH_3	H	allyl	3.39 ± 0.17	9.69	2.41 ± 0.12	6.62	0.71	1.41
28	7h	NO_2	H	H	0.97 ± 0.12	2.74	2.25 ± 0.11	6.65	2.42	0.41
29	7i	H	H	NO_2	3.59 ± 0.18	10.26	4.39 ± 0.22	12.54	1.22	0.82
30	7j	H	N–$(C_2H_5)_2$	H	0.75 ± 0.09	2.03	1.24 ± 0.15	3.49	1.7	0.58
31	Galanthamine	–	–	–	0.60 ± 0.01	2.09	5.55 ± 0.01	19.34	9.25	0.1

[a] Selectivity for AChE is defined as IC_{50} (BChE)/IC_{50} (AChE).
[b] Selectivity for BChE is defined as IC_{50} (AChE)/IC_{50} (BChE).

This strong binding interactions in addition to hydrophobic and mild polar bonding to Trp 279, Tyr 334 and Phe 331 at peripheral anionic site as well as Trp 84 and His 440 at choline binding and catalytic triad of the enzyme, formed a strong binding network to active site channel, which effectively inhibited insertion and hydrolysis of ACh substrate inside and resulted in significant inhibitory activity observed for this compound (Fig. 4).

Compound **7j** as the most active BChE inhibitor, displayed strong hydrogen bonding to His 438 (1.93 Å) at the catalytic triad of the enzyme, an excellent explanation for its remarkable BChE inhibitory activity observed for this compound. Its unique chemical structure by enabling it to fold efficiently, assisted **7j** to completely insert into the BChE active site channel. As mentioned earlier, this effect

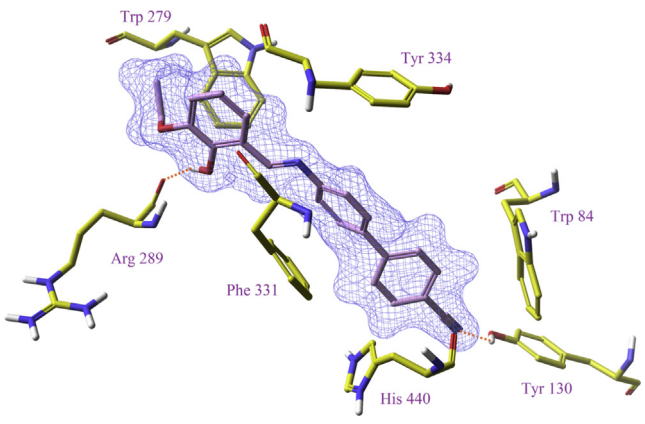


Fig. 4. Binding interaction of compound **5f** to the active site of *Tc*AChE.

inhibits BCh substrate from accessing to the catalytic region of the enzyme and interrupts its hydrolysis. Mild polar interactions with oxyanion hole composing residues such as Gly 116 and Gly 117 as well as Thr 120 in addition to hydrophobic interaction with choline binding site amino acid residues such as Trp 82 and Phe 398 were other major interactions observed for this compound (Fig. 5).

To conclude, a library of hitherto unreported Schiff bases comprising two and three aromatic cores were synthesized *via* an efficient methodology in ionic liquid [bmim]Br, ensuing excellent yields and high purity products. *In vitro* cholinesterase inhibitory results disclosed that the compounds having three aromatic cores relatively showed better AChE inhibitory activities. The most active inhibitors were also docked into the active site of AChE and BChE enzymes to disclose their binding template to cholinesterase enzymes, which strongly correlated to the *in vitro* findings.

3. Materials and methods

3.1. Chemistry

Melting points were recorded using Stuart Scientific (SMP1) apparatus and are uncorrected. The ^1H, ^{13}C and the 2D NMR spectra were recorded on a Bruker (Avance) 500 MHz NMR instrument using TMS as internal standard and DMSO-D_6 as solvent. Bruker Topspin 3.0 software was used to process the NMR data. Chemical shifts are given in parts per million (δ-scale) and the coupling constants are given in Hertz. Elemental analyses were accomplished on a Perkin Elmer 2400 Series II Elemental CHN analyzer. Crystal structure analysis was carried out using Bruker SMART APEXII CCD area-detector diffractometer.

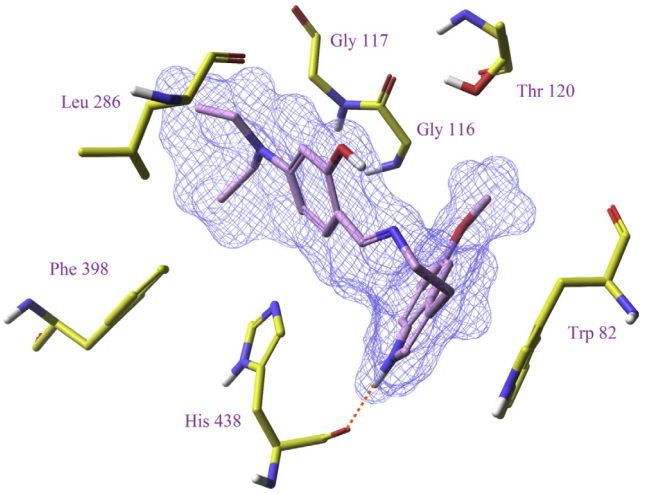


Fig. 5. Binding interaction of compound **7j** to the active site of *h*BChE.

