RESEARCH SUMMARY

1. Title: Design, Synthesis, Biological Investigations and Molecular Interactions of Triazole linked Tacrine Glycoconjugates as Acetylcholinesterase Inhibitors with Reduced Hepatotoxicity

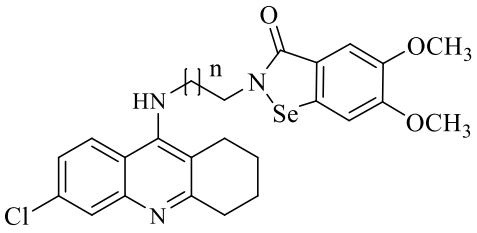
2. **Introduction**

Globally, AD has become a major public health concern and is a leading source of dementia in old age. It is a progressive neurodegenerative menace, characterized by the presence of neurofibrillary tangles formed inside the nerve cells [1], deposition of $A\beta_{1-42}$ between the nerve cells [2], oxidative stress [3], neuro-inflammation [4] and metal ion dysregulation, involved directly or indirectly in neuronal loss [5]. This complication has engulfed millions of geriatric population and to some extent adult population (aged between 55-65 years) worldwide and still seems to follow an upward trend [6, 7]. Almost, all AD victims suffer varying degrees of symptoms (depending upon stage of AD) such as cognitive, behavioral, mood, psychological [8, 9, 10]. Data of research experience and its outcomes has proved Alzheimer's as a diversified condition with high variability in age of onset and clinical decline rate [11]. Till date, there is neither any drug~~s~~ available in market nor any disease modifying therapy has been developed which can fully cure this disease. So, these are of utmost importance to develop the same. Only a limited range of medications (AChE inhibitors such as Donepezil, Galantamine, Rivastigmine, and NMDA receptor antagonist Memantine) are prescribed to Alzheimer's patient, which can provide only symptomatic relief but do not stop the progression of the disease. Presently, only Cholinesterase inhibitors and NMDA receptor antagonist are available in pharmaceutical market.

Tacrine was the first drug introduced in the market for the treatment of Alzheimer's disease, known primarily for its strong inhibition towards AChE. AChE is an enzyme responsible for the hydrolysis of liberated Acetylcholine into choline in ganglion cells. As soon as, Tacrine was launched in the market in 1993, it improved the whole clinical scenario of AD patients. But unfortunately, its prominence did not prevailed for a longer period of time due to its acute hepatotoxicity after regular dosage, reason of which is still unknown. So, it was withdrawn from the market in 2013 [12, 13, 14]. Many postulates have been linked with its acute hepatotoxicity. It has been hypothesized that the liver toxicity effect is linked to the formation of highly reactive

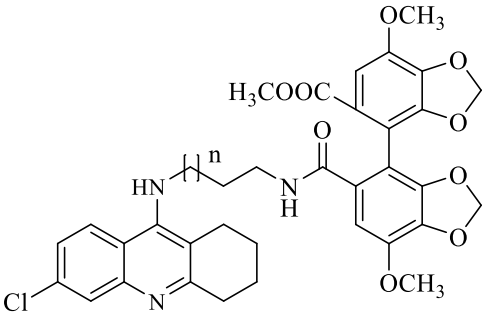
hydroxy metabolites- especially 7-hydroxy Tacrine and its metabolite, quinone methide. These metabolites formation occurs during Tacrine's metabolism by cytochrome P_{450} 1A2 (CYP1A2) [15]. CYP1A2 is a hepatic enzyme responsible for Tacrine metabolism in the liver. It consists of narrow and flat binding pocket, from which its substrate specificity (small planar molecule) could be determined [16]. Hydroxylation in Tacrine scaffold via oxidative metabolism by CYP 1A2 can occur at any one of the carbon atoms (C-1, C-2, C-4 or C-7 positions). These hydroxyl metabolites undergo consecutive rearrangement to form reactive quinine methide and its intermediates. These reactive species form covalent adducts with DNA or cellular proteins of liver cells, or induce different redox cycling, resulting in liver damage and toxic effects [17]. Alternatively, stimulation of reactive oxygen species production and reduction of glutathione may also be linked with its hepatotoxicity [18].

Despite these chronic hepatotoxicity issues, Tacrine gained huge success in Alzheimer's therapy due to its low molecular weight and its higher ability for crossing blood brain barrier. Several attempts have been made by medicinal chemists to overcome the hepatotoxicity of Tacrine by synthesizing its adducts with various pharmacophores (Figure 1) [19, 20, 21, 22]. In most of the strategies, the adducts were formed on amino functionality of Tacrine keeping other positions of pharmacophore intact for its AChE inhibitory activity. However, overall hepatotoxicity data of the above mentioned adducts (Figure 1) are either non-toxic at low concentration or hepatotoxicity still persists at high concentration.



Tacrine-ebselen hybrid

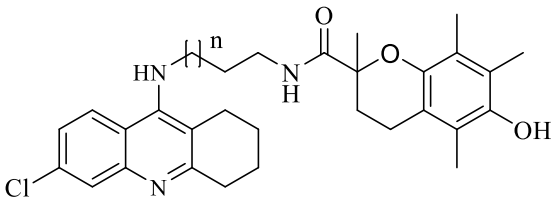
Upto 60% cell viability at 20 μM



n = 2,4,6,8,10

Tacrine-bifendate

90% cell viability at 10 μM



Tacrine-Trolox hybrid

Non-toxic at 2 μM

Figure 1: Examples of Tacrine hybrids with reduced hepatotoxicity.

Glycosides are significantly enjoying their role in the pharmaceutical world due to their sufficient potency [23]. Different types of glycosides, extracted from various plants, have been reported to produce hepato-protective effect in humans [24]. Glycosides rich fraction has proved to be hepato-protective through their strong antioxidant effect in CCl_4 induced liver damage in rats [25]. Triazole glycosides were found to be very effective in natural product modifications and various medicinal properties. Many literature reports have stated its importance as AChE inhibitors in the treatment of AD [26, 27].

Triazole as a linker has many advantages. Tethering of two or more functional moieties through 1,2,3-triazole has received much attention in drug design strategy for its scaffold has been an elegant bio-isostere of amide. Besides this, triazole ring also offers better chemical stability in the physiological environment which helps to upgrade the pharmacokinetic and toxicity properties [28]. Hybridization via click chemistry using this linker provides rapid, clean and potent pathway (Figure 2).

