

Original Research Article

(1H-pyrrolo [2,3-b] pyridine) 7-Azaindole as Cholinesterase/Glycation inhibitors

Abstract: As a part of our program to discover novel analogues of (1H-pyrrolo [2,3-b] pyridine) 7-azaindole having useful biological activities, a variety of 7-azaindole analogs **1-9** with variable substituents on phenyl ring of phenacyl moiety were synthesized and evaluated for their AChE, BChE and antiglycation Inhibitory potential. Compounds **2-5** were found to be AChE inhibitors with IC_{50} 1.34 μ M, 11.60 μ M, 0.957 μ M and 0.975 μ M respectively, while compounds **2-5** were also found to be BChE inhibitors with IC_{50} 1.25 μ M, 3.93 μ M, IC_{50} 9.18 μ M and 10.20 μ M, respectively. Compounds **2** ($IC_{50} = 1.25 \pm 0.019$ μ M) and **3** ($IC_{50} = 3.93 \pm 0.36$ μ M), showed potent BChE inhibitory potential than standard Galantamine ($IC_{50} = 8.5 \pm 0.02$ μ M). Besides this, compounds **2-9** were evaluated for glycation inhibition activity. Compound **5** (IC_{50} values 120.6 $\pm$ 0.2 μ M) showed potent antiglycation potential than standard rutin ($IC_{50} = 294.50 \pm 1.5$ μ M). The size of the substituent, electron donating or withdrawing affect of substituents as well as the position of substituent on phenyl affects the activity.

Keywords: 7-Azaindole, acetylcholinesterase, butyrylcholinesterase, synthesis, Antiglycation

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; BChE, butyrylcholinesterase; HMBC, Heteronuclear Multiple Bond Connectivity; PDB, Protein Data Bank; VDCs, voltage dependent Ca21 channels,

Introduction:

The 7-azaindole nucleus has proved to be an important model for working on its own synthesis and synthesizing its different analogues [1-4]. In the past, indole derivatives have attracted the interest of scientists for their potential biological activities [5-8]. Different azaindole compounds were reported for analgesic, blood pressure lowering and anti-inflammatory activities and acting as effective coronary vasodilator, cardiovascular and potent hypotensive agent [9]. Other indole derivatives were prepared as cyclooxygenase inhibitors and were found useful as anti-inflammatory agents [10-11]. These azaindole structures were expected to enhance the cytotoxicity towards tumor cell lines through stronger hydrogen bonding with the target enzymes. Saify *et. al.* synthesized a series of 7-azaindole derivatives having important antimicrobial response against gram positive and gram negative bacteria. As a result of our continued interest in 1H pyrrolo (2,3-b) pyridine as potential bioactive agents[14-16], 7-Azaindole derivatives were active in nervous central system and shown

affinity for serotonin receptor [17-19]. we now wish to report the investigation for acetylcholinesterase and glycation inhibition.

Alzheimer's disease is age related neurodegenerative disease and is due to progressive memory failure and extra cognitive mutilations. The cholinergic theory of Alzheimer's disease proposes that the selective loss of cholinergic neurons in AD results in a deficit of acetylcholine in specific regions of the brain that mediate learning and memory functions. The primary approach for treating AD is, therefore, aimed at rising the acetylcholine levels in the brain by using acetylcholinesterase inhibitors (AChEIs) such as donepezil, galantamine or rivastigmine. On the other hand, calcium excess seems to be the main factor initiating the processes leading to cell death. Augments β amyloid $-(A\beta)$ formation, [22] and tau hyper phosphorylation [23] calcium entry through the L-subtype of high voltage activated calcium channels causes both calcium in excess and mitochondrial disruption, which activate the apoptotic cascade and cell death [24,25]. Hence, blocking the entrance of calcium through calcium channels could be a good strategy to prevent cell death. In U.S.A alone, Alzheimer's disease related expenses surpassed \$100 billion every year per patient [26]. Recently Alzheimer's disease affected more than 35 million people throughout the world [27]. Its two feature abnormalities are plaques deposits of a protein fragment called beta amyloids and tangle, twisted strands of another protein tau [28].

In Alzheimer's disease, information transfer at the synapses begins to fail and eventually the cells die. In a brain with advanced Alzheimer's disease, there is dramatic shrinkage from cell loss and widespread debris from dead and dying neurons. Alzheimer's disease can affect different people in different ways [29], but the most common indication pattern begins with gradually worsening difficulty in identification fresh information. This is because disruption of brain cells usually begins in regions involved in forming new memories.

Acetylcholinesterase and butyrylcholinesterase (BChE, EC .1.1.8) are cholinesterases; with divergent properties dependent on the species, tissue and source. AChE catalyzes the rapid hydrolysis of the neurotransmitter acetylcholine in the cholinergic synapses [30]. This enzyme is an attractive target for rational drug design and discovery of mechanism-based inhibitors for the treatment of many neurodegenerative disorders such as Alzheimer's disease and

myasthenia gravis [31]. BChE is present in significantly higher quantities in Alzheimer's plaques than in plaques of normal age-related non-demented brains [32]. Selective inhibitors of BChE such as cymserine analogues and the dual inhibitor of AChE and BChE, rivastigmine, showed potential therapeutic benefits in AD and related dementias [33,34].

The recently solved crystal structure of the BChE enzyme [35,36] has shown to be very similar to that of *Torpedo californica* AChE. However, several aromatic amino acids, lining the gorge of the AChE, were replaced in BChE by hydrophobic analogues. Due to this, the BChE gorge becomes relatively larger and capable of accommodating bulkier and relatively nonpolar ligands. Since the discovery of physostigmine [37] as the first natural AChE inhibitor, only few other natural inhibitors have attracted the attention of neuropharmacologists [38].

For the treatment of late diabetic complications antiglycation agents is an important approach. Currently number of effective antiglycating agents is very small, the need of new antiglycating agents is still unmet. As the unpleasant incident of type-2 diabetes is increasing, its injurious effects are mostly attributed to the formation of sugar derived substances called advanced glycation end products [39] which are pathogenic mediators of almost all diabetic complications [40]. Effort has been focussed on the discovery of new inhibitors of glycation, because of their therapeutic potential [41]. Certain molecules were developed that can cleave advanced glycation end products cross links and perhaps open the possibility of reversing the steady process of diabetic complications [42]. It has been found that aged garlic extract inhibit the formation of advanced glycation end products *in vitro*. S-allylcysteine is a very important component of aged garlic extract that acts as a potent antioxidant and thus inhibit the advanced glycation end products formation [43,44]. Aminoguanidine, an inhibitor of advanced glycation end products formation [45]. Efforts have now been made to develop new and safe synthetic antiglycation agents [46]. It has demonstrated that polyamines, spermidine and spermine have potent antiglycation effects, comparable to those of aminoguanidine and carnosine [47].