*3.1.1. General procedure for synthesis of Schiff bases of series **3**(**a–j**)*

To an equimolar dry mixture of substituted benzaldehydes **1**(**a–j**) and *p*-aminohippuric acid (**2**) in a semi-micro boiling tube, 6.5 mmol of [bmim]Br added and the mixture grounded uniformly in an oil bath for 10–15 min. After completion of the reaction as evident by TLC, the reaction mixture cooled down, extracted using diethyl ether and dried over $MgSO_4$. Diethyl ether evaporated *in vacuo* to afford the crude. The Schiff bases **3**(**a–j**) thereafter recrystallized from boiling toluene in excellent yields (73–92%) and isolated [bmim]Br was employed for the next reaction using similar conditions.

*Analytical data for 4-{[(4-diethylamino-2-hydroxybenzylidene) amino]benzoyl}glycine (**3j**).* Yellow solids; Yield: 76%; mp 203–206 °C; IR (KBr) υ_{max}: 3287, 1649, 1598, 1249 cm^{-1}; Anal. for $C_{20}H_{23}N_3O_4$; Calculated(found): C: 65.03(65.35), H: 6.28(6.46), N: 11.37(11.34). ^1H NMR (500 MHz, DMSO): δ_H 1.12 (6H, t, J = 6.9 Hz, CH_3 [N-ethyl]), 3.40 (4H, q, J = 6.9 Hz, CH_2 [N-ethyl]), 3.94 (2H, d, J = 5.8 Hz, CH_2-8′), 6.08 (1H, d, J = 2.0 Hz, H-3), 6.34 (1H, dd, J = 8.9, 2.0 Hz, H-5), 7.35 (1H, d, J = 8.9 Hz, H-6), 7.40 (2H, d, J = 8.4 Hz, H-3′, H-5′), 7.92 (2H, d, J = 8.4 Hz, H-2′, H-6′), 8.76 (1H, s, H-7), 8.83 (1H, t, J = 5.8 Hz, NH). ^{13}C NMR (75 MHz, DMSO): δ_c 12.5, 41.1, 43.9, 96.7, 104.0, 108.5, 120.5, 128.6, 130.4, 134.4, 150.9, 151.8, 162.2, 163.5, 165.8, 171.3.

*3.1.2. General procedure for synthesis of Schiff bases of series **5**(**a–j**)*

To an equimolar dry mixture of substituted benzaldehydes **1**(**a–j**) and 4-(4′-aminophenyl)benzonitrile (**4**) in a semi-micro boiling tube, 6.5 mmol of [bmim]Br added and the mixture grounded uniformly in an oil bath for 10–15 min. After completion of the reaction as evident by TLC, the reaction mixture cooled down, extracted using diethyl ether and dried over $MgSO_4$. Diethyl ether evaporated *in vacuo* to afford the crude. The Schiff bases **5**(**a–j**) thereafter recrystallized from boiling toluene in excellent yields (68–93%) and isolated [bmim]Br was employed for the next reaction using similar conditions.

*Analytical data for 4′-[(2-hydroxy-4-methoxybenzylidene)amino]-biphenyl-4-carbonitrile (**5b**).* Orange solids; Yield: 86%; mp 137–139 °C; IR (KBr) υ_{max}: 2946, 2226, 1594, 1245 cm^{-1}; Anal. for $C_{21}H_{16}N_2O_2$; Calculated(found): C: 76.81(76.94), H: 4.91 (4.44), N: 8.53(8.41). ^1H NMR (500 MHz, DMSO): δ_H 3.82 (3H, s, OCH_3), 6.51 (1H, d, J = 2.4 Hz, H-3), 6.58 (1H, dd, J = 8.6, 2.4 Hz, H-5), 7.52 (2H, d, J = 8.5 Hz, H-3′, H-5′), 7.56 (1H, d, J = 8.6 Hz, H-6), 7.85 (2H, d, J = 8.5 Hz, H-2′, H-6′), 7.93 (4H, s, H-2″, 3″, 5″, 6″), 8.95 (1H, s, H-7), 13.59 (1H, s, OH). ^{13}C NMR (75 MHz, DMSO): δ_c 55.4, 100.8, 106.9, 109.8, 113.0, 118.8, 121.9, 127.2, 128.1, 132.8, 134.2, 136.0, 143.8, 148.2, 162.8, 163.2, 163.8.