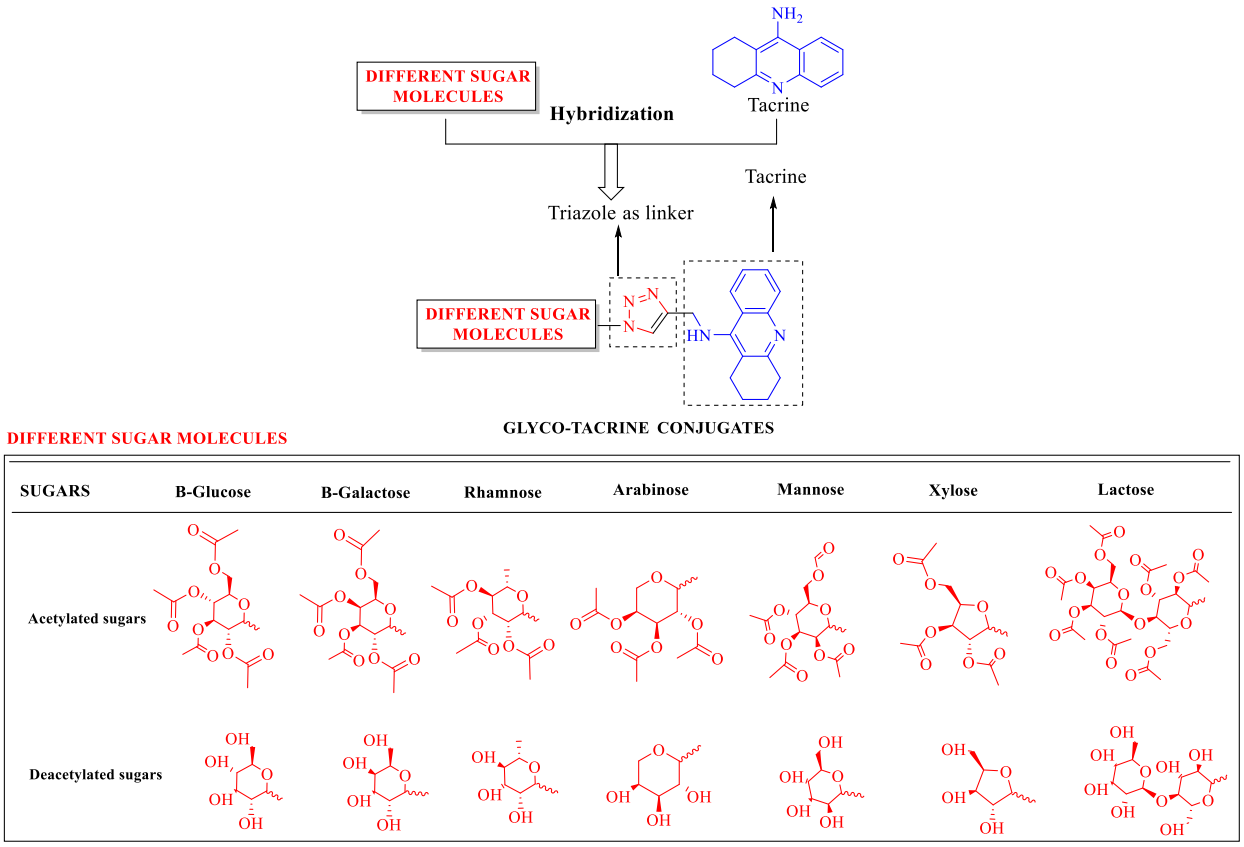


Figure 2: Overview of synthesis via molecular hybridization technique

Hepatotoxicity of the reported potent adducts mentioned in the figure 1 is due to the presence of methyl groups as linker providing flexibility to the whole molecule and ensuring binding at CYP 1A2. Use of docking in predicting toxicity due to drug metabolites is the best alternate to screen a library for potential non-hepatotoxic molecules [29, 30]. To predict the hepatotoxic potential of designed molecules, molecular dynamics were done using crystal structure of CYP 1A2 (PDB: 2HI4) at the metabolic active site. But, surprisingly, none of the designed molecules were able to be fit at the metabolic site of CYP 1A2, except **A-11** and **A-14**. Observations from molecular docking concluded that small and narrow binding pocket of enzyme would expect the small molecule as substrate for its binding. It means, rigidity of carbohydrate scaffold is preventing the orientation of glyco-conjugate towards the entry of the enzyme. Due to this, glyco-conjugate remains outside the pocket of enzyme and further not being able to bind, which automatically hinders the formation of hepatotoxic metabolites. In comparison to Tacrine's affinity for CYP 1A2, addition of rigid molecule at C-9 position of Tacrine had significantly reduced the molecule's affinity for enzyme. Undocked molecules have no affinity for CYP

1A2. Molecular modelling results convey that conformational rigidity of the glyco-conjugate is arresting its binding to the CYP 1A2, thereby minimizing the possibility of forming hepatotoxic metabolites. This conformation rigidity was missing in case of other molecules as mentioned in Figure 1. Overall, carbohydrate addition to Tacrine scaffold is more beneficial in improving hepatotoxicity of Tacrine.

So keeping in view the main disadvantage of Tacrine, the substrate specificity and pocket size of CYP-1A2, remarkable characteristics of carbohydrates, triazoles and Tacrine provide a strong rationale to design a series of non-hepatotoxic Tacrine derivatives, without disturbing much of its AChE inhibition potential (Figure 3).

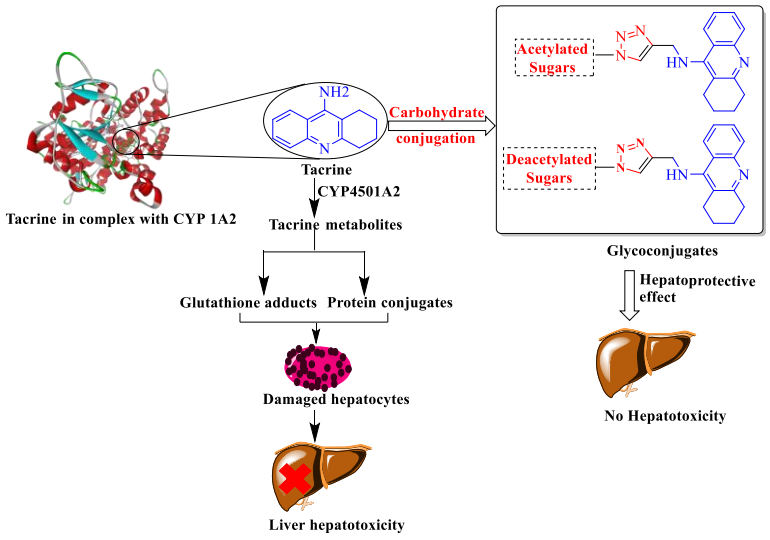


Figure 3: Design Strategy of targeted compounds

Prior to synthesis, dock score analysis was also done by using LeadIT dock tools to find out that whether the designed compounds can better fit to the active site of AChE than Tacrine or not.

Dock scores of designed molecules were evaluated and indicates that almost all of the compounds showed better dock score than Tacrine, suggesting that these compounds could perform better than Tacrine in terms of inhibitory potential. All of the designed conjugates were then synthesized using hybridization technique by implementing copper catalyzed azide-alkyne cycloaddition click reaction. Their inhibitory potential against AChE was evaluated by using Ellman's method [31]. Their derivatives without the acetyl groups on sugar moiety were also

synthesized to check the influence of hydroxy groups on inhibitory potential. In addition to this, enzyme kinetics was also performed, providing insight of the catalytic mechanism of AChE.

Further, hepatotoxic evaluation by MTT assay of the potent compound was also checked to correlate with the molecular docking results [32]. The best hit among the series was docked in the active site of AChE (PDB: 1ODC), and its binding pattern was evaluated. Then, molecular docking simulations of 20ns were carried out to elucidate the binding mode and time dependent stability of **A-1**/AChE complex.