We have previously reported a number of novel aza indole compounds [12-13]. In the present study, *in vitro* antiglycation activity of nine 7-Azaindole derivatives **2–9** were evaluated. We

report in this paper the synthesis and AChE, BChE and glycation inhibitory properties of the 7-aza indole compounds, and their mechanism of AChE and BChE inhibition.

Materials and methods

Experimental

General procedure

Reagents were procured from Aldrich Chemical Company. The entire solvents were reagents grade. Reactions were monitored by TLC using pre-coated silica gel, GF-254 and Analytical thin layer chromatography (TLC) was executed on silica gel (Kieselgel 60, 254, E. Merck) pre-coated 0.25 mm plates. Visualization was accomplished with ultraviolet light at 254 and 365 nm UVP UVLS-26 Series (Cambridge). Iodine vapors were also employed for the recognition of spots. All melting points were recorded on Gallenkamp melting point apparatus and were corrected. Silica granules from E. Merck was used for drying reaction product after workup. Ultraviolet spectra were recorded in methanol on a Hitachi U-3200 spectrophotometer. Infra Red spectra were measured on a Shimadzu IR 460 spectrophotometer using KBR disc. Mass spectra were determined on Varian Massen spectrometer MAT 311A spectrometer. Nuclear magnetic resonance spectra were recorded in DMSO-D₆ and MeOD on AVANCE AV 300 spectrometer operating at 300 MHz.

General method of preparation of compounds

To a stirred equimolar, quantity of 7-azaindole in acetone (20-25 ml) was added successively and 2-bromo-2-acetonaphthone, 1-adamantyl-bromo ethyl-ketone, 2-bromo-3-nitro-acetophenone, 2-bromo-4-nitro-acetophenone were dissolved separately in 20-25 ml of acetone. Reaction mixture was stirred for 24 hours at 52-54°C. The process of reaction is monitored through thin layer chromatography. The crude solid product was filtered and washed with acetone. The product thus obtained was purified through re-crystallization by using ethanol and ether. The pure compound was dried in desiccators over anhydrous calcium sulphate. Melting point and spectral data were obtained to confirm the structure of compound (Scheme 1).

Enzyme Inhibition Assays

Assay for acetylcholinesterase and butyrylcholinesterase inhibition

Materials: All the reactions were performed in triplicate in a 96 well micro-plate on Spectra Max 340 (Molecular Devices, U.S.A). Electric eel acetylcholinesterase (EC 3.1.1.7), horse serum butyrylcholinesterase (EC 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, 5,5'-dithio-bis (2-nitrobenzoic) acid were purchased from Sigma (St. Louis, MO, USA). Buffers and other chemicals were of analytical grades. AChE and BChE inhibiting activities were measured by slightly modifying the spectrophotometric method developed by Ellman *et al.* [48]. Acetylthiocholine Iodide and butyrylthiocholine chloride were used as substrates of the reaction and 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB) (Sigma Chemical Co) were used for the measurement of the cholinesterase activity.

Procedure Acetylcholinesterase/ butyrylcholinesterase inhibition:

AChE and BChE inhibitory activities were measured *in vitro* by a modified spectrophotometric method developed by Ellman et al. [48]. The assay procedure has been previously described [49]. All the inhibition studies were performed using a SpectraMax microplate spectrophotometer (Molecular Devices, CA, USA). Electric eel AChE (type VI-S, Sigma), and horse serum BChE (Sigma) were used as enzyme sources, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma) were used as substrates. Ellman reagent i.e. 5,5-dithiobis (2-nitro) benzoic acid (DTNB, Sigma) was used to develop the chromogenic marker for the measurement of the cholinesterase activity. Sodium phosphate buffers (1mM) at pH 7.0 and 8.0 were used to prepare the enzyme working solutions and to assay enzyme activity at 30°C, respectively. The initial rates of the enzymatic reactions were measured as the rate of change in OD/ min. (optical density/min.) and used in subsequent calculations.

According to Ellman et al., the rate of the enzymatic reaction is calculated by the following equation [48].

$$\text{Rate (mole/L/min)} = \frac{\text{change in absorbance/min.}}{13,600}$$

Assay for Antiglycation

Chemicals:

Bovine Serum Albumin (BSA) was purchased from Merck Marker Pvt. Ltd. while other chemicals {glucose anhydrous, trichloroacetic acid (TCA) sodium azide (NaN_3), dimethyl sulfoxide (DMSO), sodium dihydrogen phosphate (NaH_2PO_4), sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), and sodium hydroxide (NaOH) were purchased from Sigma–Aldrich. Sodium phosphate buffer (pH 7.4), was prepared by mixing Na_2HPO_4 and NaH_2PO_4 (67 mM) containing sodium azide (3 mM). Phosphate buffer saline (PBS) was prepared by mixing NaCl (137 mM), Na_2HPO_4 (8.1 mM), KCl (2.68 mM), and KH_2PO_4 (1.47 mM) and pH 10 was adjusted with NaOH (0.25 mM). BSA (10 mg/ml) and glucoses anhydrous (50 mg/ml) solutions were prepared in sodium phosphate buffer. Test samples were prepared in DMSO (1 mM/ml).

PROTOCOL:

This test was used to evaluate the ability of the inhibitors to inhibit the methyl glyoxal mediated development of fluorescence of BSA. assay was performed by using the method described by Rahbar *et al.*, [50,51] with slight modifications.

Triplicate samples of BSA 10 mg/ml, 14 mM MGO, 0.1M phosphate buffer (pH 7.4) containing NaN_3 (30 mM) were incubated under aseptic conditions, (in such a way that each well of 96-well plate contained 50 μL BSA solution, 50 μL MGO, and 20 μL test sample) at 37°C for 9 days in the presence or absence of various concentrations of the compounds. After 9 days of incubation, each sample was examined for the development of specific fluorescence (excitation, 330 nm; emission, 440 nm), against sample blank on a microtitre plate spectrophotometer (Spectra Max, Molecular Devices). Rutin was used as a positive control ($\text{IC}_{50} = 294\mu\text{M} \pm 1.50 \text{ SEM}$).

The percent inhibition of AGE formation in the test sample versus control was calculated for each inhibitor compound by using the following formula:

$$\% \text{ inhibition} = (1 - \text{fluorescence of test sample} / \text{Fluorescence of the control group}) * 100$$

196 **1H-pyrrolo[2,3-b]pyridine (7-azaindole) (1)**; White crystalline solid, EIMS = m/z = 118.14
197 amu, Melting point = 105-107°C.

