

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, 0.96 μ M and 0.97 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} = 8.51 $\pm$ 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 effect of substituents as well as the position of substituent on phenyl effects the activity.

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

Abbreviations: ACh, acetylcholine; AChE, Acetylcholinesterase; AD, Alzheimer's disease; BChE, butyrylcholinesterase; AGE, Advanced glycation end products; HMQC, Heteronuclear Multiple Quantum Connectivity; TLC, Thin Layer Chromatography; PDB, Protein Data Bank; VDCs, voltage dependent Ca²⁺ channels

Introduction:

7-Azaindoles are the most widely studied nitrogen analogue of the Indole ring system [1]. The 7-azaindole nucleus has proved to be an important model for working on its own synthesis and preparation of its different analogues [12-5]. The pharmacology of 7-azaindole derivatives may lead to the possibility that they may serve as parent nucleus of active or antagonistic analogues of naturally occurring Indole derivatives such as endogenously important serotonin, tryptophan and melatonin [6]. The chemistry and the biological applications of 7-azaindoles have been widely investigated, over last several years [7-8, 9]. In the past, Indole derivatives had attracted the interest of scientists for their potential biological activities like in the treatment of fungal infections of the skin, 7-azaindole were used as inhibitors of p38 MAP kinase [10-13]. 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 [14]. Azaindoles useful as inhibitors of JAK and other protein kinases [15]. New NADH models in the pyrrolo [2,3-b] pyridines series and 7-azaindole derivatives with high affinity for the dopamine D₄ receptor have been synthesized. The syntheses of 7-azaindole analogues of natural products such as melatonin, ellipticin and tryptophan have been described [16]. Other Indole derivatives were prepared as cyclooxygenase inhibitors and were found useful as anti-inflammatory agents [17,18]. 7-Azaindole is an effective drug against nervous central system and shown affinity for serotonin receptor [19-21]. These azaindole structures were expected to enhance the cytotoxicity towards tumor cell lines through stronger hydrogen bonding with the target enzymes. Synthesis of 7-azaindole derivatives of sulfonamide was reported by Saify *et. al.* [22] and these compounds also exhibit antinociceptive activity.

Saify *et. al.* synthesized a series of 7-azaindole derivatives having important antimicrobial response against gram positive and gram negative bacteria. We had previously reported a number of novel aza indole compounds [22-23,24,25,26]. Azaindole derivatives are closely related to Physostigmine and could be compared for their use in the treatment of AD. Physostigmine was one of the clinically tested AChE inhibitors and might be helpful for enhancing memory in patient of AD [27]. There was no data available that 7-Azaindole derivatives can be used as cholinesterase inhibitors. So we decided to do its cholinesterase activity. By doing so we get very promising results.

Although 7-Azaindole was synthesized but the useful biological activities was unexplored for many years. As a result of our continued interest in 1H pyrrolo (2,3-b) pyridine as potential bioactive agents [24-26]. We now wish to report the investigation for AChE/BChE and glycation inhibition.

Alzheimer's disease is age related neurodegenerative disease connected with memory failure and cognitive mutilations. The cholinergic theory of AD proposes that the selective loss of cholinergic neurons in AD results in a deficit of acetylcholine (ACh) in specific regions of the brain that mediate learning and memory functions. The primary approach for treating AD is, therefore, aimed at rising the ACh levels in the brain by using AChE 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 β) formation, [28] and tau hyper phosphorylation calcium entry [29] through the L-sub type of

high voltage, activated calcium channels causing both calcium in excess and mitochondrial disruption. This activated the apoptotic cascade and cell death [30, 31, 32]. Hence, blocking the entrance of calcium through calcium channels could be a good strategy to prevent cell death. In U.S.A alone, AD related expenses surpassed \$100 billion every year [33]. Recently AD affected more than 35 million people throughout the world [34].

In AD, information transfer at the synapses began to fail and eventually the cells died. In a brain with advanced AD, there is dramatic shrinkage from cell loss and widespread debris from dead and dying neurons. AD could affect different people in different ways, but the most common indication pattern begins with gradually worsening difficulty in identification of fresh information. This was because disruption of brain cells usually begins in regions involved in forming new memories.

AChE and BChE (BChE, EC 3.1.1.8) are cholinesterases; with divergent properties dependent on the species, tissue and source. AChE catalyzed the rapid hydrolysis of the neurotransmitter ACh in the cholinergic synapses [35]. 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 AD and myasthenia gravis [36]. BChE was present in significantly higher quantities in Alzheimer's plaques than in plaques of normal age-related non-demented brains [37]. 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 [38, 39].

The recently solved crystal structure of the BChE enzyme [40,41] had 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 became relatively larger and capable of accommodating bulkier and relatively nonpolar ligands. Since the discovery of Physostigmine [42] as the first natural AChE inhibitor, only few other natural inhibitors had attracted the attention of neuropharmacologists [43].