3. Objectives:

I. To predict the hepatotoxic potential of designed molecules using crystal structure of CYP 1A2 (PDB: 2HI4)

II. To predict the Acetylcholinesterase inhibitory potential of designed molecules using LeadIT dock tools

III. Synthesis and characterization of Glycoconjugates

IV. Biological evaluation of all synthetics against Acetylcholinesterase enzyme using Ellman's method

V. Enzyme kinetics of potent molecule, providing insight of the catalytic mechanism of AChE.

VI. Hepatotoxic evaluation by MTT assay of potent compounds

VII. Study of possible binding interactions between the most potent hybrid and the amino acid residues of active sites of AChE

VIII. Molecular docking simulations to elucidate the binding mode and time dependent stability of potent hybrid molecule

4. Materials and methods:

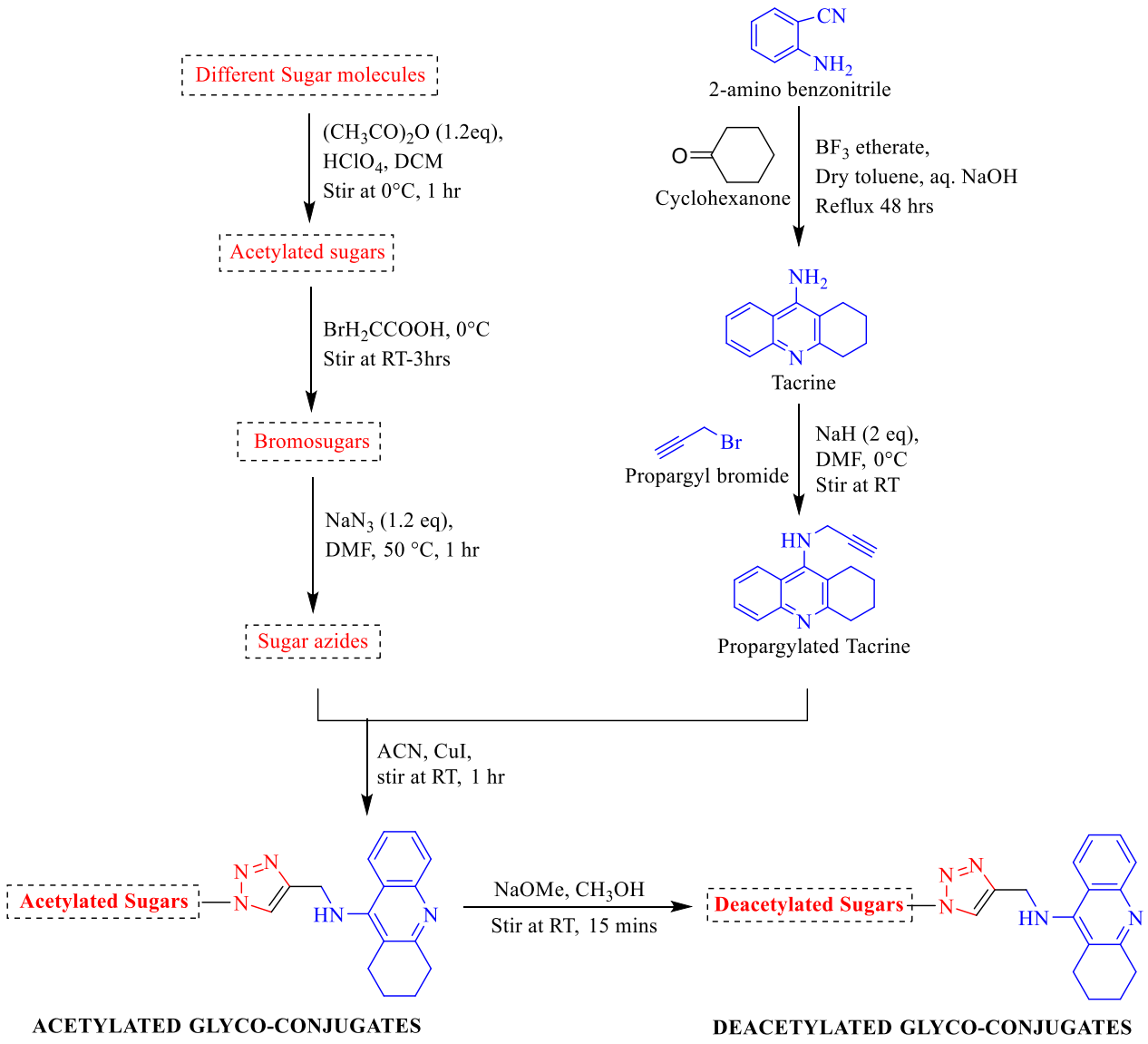
The chemical reagents were procured from CDH, Sigma-Aldrich, and Loba, India. All yields refer to isolated products after purification. Products were characterized by comparing with

authentic samples and by spectroscopic techniques, that is, ^{1}H and ^{13}C NMR, AVANCE III HD 500 MHz Bruker Biospin were used to record the NMR spectra. The spectra were recorded by dissolving in $CDCl_3$, DMSO-d_6 and MeOD relative to tetramethylsilane (TMS) (0.00 ppm). In ^{1}H NMR, chemical shifts were reported in δ values using the internal standard (TMS) with a number of protons, multiplicities (s-singlet, d-doublet, t-triplet, q-quartet, and m-multiplet), and coupling constants (*J*) in hertz (Hz). Acetylcholinesterase (#C2888) from *Electrophorus electricus* (electric eel), acetylthiocholine chloride (#A5626), 5,5′- Dithiobis(2-nitrobenzoic acid) (#D218200) was purchased from Sigma Aldrich. Sodium dihydrogen phosphate monohydrate (# 106346) and di-sodium hydrogen phosphate dihydrate (# 137036) was purchased from Merck.

5. Results and Discussion

5.1 Synthesis

All the designed conjugates were synthesized by following Scheme 1. Different sugars were subjected to acetylation using acetic anhydride to obtain acetylated ones. These were further subjected to bromination and then, azidation. Tacrine was synthesized as per reported method in the literature [33] and further propargylated using propargyl bromide taking sodium hydride as a base. Both the azidated sugars and propargylated Tacrine were further clicked using copper iodide to obtain the targeted hybrid molecules. All the reactions proceeded smoothly with different range of sugars. The structures were elucidated by NMR and mass spectrometry. All spectral data were found in accordance with supposed scaffolds.



Scheme 1: Synthesis of Tacrine-glycoconjugates (acetylated and deacetylated)

5.2 Biological investigations

5.2.1 AChE inhibitory activity:

All the synthesized hybrids were tested against AChE enzyme by using *in vitro* Ellman's method. At first, all samples along with the standard drug, Tacrine were screened at 10 µM concentration. Figure 4 depicts the sample screening at 10 µM concentration by percentage inhibition of the enzyme. At this concentration, Tacrine showed 100 % inhibition of AChE. Almost all of the compounds have shown more than 70 % inhibition amongst which four

compounds (**A-1, A-3, A-4** and **A-7**) were found to exhibit above 90% inhibition against the enzyme at 10 µM.