198 **Naphthalen-2-yl-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide(2)**: Yield = 86.2%,
199 HREI MS: m/z = C₁₉H₁₅N₂OBr, EIMS = m/z (relative intensity; %) = 367.23 amu, M. P. =
200 267 ± 2°C, UV λ_{max} (MeOH) nm = 340, 293, 250, 224, 203, IR ν_{max} (KBr) cm⁻¹ = 3755, 2923,
201 1687, 1361, 987, 823.5, 783, 545, 474.5; EIMS = m/z (relative int, %): 286 (M⁺ -HBr
202 C₁₉H₁₄N₂O, 7.4), 259(8.2), 258(62), 257(52), 155(15), 81(18), 77(22), ¹H-NMR (DMSO, D₆,
203 300 MHz), δ 8.86 (2H, d, J =7.047, H-6, 4), 8.60 (1H, d, J =6.09, H-5), 8.24(1H, d, J =7.71,
204 H-10'), 8.07-8.03 (2H, m, H-2', 8'), 7.96 (2H, d, J =3.43, H-5', 3'), 7.76(2H, m, H-6',7'),
205 6.62(2H, d, J =3.50, H-2', 3'), (2H, s, H-2").

206 **7-(2-Adamantan-1-yl-2-oxo-ethyl)-1H-pyrrolo[2,3-b]pyridinium bromide(3)**: yield = 81.5
207 %, HREI MS: m/z = C₁₉H₂₃N₂OBr, EIMS: m/z = 375.30 amu, M. P. = 287 + 2°C, UV
208 λ_{max} (MeOH) nm = 299.6, 226.0, IR ν_{max} (KBr) cm⁻¹= 3409, 2918, 2850, 2773, 1712, 1620,
209 1458, 1357, 1164, 1097, 887, 779, 727, 661, 536, 476, EIMS = m/z (relative intensity; %), 295
210 (M⁺, C₁₉H₂₃N₂O, 7.3), 294 (35), 237(2.6), 176(3), 159(15.5), 131(100), 93(18.4), 79(27), ¹H-
211 NMR (DMSO, 300 MHz): δ 8.76-8.79 (1H, d, J = 8.68 Hz, H-6), 8.48 (1H, d, J =6.15 Hz, H-
212 4), 7.95(1H, d, J =3.48 Hz, H-2), 7.66(1H, dd, J = 7.8, 1.5 Hz, H-5), 6.97 (1H, d, J = 3.57, H-
213 3), 6.06 (2H, s, H-2''), 2.07(5H, s, H-4', 6, 7', 8', 9'), 1.97(5H, s, H-10', 5', 3', 2'), 1.78-1.68
214 (6H, m, H-3', 6', 6', 7', 9'10').

215 **7-[2-(4-nitrophenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide (4)**: Yield =
216 61%, HREI MS: m/z C₁₅H₁₂N₃O₃Br, EI MS: m/z = 362.18 amu, Melting Point = 252 + 2°C,
217 UV λ_{max} (MeOH) nm: 270, 248 and 201. IR ν_{max} (KBr) cm⁻¹: 3390, 2920, 1690, 1600 and
218 1340, EIMS, m/z (relative int., %): 282 (M⁺-Br, C₁₅H₁₂N₃O₃, 8), 164(4), 160(12), 132 (11),
219 122(18), 118(100) and 77(23), ¹H-NMR (MeOD, 300 MHz) δ : 8.81 (1H, d, J = 7.84, 1.08 Hz,
220 H-6), 8.84 (2H, d, J =8.02, Hz, H-3', 5'), 8.26 (1H, d, J =8.99 Hz, H-4), 7.78 (2H, d, 8.02 Hz,
221 H-2', 6'), 7.71 (1H, d, J =3.51 Hz, H-2), 7.68 (1H, dd, J =8.99, 7.84 Hz, H-5), 7.02 (1H, d, J =
222 3.51 Hz, H-3) and 6.02 (2H, s, H-2'').

223
224 **7-[2-(3-nitrophenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide (5)**: Yield =
225 80%, HREI MS: m/z = C₁₅H₁₂N₃O₃Br, EI MS: m/z = 362.178 amu, Melting Point = 224 +
226 2°C, UV λ_{max} (MeOH) nm = 299.6, 228, 200 nm, IR ν_{max} (KBr) cm⁻¹ = 3232, 2916, 1965,

1797, 1697, 1529, 1475, 1356, 1224.9, 1172.8, 1091.5, 930, 883, 799, 598, 519, 481.2, EIMS
 $= m/z$ (relative int., %): 282(M^+ , $C_{15}H_{12}N_3O_3$, 9.8), 252(100), 236(41), 206(57), 193(6.1),
 150(15.6), 131(53.5), 118(13.3), 90(6.2), 77(17.1), 63(6.6), ^1H -NMR (MeOD, 300 MHz) δ :
 8.93 (1H, s, H-6), 8.81 (1H, d, $J = 7.79$ Hz, H-4), 8.69 (1H, dd, $J = 7.71$, Hz, H-2'), 8.52 (1H,
 m, H-5), 8.39 (1H, s, H-4'), 7.93 (1H, dd, $J = 8.02$, 3.35Hz, H-6'), 7.77(1H, d, $J = 3.35$ Hz, H-
 5), 7.67 (1H, m, H-2), 7.01 (1H, d, $J = 3.50$ Hz, H-3), 6.90 (2H, d, $J = 3.57$ Hz, H-2'').

7-[2-(3,4-dihydroxyphenyl)3-oxoethyl]-7H-pyrrolo[2,3-b]pyridine-1-ium;bromide (6):
 HREI MS: $C_{15}H_{13}N_2O_3Br$; UV: 289, 227 and 208. IR: 3100, 1670, 1595 and 1290, EIMS m/z
 (rel. int %): $M-1(C_{15}H_{12}N_2O_3)$, 269 (2), 239 (14), 193 (3), 165 (12), 137 (15), 118 (100) and
 109 (25), ^1H -NMR; D_2O (300 MHz) δ : 8.72 (1H, d, $J = 7.85$ Hz, H-6), 8.24 (1H, d, $J = 6.80$
 Hz, H-4), 7.70 (1H, dd, $J = 8.46$, 2.21 Hz, H-6), 7.67 (1H, d, $J = 3.65$ Hz, H-2'), 7.62 (1H, d, J
 $= 7.85$, 6.80 Hz, H-5), 7.55 (1H, d, $J = 2.19$ Hz, H-2), 7.06 (1H, d, $J = 8.46$ Hz, H-5), 6.97
 (1H, d, $J = 3.65$ Hz, H-3), 6.48 (2H, s, $N-CH_2$), 4.83 (1H, br.s, OH), 4.65 (1H, br.s, OH).