The development of novel antiglycation agents represents an interesting strategy for management of late diabetic complications. Currently, the number of effective antiglycating agents were very few, and the need of new antiglycating agents was still unmet. As the unpleasant incident of type-2 diabetes is increasing, its injurious effects were mostly attributed to the formation of sugar derived substances called advanced glycation end products (AGE), [44] which are pathogenic mediators of almost all diabetic complications [45]. Effort had been focussed on the discovery of new inhibitors of glycation, because of their therapeutic potential [46]. Certain molecules were developed that could cleave AGE cross links and perhaps open the possibility of reversing the steady process of diabetic complications [47]. It was found that aged garlic extract inhibited the formation of AGE *in vitro*. S-allylcysteine was a very important component of aged garlic extract that acted as a potent antioxidant and thus inhibited the AGE formation [48,49]. It has demonstrated that polyamines, spermidine and spermine have potent antiglycation effects, comparable to those of aminoguanidine and carnosine [50]. Efforts were made to develop new and safe synthetic antiglycation agents [51]. It had demonstrated that polyamines, spermidine and spermine have potent antiglycation effects, comparable to those of aminoguanidine and carnosine [52].

In the present study, *in vitro* antiglycation activity of nine 7-Azaindole derivatives 2–9 were evaluated. We reported in this paper the synthesis, AChE, and 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 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 365nm 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

7-azaindole (0.01 mol) and substituted phenacyl halide (0.01 mol) were dissolved separately in acetone (20 mL) and then mixed together in a round-bottomed flask.

Reaction mixture was stirred for 24 hours at 52-54°C. The process of reaction was monitored through. 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 desicators over anhydrous calcium sulphate. Melting point and spectral data were obtained to confirm the structure of compound (Scheme 1).

Enzyme Inhibition Assays

Procedure AChE/BChE Inhibition:

AChE and BChE inhibitory activities were measured *in vitro* by a modified spectrophotometric method developed by Ellman et al. [53]. The assay procedure was previously described [54]. All the inhibition studies were performed in triplicate 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 (1 mM final concentration of each). 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 [53].

According to Ellman et al., the rate of the enzymatic reaction was calculated by the following equation.

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

Assay for AChE and BChE Inhibition

140 μ l of sodium phosphate buffer (pH 8.0), 10 μ l of DTNB, 20 μ l of 0.2mM test compound solution and 20 μ l AChE or BChE solution were assorted and incubated for 15 min at 25°C. The reaction was then initiated by the addition of 10 μ l acetylthiocholine Iodide or butyrylthiocholine chloride, in that order. The hydrolysis of these substrates were examined spectrophotometrically by the development of yellow 5-thio-2-nitrobenzoate anion as the product of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine Iodide or butyrylthiocholine chloride, correspondingly, at a wavelength of 412 nm. Test compounds and control were dissolved in ethanol (0.2mM). All the reactions were performed in triplicate.

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 (NaN₃), dimethyl sulfoxide (DMSO), sodium dihydrogen phosphate (NaH₂PO₄), sodium chloride (NaCl), disodium hydrogen phosphate (Na₂HPO₄), potassium chloride (KCl), potassium dihydrogen phosphate (KH₂PO₄), and sodium hydroxide (NaOH) were purchased from Sigma–Aldrich. Sodium phosphate buffer (pH 7.4), was prepared by mixing Na₂HPO₄ and NaH₂PO₄ (67 mM) containing sodium azide (3mM). Phosphate buffer saline (PBS) was prepared by mixing NaCl (137mM), Na₂HPO₄ (8.1mM), KCl (2.68mM), and KH₂PO₄ (1.47mM) and pH 10 was adjusted with NaOH (0.25mM). BSA (10 mg/ml) and glucoses anhydrous (50mg/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.*, [55. 56] with slight modifications.

Triplicate samples of BSA 10mg/ml, 14mM MGO, 0.1M phosphate buffer (pH 7.4) containing NaN₃ (30mM) were incubated under aseptic conditions (in such a way that each well of 96-well plate contained 50μL BSA solution, 50μL MGO, and 20μL 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, 330nm; emission, 440nm), against sample blank on a microtitre plate spectrophotometer (Spectra Max, Molecular Devices). Rutin was used as a positive control (IC₅₀ = 294μM ± 1.50 SEM).

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

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

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

Naphthalen-2-yl-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide(2):

Yield = 86.2%, HREI MS: m/z = C₁₉H₁₅N₂OBr, EIMS = m/z (relative intensity; %) = 367.23 amu, M. P. = 267 ± 2°C, UV λ_{max} (MeOH) nm = 340, 293, 250, 224, 203, IR ν_{max} (KBr) cm⁻¹ = 3755, 2923, 1687, 1361, 987, 823.5, 783, 545, 474.5; EIMS = m/z (relative int, %): 286 (M⁺-HBr C₁₉H₁₄N₂O, 7.4), 259(8.2), 258(62), 257(52), 155(15), 81(18), 77(22), ¹H-NMR (DMSO, D₆, 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, 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'), 6.62(2H, d, J =3.50, H-2', 3'), (2H, s, H-2'').