A structure-activity relationship has been established from figure 4 which revealed that seven different types of sugars (β-Glucose, β-Galactose, Rhamnose, Xylose, Lactose, Arabinose and Mannose) have different impact on AChE inhibitory potential. In case of acetylated conjugates, Xylose and arabinose (both are stereoisomers of each other) showed enzyme inhibition above 90 percent at 10 µM. But, in case of β-Glucose (96.61 % enzyme inhibition) and β-Galactose (79.51 % enzyme inhibition), being epimers of each other, showed contrasting results in the *in-vitro* studies. **A-2** (β-Galactose-Tacrine hybrid) was found to be least potent among acetylated series of glycoconjugates while A-1 and A-4 are the most potent among acetylated series of glycoconjugates. Thus, the overall preference order of acetylated glycoconjugates for anti-AChE potential is Xylose > β-Glucose > (Rhamnose ~ Arabinose) > Mannose > Lactose > β-Galactose. Among the deacetylated series, mannose (**A-12**) takes the lead. Deacetylated series follows the following pattern: Mannose > Rhamnose > Xylose > β-Glucose > Lactose > Arabinose > β-Galactose. All acetylated forms of glyco-conjugates were found to be more potent in contrast to the deacetylated forms of glyco-conjugates. Thereafter, four most potent samples (**A-1, A-3, A-4, A-7**) showing enzyme inhibition above 90% at 10 µM from the series were selected and their IC_{50} values were determined as shown in the figure 5. Nonetheless, we have selected compound **A-1** for further detailed study based on superior anomeric purity (only β). However, the IC_{50} of other compounds was also found to be less than 10 µM. These *in-vitro* results partially resembled with the dock scores (see table S-1 in supplementary file), which were calculated initially, since, all compounds were not as potent as Tacrine, but **A-1** (acetylated β-Glucose tethered with Tacrine) was potent among the synthesized series in the *in-vitro* studies as shown in the dock scores. However, the IC_{50} of other compounds was also found to be less than 10 µM.

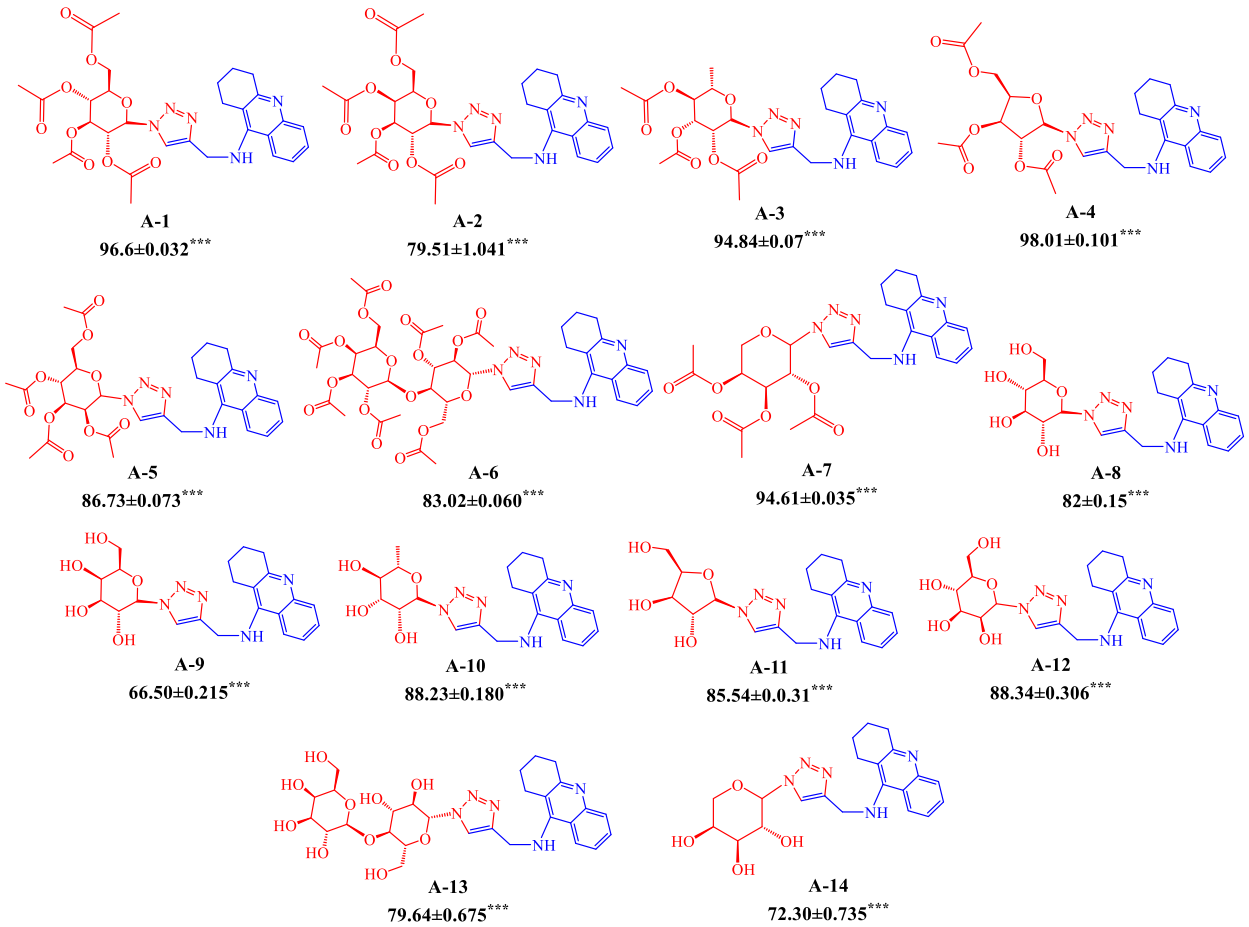


Figure 4: AChE inhibitory activity (expressed in %) of synthesized compounds (at 10 µM)

Mean ± SD, The "***" represent the p value by Bonferroni Multiple Comparisons Test *** P<0.001

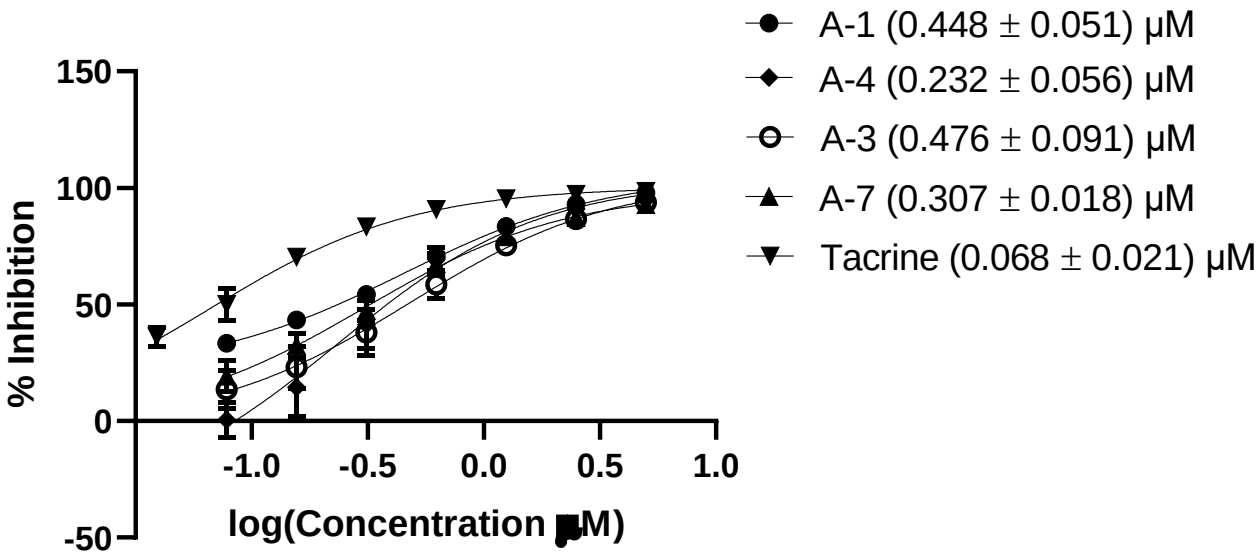


Figure 5: IC_{50} values of most promising compounds in AChE inhibitory assay

Enzyme kinetics of one of the potent sample, **A-1** was carried out, which is presented by Lineweaver-Burk plot (Figure 6). Three different concentrations of compound **A-1** were used to determine the enzyme inhibition scenario, i.e. 0.888 µM, 0.444 µM, and 0.222 µM. Enzyme kinetics analysis of A-1 revealed mixed type of inhibition for the enzyme, which was evident from increase in K_m value with respect to control. While, V_{max} value decreased in a concentration dependent manner (Table 1).