7-[2-(4-Methoxy-phenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridine-7-ium (7): Yellow
 needles, HREI MS: m/z $C_{16}H_{15}N_2O_2Br$, UV: 389.4, 284.8 and 223.0, IR: 3449.6, 3010.8,
 1680.5, 1341.5 1309.5, 1236.1, 1101.2; EIMS m/z (rel. int %): 266 (M^+-Br , $C_{16}H_{15}O_2N_2$, 31),
 238(55), 149(2), 135(100), 131(10), 118(6) and 107(12), ^1H -NMR: 8.78-8.75 (1H, d, $J = 7.77$
 Hz, H-6), 8.45-8.43 (1H, d, $J = 6.17$ Hz, H-4) 8.14-8.11 (2H, d, $J = 8.88$ Hz, H-5) 7.73-7.72
 (1H, d, $J = 3.528$ Hz, H-2) 7.66-7.62 (1H, m, H-6) 7.16-7.13 (2H, d, $J = 8.92$ Hz, H-3, H-5),
 6.99-6.98 (1H, d, $J = 3.55$ Hz, H-2), 6.43-6.41 (1H, d, $J = 5.72$ Hz, H-3), 3.93 (1H, s, H-4),
 3.30-3.29 (1H, s, H-1).

7-(4-Oxo-4-thiophen-2-yl-butyl)-1H-pyrrolo[2,3-b]pyridin-7-ium; chloride(8): UV: 389.4,
 286.2, 263.8, 222.2 and 204.0; IR: 3075.8, 2469, 1496.5, 1510.5, 1359.2, 1310.9, 1246.2;
 EIMS: 270(M^+-Cl , $C_{15}H_{15}ON_2$, s, 19) 185(2), 159(28), 132(50), 126(48), 118(100), 111(87),
 83(9); ^1H -NMR: δ 8.68-8.65 (1H, d, $J = 7.934$ Hz, H-6), 8.55-8.53 (1H, d, $J = 6.32$ Hz, H-4),
 7.87-7.80 (1H, m, H-2, H-4), 7.58-7.53 (1H, t, $J = 7.015$ Hz, H-5), 7.20-7.17 (1H, t, $J = 4.38$
 Hz, H-3), 6.97- 6.95 (2H, d, $J = 3.48$ Hz, H-2, H-3), 3.3-3.2 (1H, m, H-1), 2.47 – 2.43(2H, m,
 H-2, H-3).

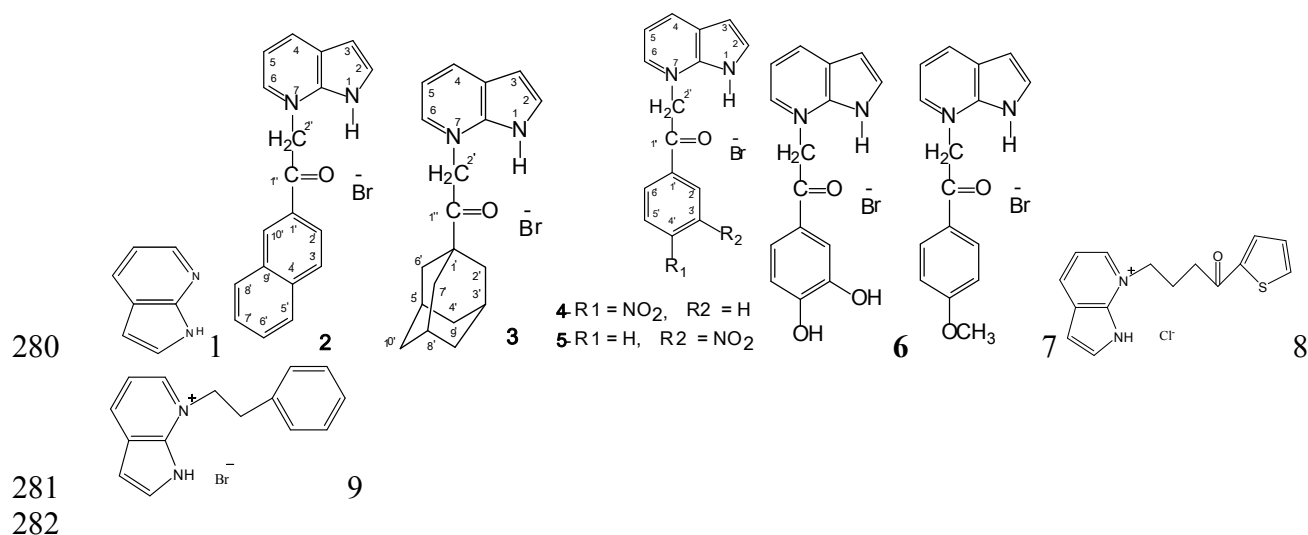
258 **7-Phenethyl-1H-pyrrolo[2,3-b]pyridin-7-ium; bromide (9):** UV (MeOH) nm: 390.0, 293.0,
 259 224.0, 203.4, 198.6; IR $\nu_{\max}$ (KBr) cm^{-1} 51.5, 1647.6, 1495.0, 1357.4, 1307.8 and 1189.1;
 260 EIMS (relative int, %): 223 ($\text{M}^+ - \text{Br}$, $\text{C}_{15}\text{H}_{14}\text{N}_2$ (10), 132(3), 118(100), 104(8), 91(7), 77(6);
 261 $^1\text{H-NMR}$ (MeOD, 300 MHz): δ 8.63-8.61(1H, d, $J = 7.76$ Hz, H-6), 8.20-8.18 (1H, d, $J = 6.17$
 262 Hz, H-4), 7.74-7.73 (1H, d, $J = 3.52$ Hz, H-5), 7.45-7.40 (2H, m, H-3, H-5'), 7.2-7.18 (2H, t, J
 263 = 6.33 Hz, H-1, H-2), 7.02-6.99 (2H, m, H-2, m, H-2, H-3), 6.93-6.92 (1H, d, $J = 3.51$ Hz, H-
 264 1), 5.02-4.97 (1H, t, $J = 6.92$ Hz, H-1'), 4.57 (1H, s, H-2').

265 266 Results and discussion

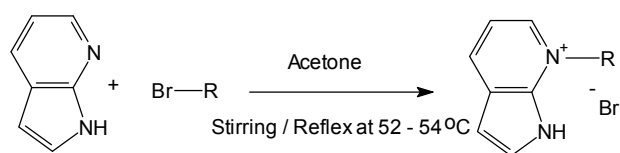
267 The compounds herein described were synthesised as a part of study complementary to anti-
 268 cholinesterase antiglycation investigation of fused pyrrolo pyridine system. All the 7-aza
 269 indole were prepared under the conditions recorded in experimental section[12-16].

270
 271 The $^1\text{H-NMR}$ spectra were specially helpful in deciding the substitution pattern at the
 272 different positions of phenacyl part of the molecule. The molecular ion peaks of the
 273 compounds were obtained from EIMS spectra [15-16]. The spectral data of compounds **4, 5**
 274 was reported previously by us [20,21].

275
 276
 277 A series of 7-azaindole derivatives were prepared by quaternization with phenethyl bromide
 278 and substituted phenacyl halides, the side chain thus possessing certain features common to
 279 those present in sympathomimetic amines and related compounds.