7-(2-Adamantan-1-yl-2-oxo-ethyl)-1H-pyrrolo[2,3-b]pyridinium bromide(3):

yield = 81.5 %, HREI MS: m/z = $C_{19}H_{23}N_2OBr$, EIMS: m/z = 375.30 amu, M. P. = $287 + 2^{\circ}C$,
UV λ_{max} (MeOH) nm = 299.6, 226.0, IR ν_{max} (KBr) cm^{-1} = 3409, 2918, 2850, 2773, 1712,
1620, 1458, 1357, 1164, 1097, 887, 779, 727, 661, 536, 476, EIMS = m/z (relative intensity;
%), 295 (M^+ , $C_{19}H_{23}N_2O$, 7.3), 294 (35), 237(2.6), 176(3), 159(15.5), 131(100), 93(18.4),
79(27), ^1H -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-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-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 (6H, m, H-3', 6', 6', 7', 9', 10').

7-[2-(4-Nitrophenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide (4):

Yield = 61%, HREI MS: m/z $C_{15}H_{12}N_3O_3Br$, EI MS: m/z = 362.18 amu, Melting Point = $252 + 2^{\circ}C$, UV λ_{max} (MeOH) nm: 270, 248, 201. IR ν_{max} (KBr) cm^{-1} : 3390, 2920, 1690, 1600 and
1340, EIMS, m/z (relative int., %): 282 ($M^+ - Br$, $C_{15}H_{12}N_3O_3$, 8), 164(4), 160(12), 132 (11),
122(18), 118(100) and 77(23), ^1H -NMR (MeOD, 300 MHz) δ : 8.81 (1H, d, J = 7.84, 1.08 Hz,
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,
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 =
3.51 Hz, H-3) and 6.02 (2H, s, H-2'').

7-[2-(3-Nitrophenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide (5):

Yield = 80%, HREI MS: m/z = $C_{15}H_{12}N_3O_3Br$, EI MS: m/z = 362.178 amu, Melting Point =
 $224 + 2^{\circ}C$, 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, 3.35 Hz, 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).

7-Phenethyl-1H-pyrrolo[2,3-b]pyridin-7-ium; bromide (9): UV (MeOH) nm: 390.0, 293.0, 224.0, 203.4, 198.6; IR ν_{max} (KBr) cm^{-1} 51.5, 1647.6, 1495.0, 1357.4, 1307.8 and 1189.1; EIMS (relative int, %): 223 (M^+-Br , $C_{15}H_{14}N_2$ (10), 132(3), 118(100), 104(8), 91(7), 77(6); ^1H -NMR (MeOD, 300 MHz): δ 8.63-8.61(1H, d, $J = 7.76$ Hz, H-6), 8.20-8.18 (1H, d, $J = 6.17$

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 = 6.33 Hz, H-1, H-2), 7.02-6.99 (2H, m, H-2, H-3), 6.93-6.92 (1H, d, J = 3.51 Hz, H-1), 5.02-4.97 (1H, t, J = 6.92 Hz, H-1"), 4.57 (1H, s, H-2").

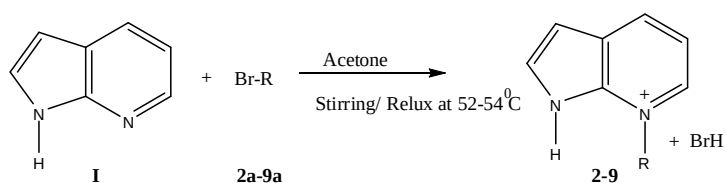
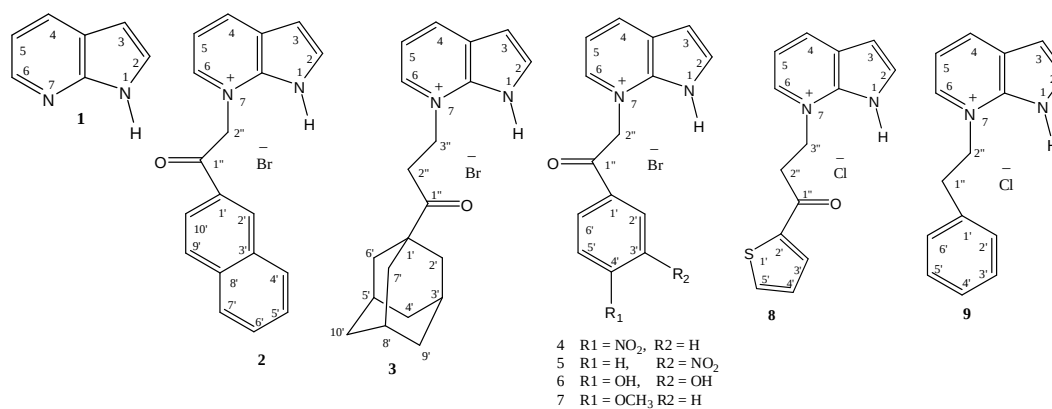
Results and discussion