Table 1. Effect of A-1 inhibition on K_m and V_{max} of AChE

A-1 [µM]	**K_m [µM]**	**V_{max} µM.min^{-1}**
0	226.5	0.00159
0.22	274.6	0.00081
0.44	312.1	0.000526
0.88	343.8	0.000321

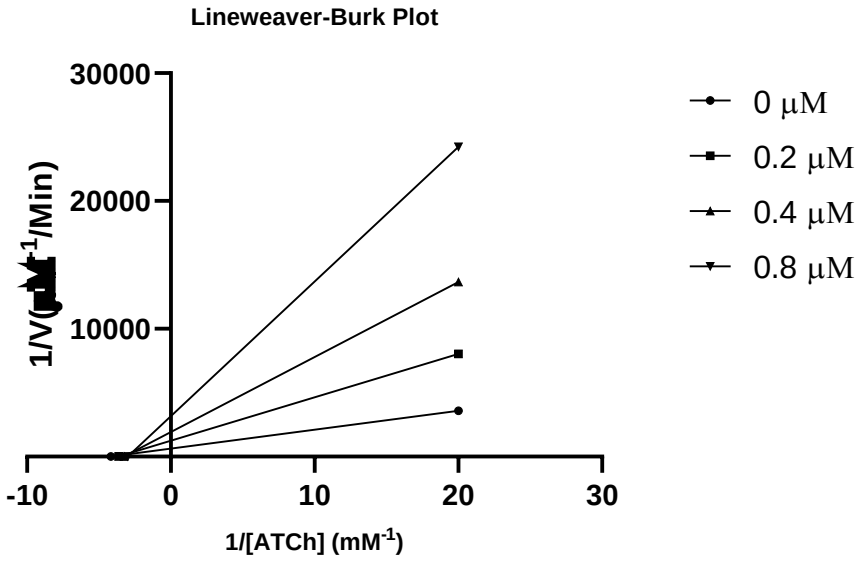


Figure 6: Lineweaver-Burk Plot of **A-1.**

5.2.2 *In vitro* hepatotoxicity studies

As mentioned before, hepatotoxicity was the biggest drawback and the key reason behind the withdrawal of Tacrine from the market to use as a drug to treat AD. Being the Tacrine hybrids, hepatotoxicity analysis is the mandatory biological aspect. To this end, MTT assay was carried out on HepG2 cell lines to evaluate cell viability of selected synthetic derivatives across the wide range of concentration (200 μM – 1.58 μM). Selection of tested derivatives was done in accordance with their anti-AChE activities. Influence of selected and reference compounds on HepG2 cell viability is summarized in figure 7. Results were observed after 24 hours of incubation period in case of Tacrine and selected synthetics. Tacrine, as expected, showed toxic effect on HepG2 cell in a dose dependent manner, showing the inflexion point at the concentration of 100 μM. No toxic effect of selected synthetic derivatives was found than that exerted by Tacrine after the incubation period of 24 hours. Since Tacrine is mainly metabolized by CYP 1A2, but its derivatives have shown more affinity towards CYP 3A4 rather than CYP 1A2. So, it could be postulated that glyco-conjugates might have been metabolized by CYP 3A4 or other drug metabolizing enzyme due to its large substrate specificity and binding

pocket.

These *in-vitro* results corroborated with computational studies performed initially using X-ray crystal structure of CYP 1A2. In agreement with the rationale of their design, selected Tacrine-sugar derivatives were not at all toxic even at the highest concentration taken (200 µM) than that of Tacrine itself (35 % cell viability at 200 µM). This could serve as the reason for the further development of these hybrids.

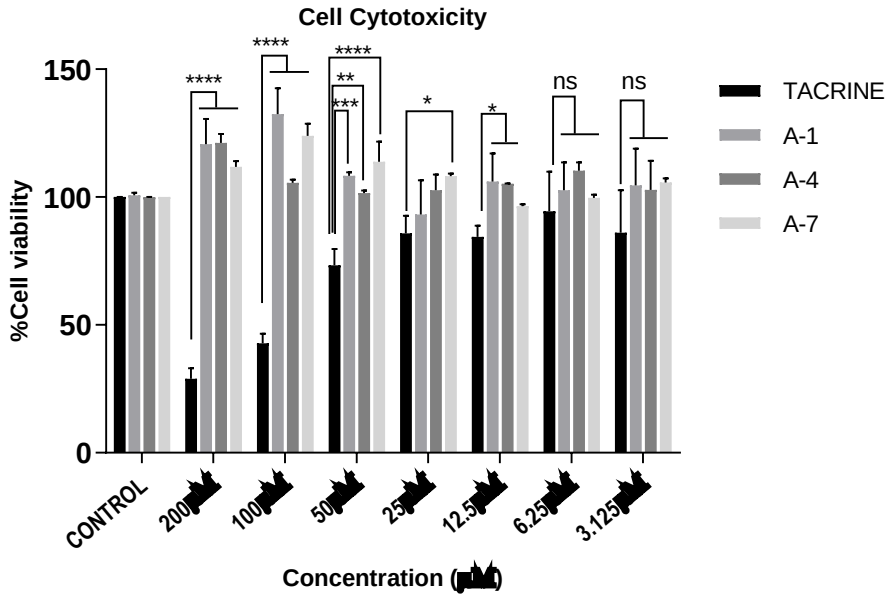


Figure 7: Cell viability determined by MTT assay after the treatment of HepG2 with different concentrations of Tacrine, **A-1**, **A-4** and **A-7**. *p<0.01, **p<0.01, ***p<0.001, with respect to Tacrine treated cells. Error bars represent SEM.

5.3 Docking Studies:

For in depth perception to figure out the plausible molecular mechanism of one of the potent anti- AChE compound **A-1**, molecular modelling studies were performed on AChE. Validation of the docking protocol was done by reproducing the X-ray derived conformation of Tacrine at the AChE binding site (PDB entry: 1ODC). **A-1** was docked at the active site of the AChE using Discovery Studio 2020 after the three-dimensional (3D) coordinates of Tacrine derivative were extracted (Figure 8a and 8b). The best fit conformation of Tacrine derivative with a root-mean-

square deviation of 0.945 was reproduced, further assuring the authenticity of the selected protocol. Therefore, LeadIT was selected as a fitness function for the dock score analysis and later Discovery Studio 2020 was used for visualization of docking pose. Many conformations of **A-1** were developed and were categorized according to their dock score. The conformation with the lower dock Score (-20) was opted for further debate.