The parent compound (**1**) investigated for acetylcholinesterase and butyrylcholinesterase activity showed insignificant activity. Compounds **1-5** showed significant activity. Compounds **3** and **2** exhibited better results as compared to standard Galanthamine. In the bio-screening of the compounds for AChE inhibition [57], compounds **4** nitro-phenacyl (para-nitro derivative) and **5** nitro-phenacyl (meta-nitro derivative) were found most active with almost same IC_{50} values $0.957 \mu M$ and $0.975 \mu M$ respectively. The result indicates that NO_2 substitution in phenacyl ring at different position (meta for compound **4** and para for compound **5**) is not making any significant change in the activity. Napthyl derivative with IC_{50} value $1.34 \mu M$ was the next to the nitro derivative while adamantyl derivative showed lowest activity among all the compound with IC_{50} value $11.60 \mu M$. Compounds **6-9** were also studied for the inhibition of AChE and BChE inhibitory activities using same methodology and found to be inactive. Results of acetylcholinesterase and butyrylcholinesterase activity are represented in Table 1.

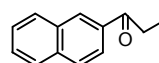


Reactants

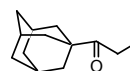
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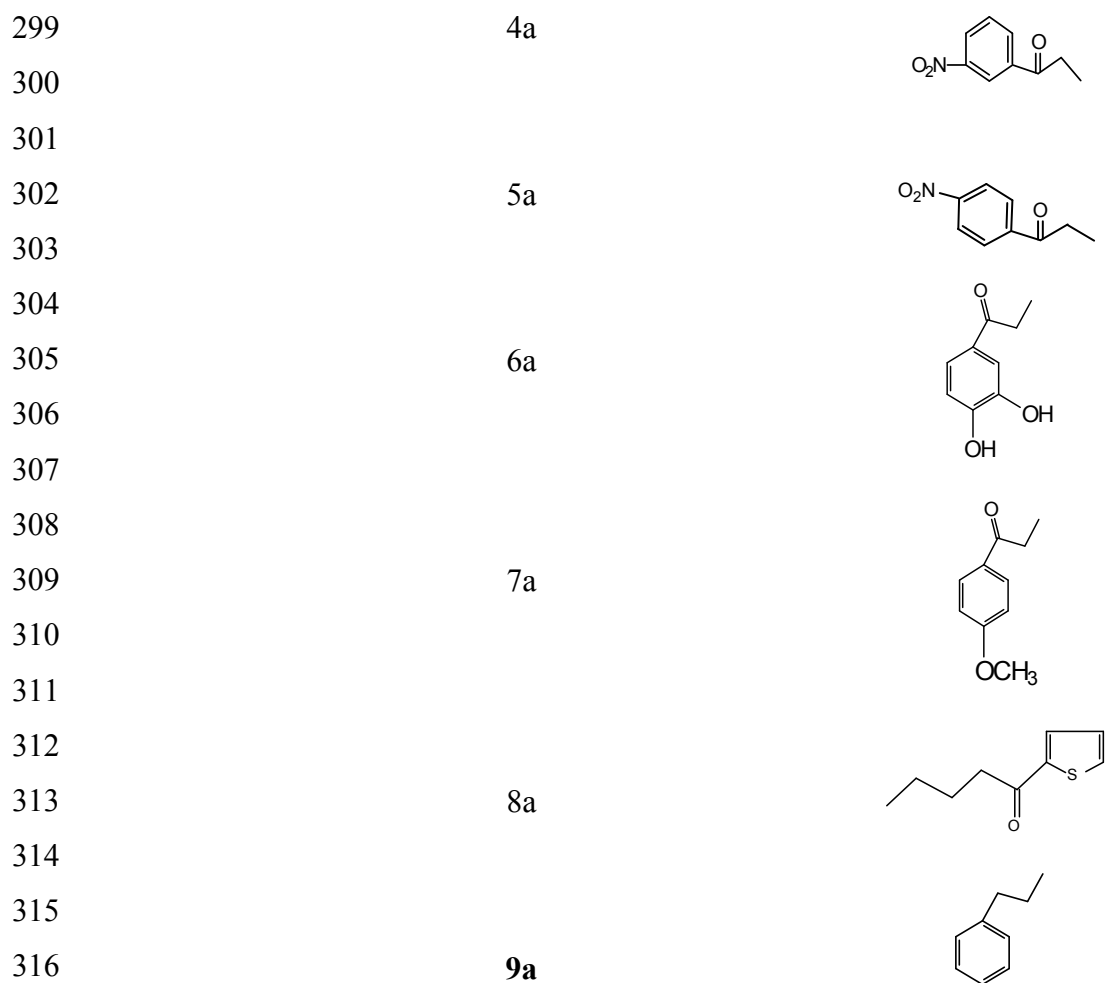
R

2a



3a





Scheme 1: Synthesis of (1H-pyrrolo [2,3-b] pyridine) 7-azaindole derivatives **1-9**

Table-1: *In vitro* quantitative inhibition of acetylcholinesterase and butyrylcholinesterase activity of (1H-pyrrolo [2,3-b] pyridine) 7-azaindole derivatives **1-9**.

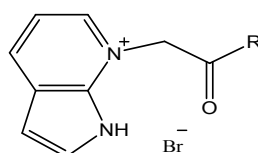
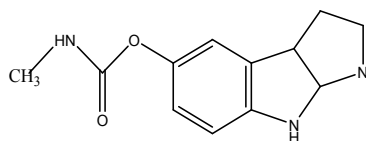
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Compound no.	Antiglycation % inhibition IC ₅₀ ±SEM [μ M]	Acetylcholinesterase IC ₅₀ ± SEM [μ M]	Butyrylcholinesterase IC ₅₀ ± SEM [μ M]
2	51.19 % inhibition	1.34 ± 0.05	1.25 ± 0.019
3	47.58 % inhibition	11.60 ± 0.173	3.93 ± 0.36
4	9.2% inhibition	0.957 ± 0.009	9.185 ± 0.250
5	78.2 % inhibition. 120.6±0.2 μ M	0.975 ± 0.0518	10.203 ± 0.282
6	64.26% inhibition, 524.93±1.19 μ M	-	-
7	58.89% inhibition, 799.27±6.7 μ M	-	-
8	67.95% inhibition, 476.56±9.7 μ M	-	-
9	50.52% inhibition, 984.98±3.5 μ M	-	-
Standard Galantamine	-	0.5 ± 0.001	8.5 ± 0.02
Rutin	86.0 % inhibition, 294.50± 1.5 μ M	-	-

330 In the investigation for BChE inhibiting activity opposite results have been observed as
 331 compared to acetylcholine esterase. Compound **2** containing naphthyl derivative displayed
 332 maximum inhibition (IC₅₀ 1.25 μ M), while the compound **3** (adamantyl derivative) with IC₅₀
 333 value 3.93 μ M was the second potent inhibitor of BChE. Compound **4** and **5** (nitrophenacyl
 334 derivatives) showed almost same level of inhibition with IC₅₀ 9.18 μ M and 10.20 μ M
 335 respectively. Approximately similar inhibition of BChE and AChE by compound **4** and **5**
 336 suggested that though the presence of NO₂ functional group imparting inhibitory activity but
 337 the position of NO₂ in the phenacyl ring is not important for any significance difference in the
 338 activity.