Chemistry

7-azaindole (0.01 mol) and substituted phenacyl halide (0.01 mol) were dissolved separately in acetone (20 mL) and then mixed together in a round-bottomed flask (Scheme-1). Reflux reaction mixtures for about 24 h. The reaction completion was monitored by TLC. After completion of reaction, the precipitates were filtered, and washed with warm acetone. The obtained products were re-crystallized from methanol. The pure compounds were dried in vacuum over anhydrous calcium sulfate. The structures of synthetic derivatives were elucidated by different spectroscopic techniques including, UV, IR, EI-MS and ^1H -NMR. All compounds gave satisfactory elemental analysis. The compounds herein described were synthesised as a part of study complementary to AChE, BChE and antiglycation inhibition investigation of fused pyrrolo pyridine system. All the 7-azaindole were prepared under the conditions recorded in experimental section [22-26].

The ^1H -NMR spectra were specially helpful in deciding the substitution pattern at the different positions of phenacyl part of the molecule. The molecular ion peaks of the compounds were obtained from EIMS spectra [25-26]. The spectral data of compounds **4**, **5** was reported previously [57,58, 9, 59]. Two-dimensional NMR techniques such as HMQC was used to obtain more structural information [60]. The one-bond $^{13}\text{C}/^1\text{H}$ couplings were determined by the HMQC spectrum (experimental part). The C-2 to C-6 and C-1' to C-6' protons of compounds **1-9** resonating at different chemical shifts showed cross-peaks with their respective carbons in the HMQC spectrum (experimental part).

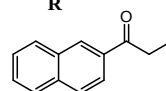
A series of 7-azaindole derivatives were prepared by quaternization with phenethyl bromide and substituted phenacyl halides [61,62,63], the side chain thus possessing certain features common to those present in sympathomimetic amines and related compounds (Scheme 1).



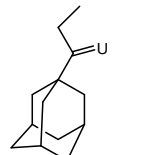
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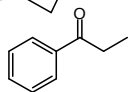
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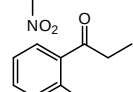
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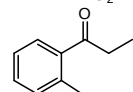
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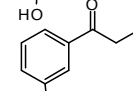
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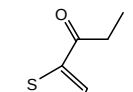
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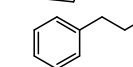
7a



8a



9a



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

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

Compound no.	Antiglycation % inhibition and 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 ± 0.02
3	47.58 % inhibition	11.60 ± 0.17	3.93 ± 0.36
4	9.21% inhibition	0.96 ± 0.01	9.18 ± 0.25
5	78.21 % inhibition. 120.61 ± 0.21	0.98 ± 0.05	10.20 ± 0.28
6	64.26% inhibition, 524.93 ± 1.19	-	-
7	58.89% inhibition, 799.27 ± 6.71	-	-
8	67.95% inhibition, 476.56 ± 9.71	-	-
9	50.52% inhibition, 984.98 ± 3.50	-	-
Standard Galanthamine	-	0.5 ± 0.001	8.51 ± 0.02
Rutin	86.0 % inhibition, 294.50 ± 1.50	-	-

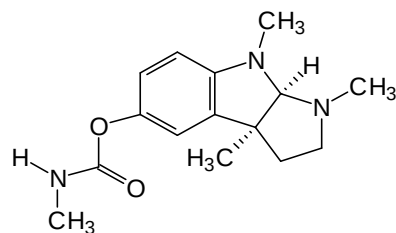
The 7-azaindole, **2-9** were screened for their inhibition potential on BChE, AChE, and glycation enzymes. These compounds were found to be specific concentration dependent inhibitors of glycation, AChE and BChE (Table I). Since none of the known AChE inhibitors is structurally similar to the compounds under study. Galanthamine was randomly selected as standard to compare the potency of the test compounds against both enzymes AChE and BChE. Galanthamine is a natural and reversible AChE and BChE inhibitor, which has a wide range of clinical applications.

The parent compound (**1**) investigated for AChE and BChE activity showed insignificant activity. Compounds **2-5** showed significant activity. Compounds **3** and **2** exhibited potent results as compared to standard Galanthamine for BChE inhibition. In the bio-screening of the compounds for AChE inhibition [64], compounds **4** (nitro-phenacyl, para-nitro derivative) and **5** nitro-phenacyl (meta-nitro derivative) were found most active with almost same IC_{50} values $0.96\mu M$ and $0.975\mu M$ respectively. The result indicated that NO_2 substitution in phenacyl ring at different position (meta for compound **4** and para for compound **5**) was not making any significant change in the activity. Naphthyl 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, BChE and antiglycation activity were represented in Table 1.

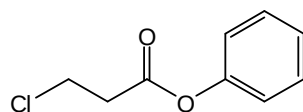
Compound **2** was found to be the most potent inhibitor of BChE, while compounds **4** and **5** were equally potent against AChE. On the other hand, compounds **5** and **4** were found to be less selective toward the BChE, while compound **4** was found to be a selective inhibitor of AChE (Table I.) It was mentioned earlier that several amino acids of the AChE gorge has been replaced in BChE by hydrophobic and/or smaller amino acids residues to enable BChE to accommodate bulkier ligands. The selectivity of compounds **2** and **3** toward BChE could be, therefore, explained in part by their ability to easily go through the relatively larger aromatic gorge of BChE [65]. Hydrophobic interactions, conversely, which are more prominent in the case of BChE, might play an important role in the selectivity of these compounds toward the enzyme.