Molecular docking results revealed that **A-1** was stabilized in the binding pocket by various electrostatic forces and was oriented at the active site of AChE in somewhat similar manner as that of native ligand of crystallography (Tacrine derivative) (Figure 8). Usually, as reported in literature, Tacrine molecule normally occupies catalytic active site (CAS) of AChE, but, in this case, **A-1** in AChE is oriented in such a way that whole A-1 molecule occupies Peripheral anionic site (PAS), thus blocking the Peripheral anionic site. Since, PAS is located at the opening of the gorge, all the substrates are entered through this domain and binding of any ligand at this site, may block the entry of acetylcholine in the Catalytic Site and indirectly inhibiting AChE.

The whole Tacrine scaffold of **A-1** is oriented in such a way that is being sandwiched between aromatic areas of two amino acid residues (Phe 331 and Tyr 334). Significant interactions between **A-1** and AChE include hydrophobic interactions between aromatic portions of Phe 330, Phe 331 and Tyr 334 and cyclohexane ring of Tacrine moiety, hydroxyl group of Tyr 70 and acetate group of acetylated β-Glucose moiety. Pi-pi stacking was also observed between indole portion of Trp 279 and triazole portion of **A-1**. Acetylated β-Glucose part is placed in the vicinity of Leu 282, Asn 280, Val 277, Ile 275, Asp 276, Glu 278. The carbohydrate moiety orients to Trp 279 and makes hydrogen bond with the oxygen of carbonyl moiety present at C-3 position. Same interactions were observed with Phe331and hydrogen present at NH^+ of pyridine moiety. Therefore, Molecular docking figures of compound **A-1** within the binding pocket of enzyme AChE provide strong rational for binding of the inhibitor with the amino acid residues associated with the peripheral anionic site and not to the catalytic active site. These results are clearly depicting that irrespective of the enzyme bound to the acetylcholine (substrate) through its catalytic active site the compound can bind to enzyme AChE through peripheral anionic site thus corroborating the mixed type inhibition and can undergo further development in drug discovery process.

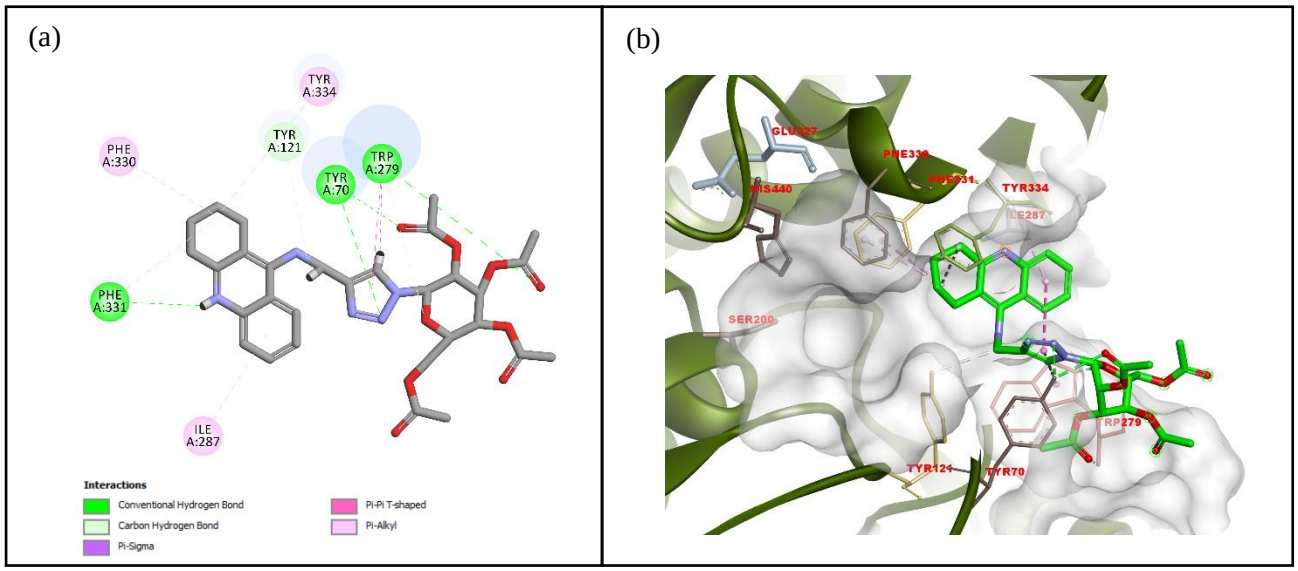


Figure 8: (a) 2D Docking conformation of **A-1** at the active site of AChE; (**b**) 3D Representation of **A-1** at the active site of AChE;

5.4 Molecular dynamic simulations

To study the time dependent stability of **A-1** compound in complex with AChE (PDB entry: 1ODC), a 20 ns molecular dynamic study was performed. The best-docked pose of **A-1** compound was selected for this purpose and this pose was also taken as reference for the calculation of various statistical parameters such as RMSD and RMSF. The results of simulation revealed that the RMSD value of the A-1/AChE complex remained below 3 Å, which lies within acceptable range. The complex got stabilized with an RMSD of approximately 0.9 Å and showed minute fluctuations till 20 ns as shown in Figure 9(a). These results indicated that the **A-1**/AChE complex reached a steady state at the end of the MD simulation process and no major conformational changes were observed during the simulation. In addition, RMSF plot of the protein helped to understand the local changes and fluctuations in the amino acid residues of the protein w.r.t. ligand (contacts shown as green vertical lines) as shown in the figure 9b. The plot (see Figure 9(b) revealed that range of RMSF value fluctuated between 0.8 – 2.0 Å which lies within the acceptable range and hence, protein residues did not undergo major orientation during the simulation.

Later, type of interaction and their pattern between **A-1** and AChE (Figure 9c) was studied. This analysis revealed that the amino acids (TRP 84, TYR 70, TYR 121, TRP 279, PHE 330 and PHE

331 surrounding the whole **A-1** molecule are mainly hydrophobic. Among these, TYR 121 and PHE 331 account for significant contribution (86 % and 82 % respectively) in the whole MD trajectory, wherein, pi-pi stacking occurs between benzene ring of Tacrine moiety and benzene ring of PHE 331 and hydrophobic interaction occurs between keto oxygen of acetylated portion of β-glucose at 4^{th} position and TYR 121. In addition to these interactions, weaker pi-cation interactions were also observed between NH^{+} of pyridine moiety and Trp 84, contributing 71 %. From the docking analysis, we observed that hydrophobic interactions have grabbed the significant role in ligand binding. The pi-pi interactions are observed between benzene ring of Tacrine moiety and benzene ring of Phe 331 and Phe 330, contributing 82 % and 53 %. These all observations add support to the stability of **A-1**/AChE complex.