339

Good results of the synthesized compounds for AChE inhibitory activity can be explained by correlating the structures of synthesized molecules of 7-azaindole with physostigmine.

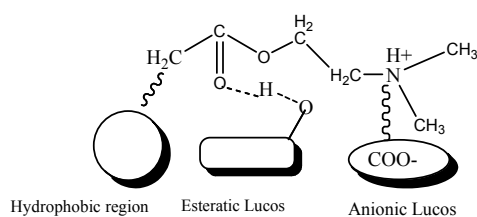


7-Azaindole derivative

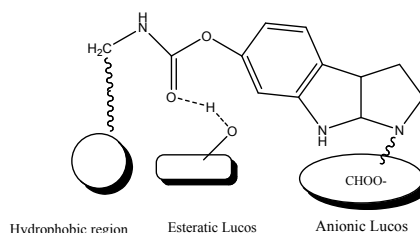
Physostigmine

R= phenacyl, naphthyl and adamantyl

Physostigmine is one of the clinically tested AChE inhibitor and may be helpful for enhancing memory in patient of Alzheimer's disease [52]. Azaindole derivatives are closely related to Physostigmine and can be compared for their use in the treatment of Alzheimer's disease. Fig. 1 showing active binding site regions [53] on the enzyme surface for acetylcholine, explaining possible three major binding areas like ionic, esteratic and possible hydrophobic region. Physostigmine (known potent ligand) and synthesized compounds showing the presence of same common essential features necessary for binding fig. 2 and 3.



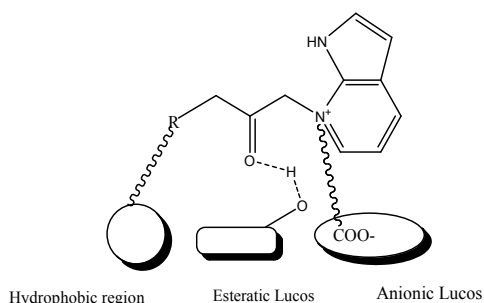
Hydrophobic region Esteratic Lucos Anionic Lucos



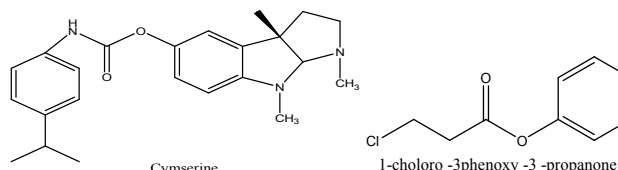
Hydrophobic region Esteratic Lucos Anionic Lucos

Fig.1: Acetylcholine with active binding site

Fig. 2: Physostigmine



Hydrophobic region Esteratic Lucos Anionic Lucos

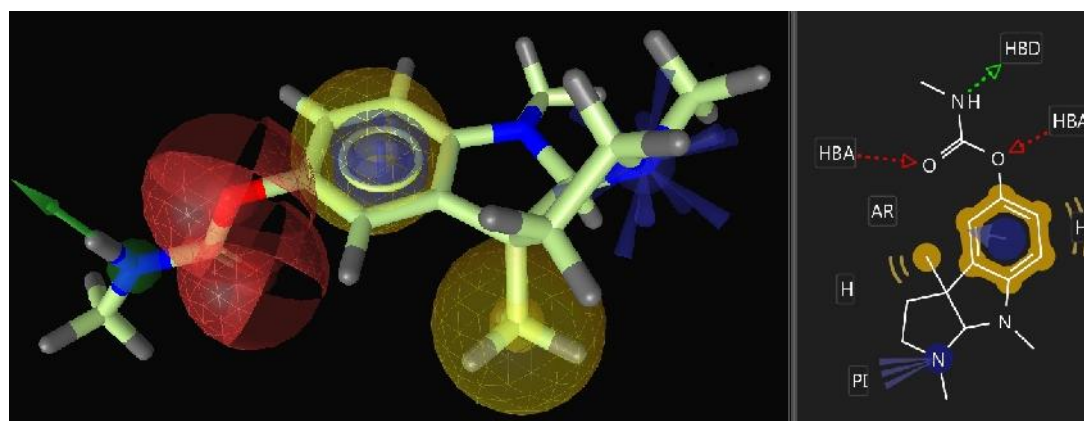


Cymserine

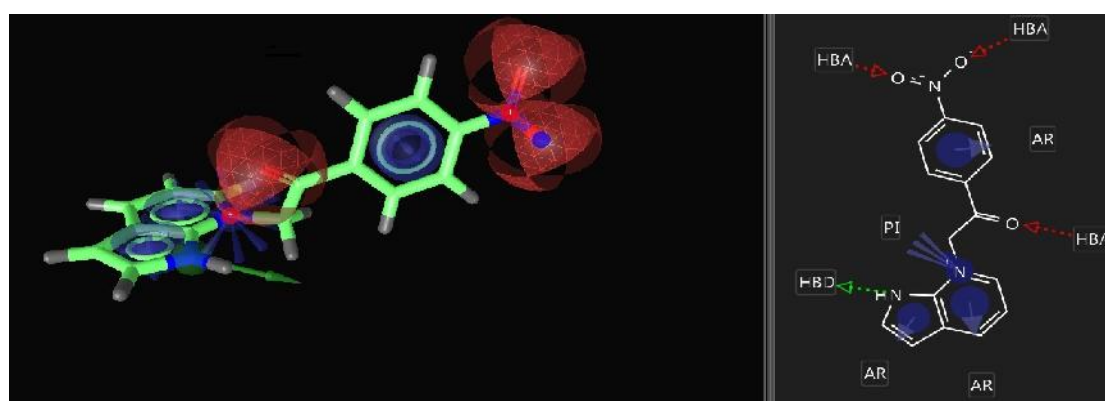
1-choloro -3phenoxy -3 -propanone

Fig. 3: Synthesized derivative of 7 Azaindole

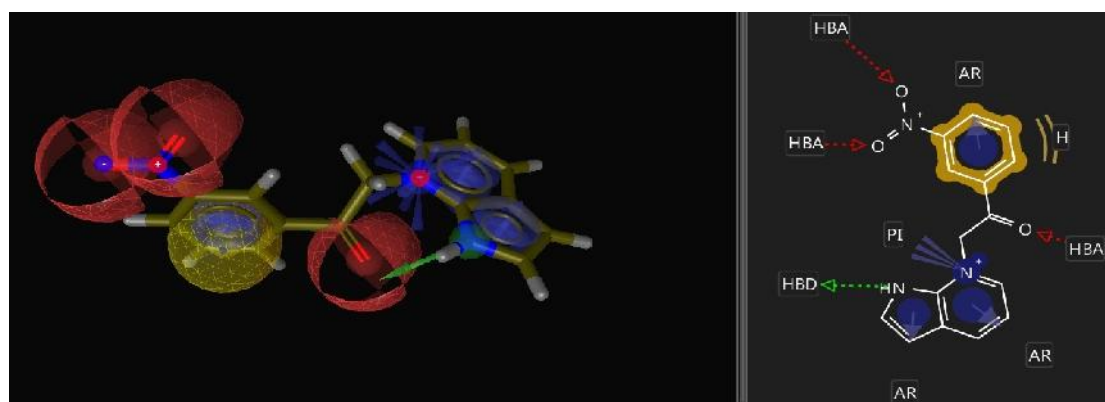
R = phenacyl, naphthyl and adamantyl



(a)