*3.1.3. General procedure for synthesis of Schiff bases of series **7**(**a–j**)*

To an equimolar dry mixture of substituted benzaldehydes **1**(**a–j**) and 5-methoxy tryptamine (**6**) in a semi-micro boiling tube, 6.5 mmol of [bmim]Br added and the mixture grounded uniformly in an oil bath for 10–15 min. After completion of the reaction as evident by TLC, the reaction mixture cooled down, extracted using diethyl ether and dried over $MgSO_4$. Diethyl ether evaporated *in vacuo* to afford the crude. The Schiff bases **7**(**a–j**) thereafter recrystallized from boiling toluene in excellent yields (72–89%) and isolated [bmim]Br was employed for the next reaction using similar conditions.

*Analytical data for 3-hydroxy-8-{(5-methoxy-1H-indol-3-yl)ethylimino]methyl}phenol (**7c**).* Yellow solids; Yield: 79%; mp 153–155 °C; IR (KBr) υ_{max}: 3364, 1580, 1227, 1064 cm^{-1}; Anal. for $C_{18}H_{18}N_2O_3$; Calculated(found): C: 69.66(69.61), H: 5.85(5.72), N: 9.03(9.06). ^1H NMR (500 MHz, DMSO): δ_H 3.06 (2H, t, J = 6.8 Hz, CH_2-10′), 3.75 (3H, s, OCH_3), 3.80 (2H, t, J = 6.8 Hz, CH_2-11′), 6.56 (1H, t, J = 7.7 Hz, H-5), 6.73 (1H, dd, J = 8.7, 2.4 Hz, H-7′), 6.76 (1H, dd, J = 7.9, 1.5 Hz, H-4), 6.81 (1H, dd, J = 7.9, 1.5 Hz, H-6), 7.07 (1H, d, J = 2.4 Hz, H-5′), 7.13 (1H, s, H-2′), 7.24 (1H, d, J = 8.7 Hz, H-8′), 8.39 (1H, s, H-7), 10.69 (1H, s, NH). ^{13}C NMR (75 MHz, DMSO): δ_c 26.4, 55.2, 57.0, 100.1, 111.1, 111.2, 112.0, 116.5, 116.9, 117.2, 121.7, 123.6, 127.3, 131.3, 146.3, 153.0, 153.6, 165.8.

3.2. In vitro cholinesterase enzymes inhibitory assay

Cholinesterase enzymes inhibitory activity was evaluated using modified Ellman's method as described by Ahmed and Gilani. Galanthamine was used as positive control. Solutions of test samples and galanthamine were prepared in DMSO at an initial concentration of 1 mg/mL (1000 ppm). The concentration of DMSO in final reaction mixture was 1%. At this concentration, DMSO has no inhibitory effect on both AChE and BChE enzymes.

For acetylcholinesterase (AChE) inhibitory assay, 140 μL of 0.1 M sodium phosphate buffer of pH 8 was first added to a 96-wells microplate followed by 20 μL of test samples and 20 μL of 0.09 units/mL acetylcholinesterase enzyme. After 15 min of incubation at 25 °C, 10 μL of 10 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) was added into each well followed by 10 μL of 14 mM acetylthiocholine iodide. Absorbance of the colored end-product was measured using BioTek PowerWave X 340 Microplate Spectrophotometer at 412 nm for 30 min after the initiation of enzymatic reaction. For butyrylcholinesterase (BChE) inhibitory assay, the same procedure described above was followed, except for the use of enzyme and substrate, instead of which, butyrylcholine esterase from equine serum and S-butyrylthiocholine chloride were used.

Each test was conducted in triplicate. Absorbance of the test samples was corrected by subtracting the absorbance of their respective blank. Percentage inhibition was calculated using the following formula:

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

3.3. Molecular docking studies

Using Glide™, (version 5.7, Schrödinger, LLC, New York, NY, 2011), the most active compounds were docked onto the active site of *Tc*AChE derived from the crystal structures of the enzyme in-complex with the anti-Alzheimer's drug, donepezil (PDB ID: 1EVE) and to *h*BChE derived from the crystal structure of the complex of the enzyme with its substrate BCh (PDB code: 1P0P).

Water molecules and hetero groups were deleted from the enzyme beyond the radius of 5 Å of the reference ligand (donepezil or BCh), and the resulting protein structure refined and minimized by Protein Preparation Wizard™ using the OPLS-2005 force field. The Receptor Grid Generation program was used to prepare *Tc*AChE and *h*BChE grid and all the ligands were optimized by LigPrep™ using the OPLS-2005 force field to generate the lowest energy state of the respective ligands. Docking stimulations were carried out on bioactive compounds to generate 5 poses per ligand, and the best pose (with the highest score) displayed for each.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2014.10.005.

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