In the investigation for BChE inhibiting activity opposite results were observed as compared to AChE. Compound **2** containing naphthyl derivative displayed maximum inhibition (IC_{50} $1.25\mu M$), while the compound **3** (adamantyl derivative) with IC_{50} value $3.93\mu M$ was the second potent inhibitor of BChE. Compounds **4** and **5** (nitrophenacyl derivatives) showed almost same level of inhibition with IC_{50} $9.18\mu M$ and $10.20\mu M$ respectively. Approximately similar inhibition of BChE and AChE by compounds **4** and **5** suggested that though the presence of NO_2 functional group imparted inhibitory activity but the position of NO_2 in the phenacyl ring was not important for any significant difference in the activity.

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



Physostigmine



1-Chloro-3-phenoxy-3-propanone

Physostigmine was one of the clinically tested AChE inhibitor and might be helpful for enhancing memory in patient of AD [27]. Azaindole derivatives are closely related to Physostigmine and could be compared for their use in the treatment of AD. showed active binding site regions [67] on the enzyme surface for ACh, explaining possible three major binding areas like ionic, esteratic and possible hydrophobic region Fig. 1. Physostigmine (known potent ligand) and synthesized compounds showed the presence of same common essential features necessary for binding (Figs. 2 and 3).

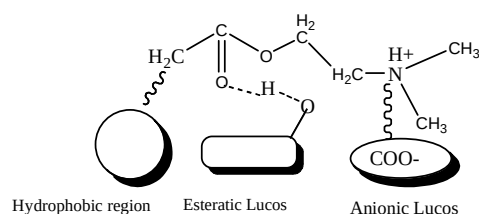


Fig.1: Acetylcholine with active binding site

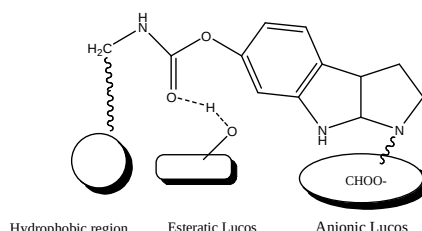


Fig. 2: Physostigmine with active binding site

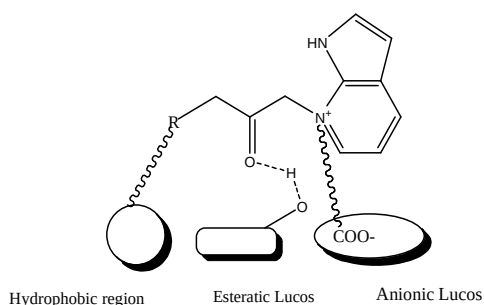
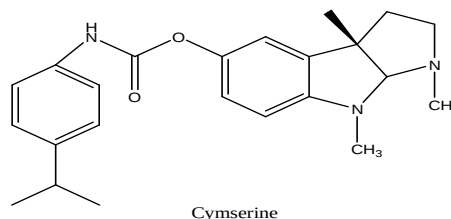
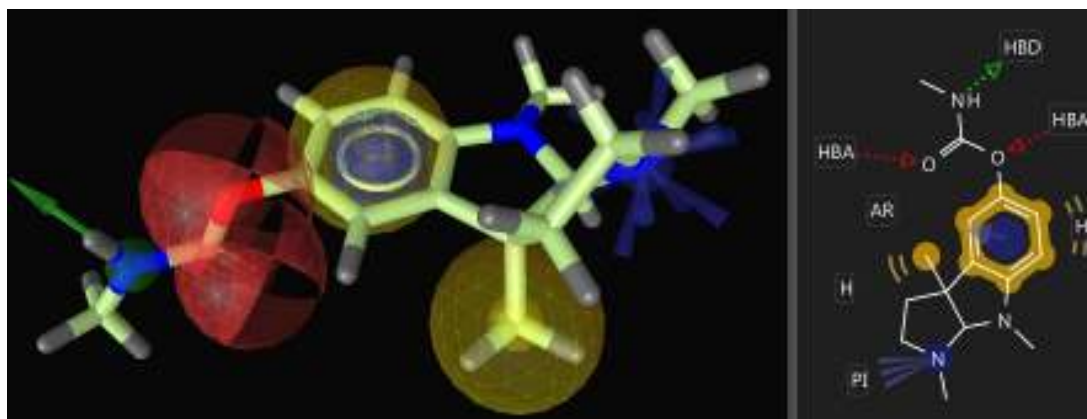