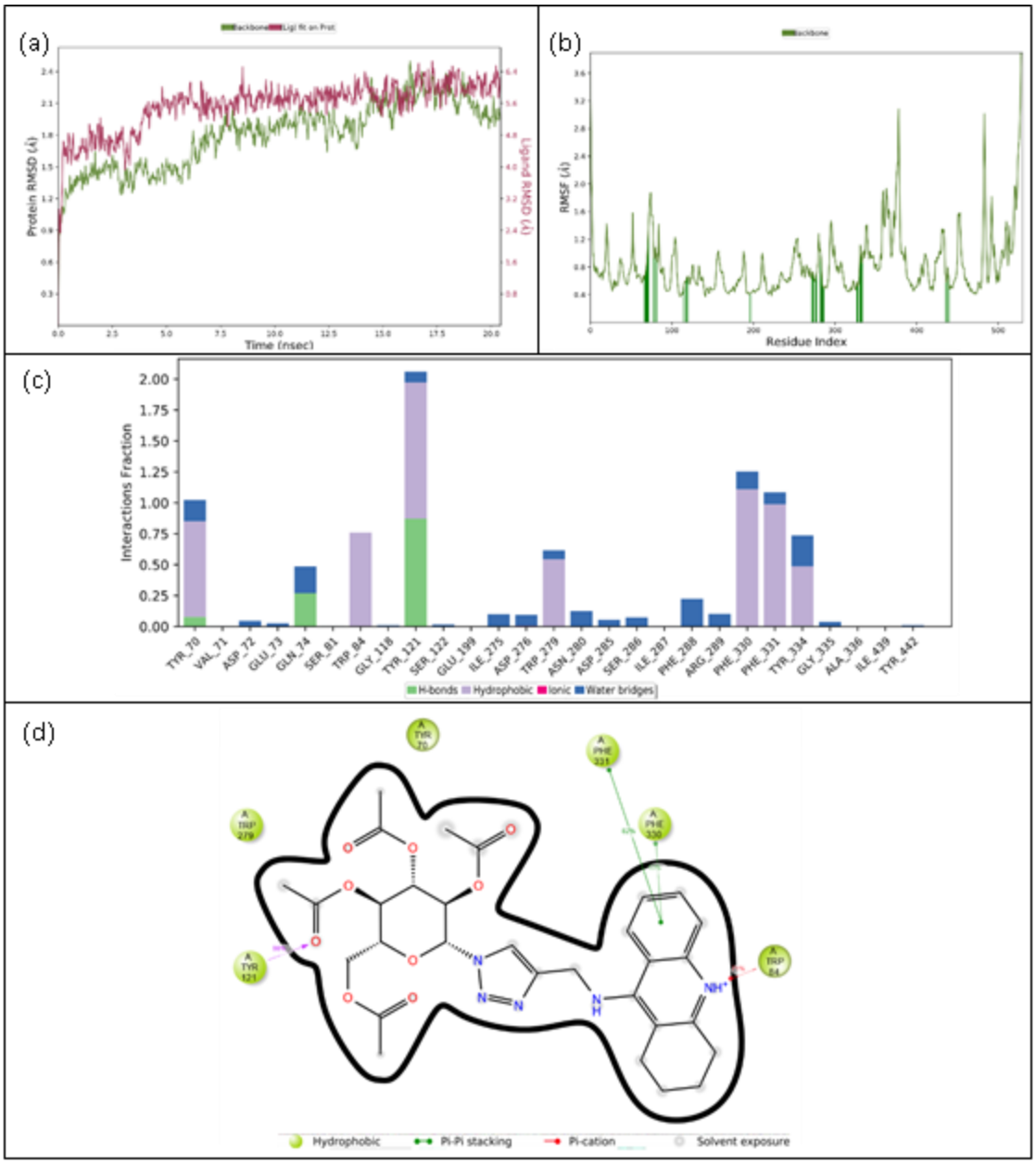


Figure 9: (a) The root-mean-square fluctuation (RMSD) curve of **A-1**/AChE (Pink); **(b):** The root mean-square fluctuation (RMSF) curve of **A-1**/AChE (Green) **(c):** Histogram showing type of protein-ligand interaction **(d):** 2D ligand interaction diagram of compound **A-1** with AChE.

5.4. Conclusion:

In summary, we have described the design and synthesis of glyco-conjugates (**A-1 to A-14**), targeting AChE aimed with the reduced hepatotoxicity. Initially, we have assessed the affinity of Tacrine derivatives towards CYP 1A2 to predict the probable risk of hepatotoxic metabolite formation and fortunately, these compounds were unable to be docked at the active metabolic

site of enzyme, thereby, eliminating the step of metabolite formation. Then, dock scores of all conjugates were calculated towards AChE to predict the AChE inhibitory activity. All the fourteen synthetic compounds were initially screened against AChE at 10 µM concentration. Among the series, four compounds **A-1, A-3, A-4** and **A-7** were found to show excellent inhibition against the enzyme and **A-1** was found to be one of the potent inhibitors with an IC_{50} value of 0.448 µM. Biological results revealed that different sugars (both acetylated and deacetylated) and their stereochemistry have different impact on AChE inhibitory activity. Acetylated compounds were more potent in enzyme inhibition than deacetylated ones. Further, hepatotoxicity analysis of three potent compounds was carried out by using MTT assay, which were found totally non-toxic, after the 24 hours of incubation period. These results supported *in-silico* studies performed initially. Molecular modeling results of one of the potent compound **A-1** displayed significant protein-ligand interactions and found to occupy the binding site via pi-pi interactions, conventional hydrogen bond and Carbon-Hydrogen bonded interactions with the key amino acids residues. In addition to this, stability of the docking complex (**A-1**/AChE) was monitored in terms of RMSD, RMSF and ligand-protein interaction, which goes in favor of ligand/enzyme complex. From the above findings, it was concluded that computational predictions and *in-vitro* observations should be considered together to avoid toxicity issues at later stage in drug development process. Thus, compound **A-1** can be used as principle template to further explore the mechanisms (of different targets involved) in AD and thus, stands as an adequate chemical probe to be launched in an AD drug discovery program.

6. Impact of the research in the advancement of knowledge or benefit to mankind:

The overall study is targeted at the synthesis of triazole tethered glycoconjugates and evaluation of the synthesized hybrids against Acetylcholinesterase along with hepatoxicity assay. The work utilizes the principles and techniques of chemistry to explore solutions to a problem of relevance to biology or that are directly inspired by some biological observation. The research work stands at the interface of chemistry and biology and involves expertise from medicinal chemist as well as pharmacologist, thereby exemplifying its interdisciplinary relevance. Keeping in view the limitations of Tarine having hepatotoxicity as main liability associated with it, these hybrids can give new dimensions to the molecules with reduced hepatotoxicity and can thus be extremely beneficial for the society which at the moment is striving hard to fight with this dreadful disease.

7. References:

[1] H. Zempel, E. Mandelkow, Lost after translation: missorting of Tau protein and consequences for Alzheimer disease, Trends Neurosci. 37 (2014) 721–732.

[2] M.P. Murphy, H. LeVine III, Alzheimer's Disease and the β-Amyloid Peptide, J. Alzheimer's Dis. 19 (1) (2010 January) 311.

[3] Z. Chen, C. Zhong, Oxidative stress in Alzheimer's disease, Neurosci. Bull. 30 (2) (2014 April) 271–281.

[4] K. Hensley, Neuroinflammation in Alzheimer's Disease: Mechanisms, Pathologic Consequences, and Potential for Therapeutic Manipulation, J. Alzheimer's Dis. 21 (1) (2010) 1–14.

[5] P. Francotte, E. Graindorgea, S. Boveriea, P. de. Tullioa, B. Pirottea, New Trends in the Design of Drugs against Alzheimer's Disease, Curr. Med. Chem. 11 (2004) 1757–1778.

[6] E.V. Strauss, M. Viitanen, D.D Ronchi, B. Winblad, L. Fratiglioni, Aging and the occurrence of dementia: findings from a population-based cohort with a large sample of nonagenarians, Arch. Neurol. 56 (1999) 587-592.