(b)



(c)

Fig. 4: Showing 2D and 3D pictures Pharmacophoric region (a) Physostigmine (b) compound 4 (c) compound 5.

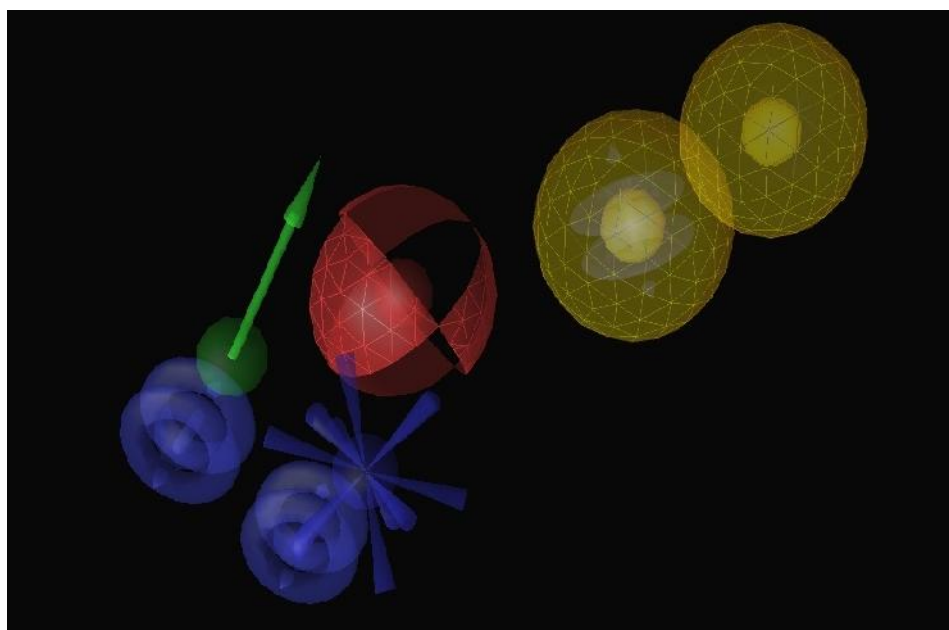
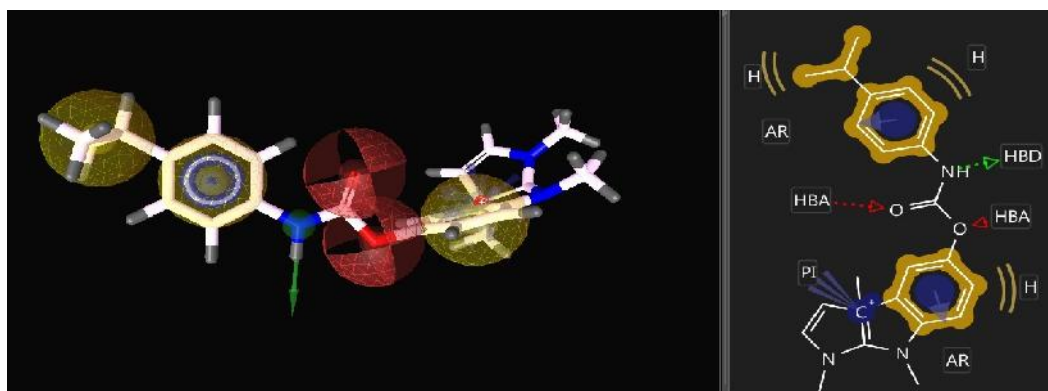
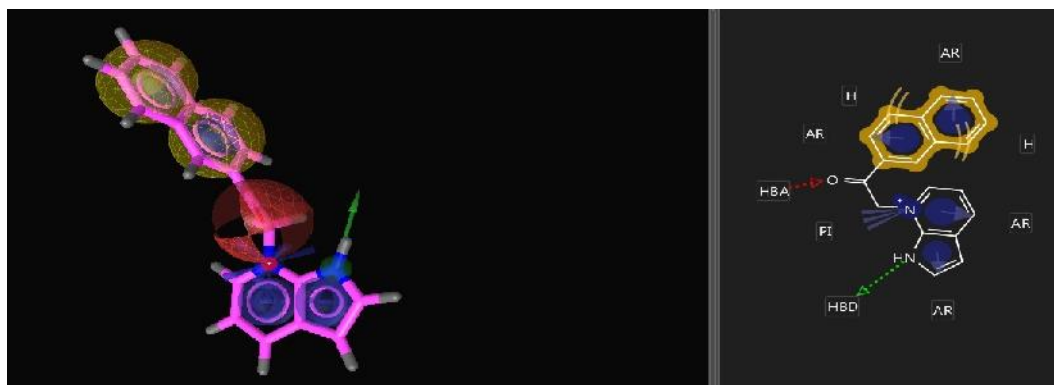


Fig. 5: Showing 3D Picture showing Shared features in Physostigmine, compound 4 and compound 5.

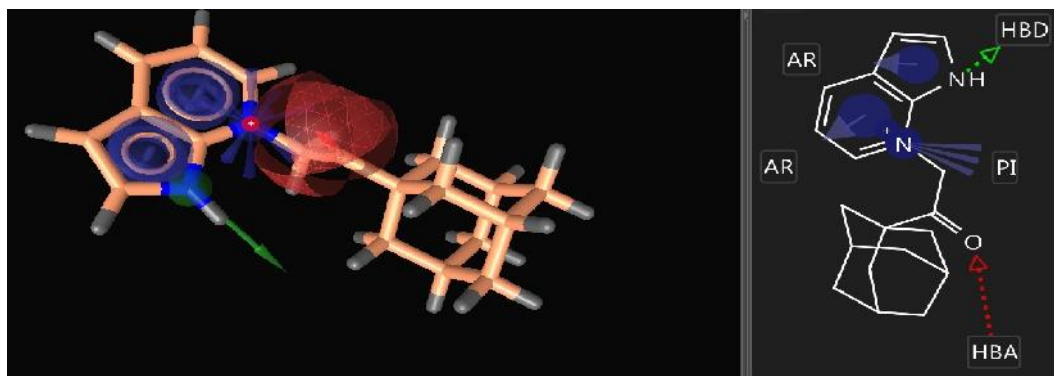
Red = HBA :Hydrogen Bond Acceptor, Green = HBD: Hydrogen Bond Donor, Blue =AR :Aromatic Ring, Yellow=H :Hydrophobic region



(a)



(b)



(c)

Fig.6: Showing 2D and 3D pictures Pharmacophoric region (a) Cymserine (b) compound 2 (c) compound 3.