Fig. 3: Synthesized derivative of 7-azaindole

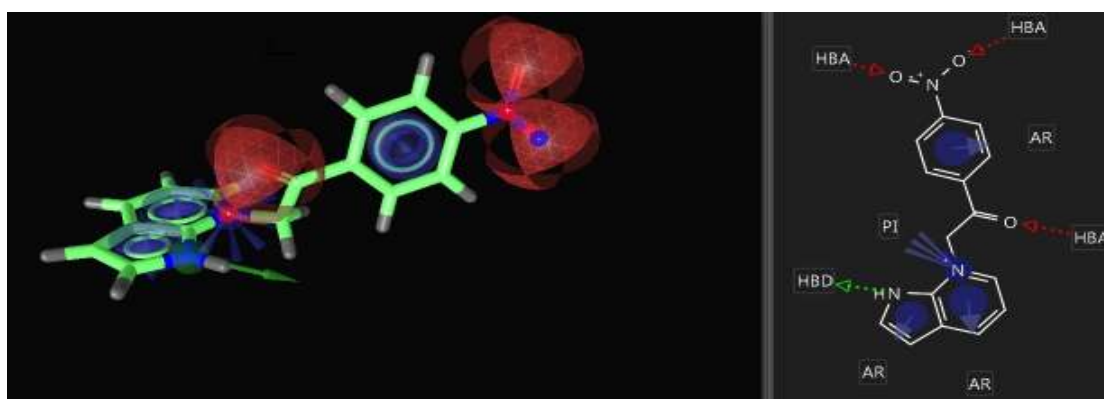
R = phenacyl, naphthyl and adamantyl



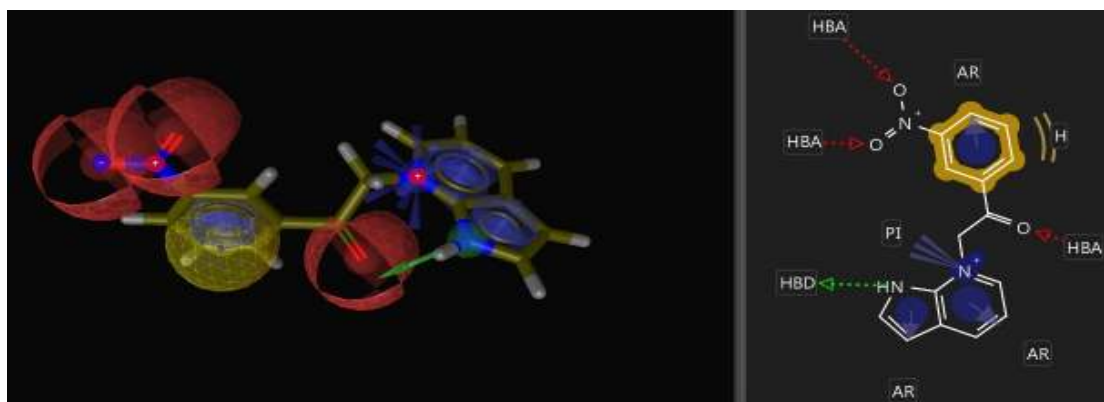
Cymserine



(a)



(b)



(c)

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

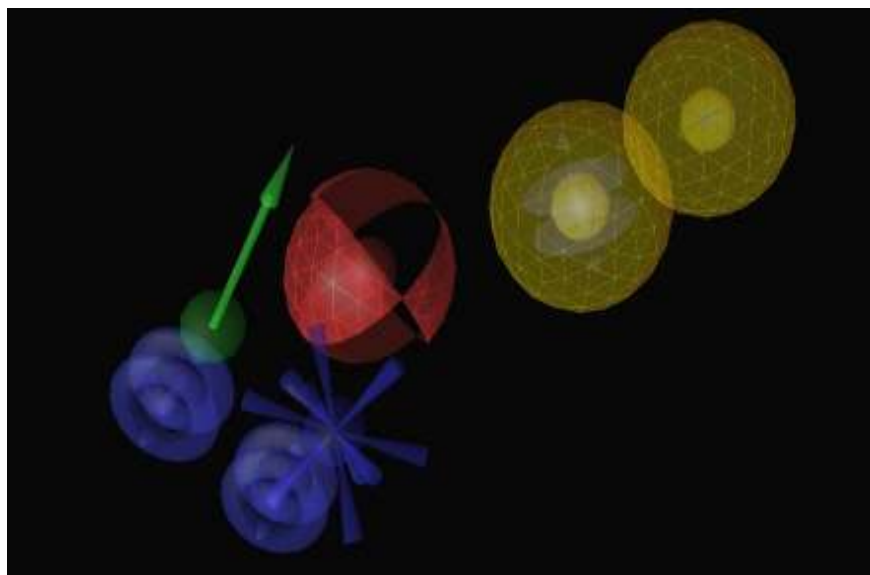
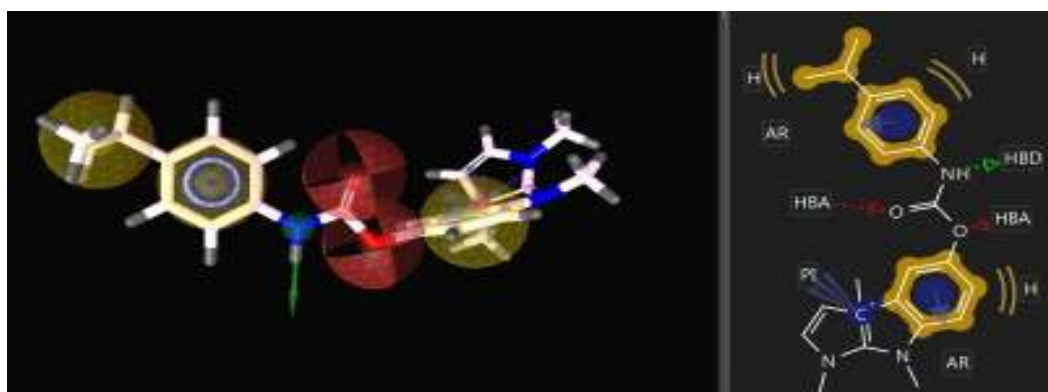