[7] M.M. Corrada, R. Brookmeyer, D. Berlau, A.P. Hill, C.H. Kawas, Prevalence of dementia after age 90: results from the 90+ study. Neurology. 71 (2008) 337-343.

[8] A. Dong, J. B. Toledo, N. Honnorat, J. Doshi, E. Varol, A. Sotiras, D. Wolk, J. Q. Trojanowski, C. Davatzikos, Heterogeneity of neuroanatomical patterns in prodromal Alzheimer's disease: links to cognition, progression and biomarkers, Brain. Epub ahead of print, 140 (3) (2016) 735–747.

[9] B. Lam, M. Masellis, M. Freedman, D. T. Stuss, S. E. Black, Clinical, imaging, and pathological heterogeneity of the Alzheimer's disease syndrome. Alzheimer's Res. Ther. 5 (1) (2013).

[10] L. Joseph, D. B. Wolfson, P. Belisle, J. 0. Brooks, III, J. A. Mortimer, J. R. Tinklenberg, J. A. Yesavage, Taking account of between-patient variability when modeling decline in Alzheimer's disease, Am. J. Epidemiol. 149 (10) (1999) 963–73.

[11] A.A. Report, 2020 Alzheimer's disease facts and figures, Alzheimer's Dement. 16 (2020) 391–460.

[12] A. Romero, R. Cacabelos, M. J. Oset-Gasque, A. Samadi, J. Macro-Contelles, Novel

Tacrine- related drugs as potential candidates for the treatment of Alzheimer's disease. Bioorg. Med. Chem. Lett. 23 (2013) 1916-22.

[13] Minarini, A. Milelli, E. Simoni, M. Rosini, M. L. Bolognesi, C. Marchetti, V. Tumiatti, Multifunctional Tacrine derivatives in Alzheimer's disease, Curr. Top. Med. Chem. 13 (15) (2013) 1771–86.

[14] Musial, M. Bajda, B. Malawska, Recent developments in cholinesterases inhibitors for Alzheimer's disease treatment, Curr. Med. Chem. 14 (25) (2007) 2654–79.

[15] Spaldin V, Madden S, Adams DA, Edwards RJ, Davies DS, Park BK. Determination of human hepatic cytochrome P450 1A2 activity in vitro use of tacrine as an isoenzyme-probe. Drug Metab Dispos 1995; 23: 929–934.

[16] Dutkiewicz, Z.; Mikstacka, R. Structure-Based Drug Design for Cytochrome P450 Family 1 Inhibitors. Bioinorg. Chem. Appl. 2018, 2018, 1−21.

[17] Sameem, M. Saeedi, M. Mahdavi, A. Shafee, A review on Tacrine-based scafolds as multi-target drugs (MTDLs) for Alzheimer’s disease, Eur. J. Med. Chem. 128 (2017) 332–345.

[18] J. Patocka, D. Jun, K. Kuca, Possible role of hydroxylated metabolites of Tacrine in drug toxicity and therapy of Alzheimers disease, Curr. Drug Metab. 9 (4) (2008) 332–335.

[19] M. Girek, P. Szymański, Tacrine hybrids as multi-target-directed ligands in Alzheimer's disease: influence of chemical structures on biological activities, Chemical Papers 73 (2019) 269-289.

[20] F. Mao, J. Chen, Q. Zhou, Z. Luo, L. Huang, X. Li, Novel tacrine-ebselen hybrids with improved cholinesterase inhibitory, hydrogen peroxide and peroxynitrite scavenging activity, Bioorg. Med. Chem. Lett. 23 (24) (2013) 6737–6742.

[21] J. Cen, H. Guo, C. Hong, J. Lv, Y. Yang, T. Wang, D. Fang, W. Luo, C. Wang, Development of tacrine-bifendate conjugates with improved cholinesterase inhibitory and pro-cognitive efficacy and reduced hepatotoxicity, Eur. J. Med. Chem. 144 (2018) 128–136.

[22] E. Nepovimova, J. Korabecny, R. Dolezal, K. Babkova, A. Ondrejicek, D. Jun, V. Sepsova V, Horova A, Hrabinova M, Soukup O, Bukum N, Jost P, Muckova L, Kassa J, Malinak D, Andrs M, Kuca K (2015) Tacrine-trolox hybrids: a novel class of centrally active, nonhepatotoxic multi-target-directed ligands exerting anticholinesterase and antioxidant activities with low in vivo toxicity. J Med Chem 58:8985–9003.

[23] A. Pałasz, D. Cież, B. Trzewik, K. Miszczak, G. Tynor, B. Bazan, In the Search

of Glycoside-Based Molecules as Antidiabetic Agents, Topics CURR. CHEM. 377 (4) (2019) 19.

[24] P. Lam, F. Cheung, H.Y. Tan, N. Wang, M.F. Yuen, Y. Feng, Hepatoprotective effects of Chinese Medicinal Herbs: a focus on anti-inflammatory and anti-oxidative activities, Int. J. Mol. Sci. 17 (2016) 465.

[25] S. Bhawna, S.U. Kumar, Hepatoprotective activity of some indigenous plants, Int. J. Pharm. Tech. Res. 4 (2009) 1330-1334.

[26] L. Yin, L. Wang, X.J. Liu, F.C. Cheng, D.H. Shi, Z.L. Cao and W.W. Liu, Synthesis and bioactivity of novel C2-glycosyl triazole derivatives as acetylcholinesterase inhibitors Heterocycl. Commun. 23 (3) (2017) 231–236.

[27] W.W. Liu, Q.X Li, D.H. Shi, Synthesis, characterization, and biological evaluation of some novel glycosyl 1,3,4-thiadiazole derivatives as acetylcholinesterase inhibitors. Heterocycles 91 (2015) 275–286.

[28] G. Wua, Y. Gaoa, D. Kanga, B. Huanga, Z. Huoa, H. Liuc, V. Poongavanamb, P. Zhana, X. Liua, Design, Synthesis and Biological Evaluation of Tacrine 1,2,3-Triazole Derivatives as Potent Cholinesterase Inhibitors, Med. Chem. Comm. 9 (2018) 149-159.

[29] L. Afzelius, C. M. Masimirembwa, A. Karlen, Discriminant and quantitative PLS analysis of competitive CYP2C9 inhibitors versus non-inhibitors using alignment independent GRIND descriptors. J. Comput. Aided. Mol. (2002) 16 (7) 443-58.

[30] M. P. Gleeson, A. M. Davis, K. K. Chohan, Generation of in-silico cytochrome P450 1A2, 2C9, 2C19, 2D6, and 3A4 inhibition QSAR models. J. Comput. Aided Mol. (2007) 21 (10-11) 559-73

[31] G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem Pharmacol. 7 (1961) 88–90.

[32] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J Immunol Methods. 65 (1-2) (1983) 55-63.

[33] M. T. Mc Kenna, G. R. Proctor, L. C. Young, and A. L. Harvey. Novel Tacrine Analogues for Potential Use against Alzheimer's Disease: Potent and Selective Acetylcholinesterase Inhibitors and 5-HT Uptake Inhibitors, J. Med. Chem. 40 (1997) 3516-3523.

Harmandeep Kaur Gulati
Department of Pharmaceutical Sciences
Guru Nanak Dev University,
Amritsar, India - 143005