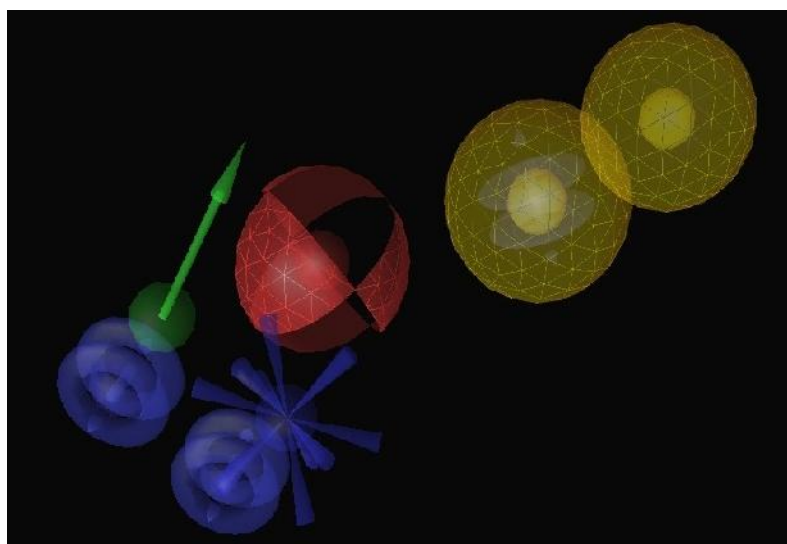


Fig.7 3D Picture showing Shared features in cymserine, compound 2 and compound 3.

Red = HBA: Hydrogen Bond Acceptor, Green = HBD: Hydrogen Bond Donor, Blue
=AR: Aromatic Ring, Yellow = H: Hydrophobic region

Though all the compounds displaying good activity but highly significant inhibiting activity of compound **4** and **5** against AChE can be justified by the fact that pharmacophoric regions in the physostigmine and derivatives **4** and **5** displayed in fig. **4** showing the presence of similar areas required for binding with cholinestase enzyme. Common structural features essential to bind the receptor's active sites and are responsible for highly encouraging results are, charged nitrogen atom in Indole nucleus to interact with anionic site; while the carbonyl portion connecting indole and aryl moiety of the derivatives is satisfying the estratic portion and the terminal aryl part along with hydrogen bond acceptor of the analog is there to fit in the possible extra active side hydrophobic site. More over 1-chloro-3-phenoxy-3-propanone has been reported as potent reversible acetylcholine inhibitor [54] is closely resembles to phenacyl portion of the derivative. Therefore acetophenone derivatives of 7-azaindole exhibit good inhibitory activity for acetylcholinesterase inhibitor.

Fig .**5** displaying shared pharmacophoric regions of physostigmine and compound **4** and **5** which are important for binding with receptor. Excellent match of these regions can be taken as a good explanation of pronounced effect.

Phenacyl derivatives exhibiting same and better activity as compared to adamantyl and naphthyl derivatives. The major difference in the terminal adamantyl and naphthyl groups showing that for AChE inhibition strong hydrophobic interaction is at the terminal position is not mandatory. Significant activity of synthesized compounds to bind and inhibit BChE enzyme can be explained by comparing the structures with Cymserine. Cymserine is the known molecule as butyrylcholinesterase inhibitor and selectively bind with this enzyme. Physostigmine and cymserine are structurally same with the difference of prominent terminal lipophyllic region in cymserine which may be responsible for its selective binding with butyrylcholine esterase enzymes [55].

Regarding the BChE inhibiting activity, naphthyl and adamantyl derivatives (compound **2**, **3**) proved potent inhibitors (IC_{50} Values $1.25 \mu M$ and $3.93 \mu M$ respectively) as compared to

nitrophenacyl derivative (compound **4** and **5**) with IC_{50} values $9.185 \mu M$ and $10.20 \mu M$ respectively. The prominent effect may be the result of the presence of naphthyl and adamantyl moieties providing strong lipophilic area as compared to phenacyl ring. These results may be justified by comparing the cymserine structure with synthesized analogues which are fulfilling the requirement of terminal strong lipophilic region which may be involved in selective binding with BChE.

Fig. **6** representing the same binding regions in cymserine and derivative **2** and **3**. These 3 structures were checked to show the shared pharmacophoric regions showing excellent match of the active areas present and which may be involved in good binding with BChE enzymes. Fig. **7** showing shared pharmacophoric regions of Cymserine and compound **2** and **3** which are imperative for binding with receptor. Admirable match of these areas can be taken as a good elucidation of marked effect.

Compounds **1–9** [56] exhibited a varying degree of antiglycation activity ranging between 120.6 and $984.9 \mu M$, and IC_{50} values compared with standard rutin ($IC_{50} = 294.46 \pm 1.50 \mu M$) (Table **1**). Compounds **5** ($IC_{50} = 120.6 \pm 0.2 \mu M$), showed an excellent antiglycation activity better than the standard (rutin, $IC_{50} = 294.50 \pm 1.5 \mu M$). Compound **8** ($IC_{50} = 476.56 \pm 9.7 \mu M$) showed antiglycation activity close to standard (rutin, $IC_{50} = 294.46 \pm 1.50 \mu M$). Compounds **6** ($IC_{50} = 524.93 \pm 1.19 \mu M$), **7** ($IC_{50} = 799.27 \pm 6.7 \mu M$) and **9** ($IC_{50} = 9984.98 \pm 3.5 \mu M$) exhibited good antiglycation potential. Compounds **2** (51.19 % inhibition), **3** (47.58 % inhibition), and **4** (9.2% inhibition) exhibited moderate antiglycation potential[58]. Nevertheless, compounds **2–4** showed less than 50% inhibition, therefore they were not further evaluated for their IC_{50} values. Compound **5** is the most active analog of the series with IC_{50} value of $120.6 \pm 0.2 \mu M$.

Declaration of interest

The authors report no conflicts of interest.

Conclusion

Over all result are significant and encouraging and because of their acetylcholinesterase and butyrylcholinestrases inhibition potential they can be selected as a useful lead molecules for

designing effective therapeutic agent to treat Alzheimer disease. Structure activity relationship studies with the standard molecules provided justification for their activity and given better understanding of their inhibiting potential depending upon their pharmacophoric regions.

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