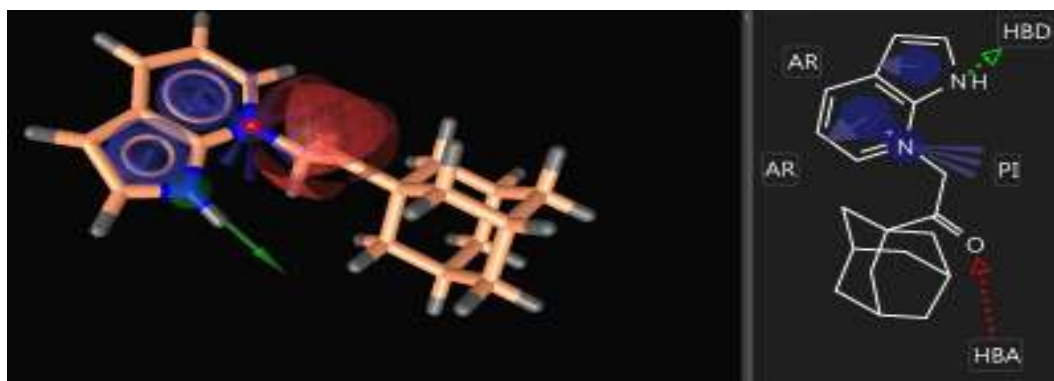
Fig. 5: 3D picture showing shared features in **Physostigmine**, compound **4** and compound **5**.
 Red = HBA: Hydrogen Bond Acceptor, Green = HBD: Hydrogen Bond Donar, Blue = AR: Aromatic Ring,
 Yellow = H: Hydrophobic region



(a)



(b)



(c)

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

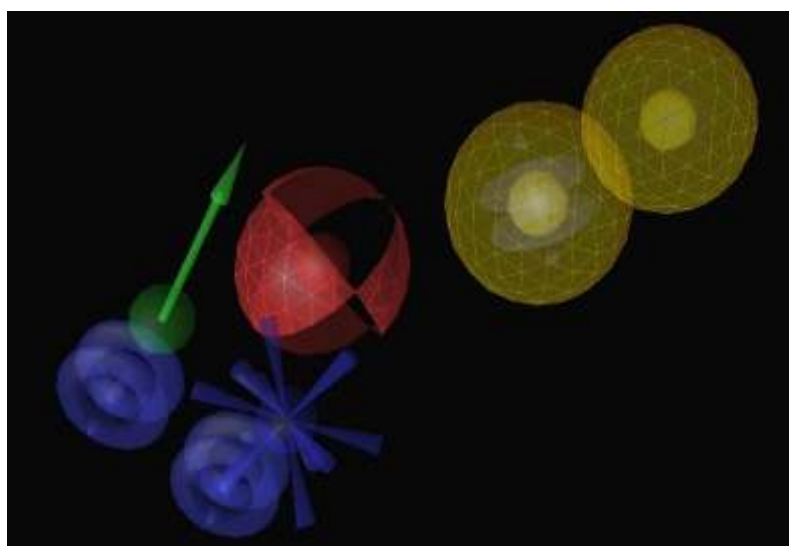


Fig.7: 3D Picture showing shared features in Cymserine, compound 2 and compound 3.

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, showed the presence of similar areas required for binding with cholinesterase 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 ACh inhibitor [68] is closely resembles to phenacyl portion of the derivative. Therefore acetophenone derivatives of 7-azaindole exhibit good inhibitory activity for AChE inhibitor. Fig .5 displayed pharmacophoric shared regions of Physostigmine and compounds 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 BChE inhibitor and selectively bind with this enzyme. Physostigmine and cymserine are structurally same with the difference of prominent terminal lipophilic region in cymserine which may be responsible for its selective binding with butyrylcholine esterase enzymes [69].

Regarding the BChE inhibiting activity, naphthyl and adamantyl derivatives (compounds 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. All compounds were found to be specific concentration- dependent inhibitors of AChE and BChE (Table I) and were found active at a concentration of $0.2mM$ against enzymes. Fig. 6 represented the same binding regions in Cymserine and derivative 2 and 3. These three 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. Figs. 7, 8 showed shared pharmacophoric regions of Cymserine and compounds 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.

Due to wide range biological importance of 7-azaindole [66], here we reported the potential of 7-azaindole against glycation [70]. 7-Azaindole derivatives 1–9 were also screened for the inhibition of glycation enzymes according to literature protocol. Compounds 1–9 [71] exhibited a varying degree of antiglycation activity ranging between 120.6 and 984.9 μ M, and IC_{50} values compared with standard rutin ($IC_{50} = 294.46 \pm 1.50 \mu M$) (Table 1). Out of nine compounds, one compound showed potent antiglycation activity than the standard rutin, while the remaining compounds showed good glycation inhibition.

Compound 5 ($IC_{50} = 120.6 \pm 0.21 \mu M$), showed an excellent antiglycation activity potent than the standard rutin ($IC_{50} = 294.50 \pm 1.50 \mu M$). Compound 8 ($IC_{50} = 476.56 \pm 9.71 \mu M$) 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 ($IC_{50} = 799.27 \pm 6.71 \mu M$) and 9 ($IC_{50} = 9984.98 \pm 3.50 \mu M$) exhibited good antiglycation potential. Compounds 2, 3 and 4 inhibited the reaction 51.19%, 47.58 % and 9.2% respectively, so exhibited moderate antiglycation potential [72]. 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 $120.6 \pm 0.2 \mu M$.

Limited SAR studies of the synthetic compounds indicated that the activity is markedly dependent on the inductive and mesomeric effects of various substituents presents on the phenyl ring of the phenacyl moiety (Table-1). Compound 5 ($120.61 \pm 0.21 \mu M$) having *para* nitro substituted phenyl ring showed potent glycation inhibitory potential than the standard rutin ($IC_{50} = 294.46 \pm 1.50 \mu M$), however, compound 4 having *meta* nitro substituted phenyl showed 19.21% inhibition. The difference in activities might be due to change in position of substituent. The compound 7 having *para* methoxy substituted phenyl ($IC_{50} = 799.27 \pm 6.71 \mu M$), 6 having *meta*, *para* dihydroxy substituted phenyl ($IC_{50} = 524.93 \pm 1.19 \mu M$), and 8 having sulfur containing five member ring ($IC_{50} = 476.56 \mu M$) showed good glycation inhibition. The difference in activity might be due to numbers as well as nature of substituents. The presence of phenyl group at *para* position as in compound 9 ($984.98 \pm 3.50 \mu M$), and the activity is significantly declines the inhibition due to unsubstituted analogue 2 (51.19 % inhibition) and 3 (47.58 % inhibition). This activity difference may be due to substitution effects. The activity decreased due to presence of electron donating group via

inductive like methoxyl or through mesomeric like hydroxyl groups as in compounds **6** ($IC_{50} = 524.93 \pm 1.19\mu M$) and **7** ($799.27 \pm 6.71\mu M$).

Declaration of interest

The authors report no conflicts of interest.

Conclusion

There were nine close derivatives of 7-azaindole and out of these four exhibited useful anti cholinesterase activity i-e Naphthalen-2-yl-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide (**2**) 7-(2-Adamantan-1-yl-2-oxo-ethyl)-1H-pyrrolo[2,3-b]pyridinium bromide(**3**), 7-[2-(4-nitrophenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide (**4**): 7-[2-(3-nitrophenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide (**5**). and two exhibited useful anti glycation activity i-e 7-[2-(3-nitrophenyl)-2-oxo-ethyl]-1H-pyrrolo[2,3-b]pyridinium;bromide (**5**). and 7-(4-Oxo-4-thiophen-2-yl-butyl)-1H-pyrrolo[2,3-b]pyridin-7-ium; chloride (**8**):

Results indicated that compound **5** ($120.61 \pm 0.21\mu M$) having *para* nitro substituted phenyl ring showed potent glycation inhibitory potential, however, compound **4** (19.21% inhibition) having *meta* nitro substituted phenyl. The difference in activities might be due to change in position of substituent. Compound **8** having sulfur containing five member ring ($IC_{50} = 476.56\mu M$). The difference in activity might be due to numbers as well as nature of substituents.

Over all result are significant and encouraging and because of their AChE and BChE inhibition potential they can be selected as a useful lead molecules. All the results of this study indicated the azaindole compounds **2-4** as interesting leads for anti-Alzheimer's drug development being cholinesterase inhibitors, and compounds **5** and **8** can serve as potential lead compound with promise for further research of drug development in the field of glycation enzyme inhibition. The potency of these compounds, especially compounds **2, 3, 4** and **5**

suggests that this is a new class of inhibitors provide a solid base for using them as lead molecules, for designing effective therapeutic agent to treat glycation and AD. Present study opens-up an avenue for further research on 7-azaindole derivatives for their evaluation against acetylcholinesterase, butyrylcholinestrerase and glycation enzymes.

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