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Review paper
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Benzene-1,3-diol derivatives as the inhibitors of butyrylcholinesterase: An emergent target of Alzheimer's disease

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Abstract: Molecular docking is a powerful and significant approach for the identification of lead molecules on the basis of virtual screening. With this a large number of compounds can be tested and based on the scoring function and ranking, the conclusion can be made that how the selected compounds can inhibit the targeted protein/receptor. By keeping in view, the importance of selective inhibitors of cholinesterase in the treatment of Alzheimer disease, here we are focused on the determination of the mechanism of binding interactions of few benzene-1,3-diol derivatives within the active site of both acetyl-cholinesterase (AChE) and butyrylcholinesterase (BChE). All the selective ligands were found to have a greater binding affinity with the BChE as compared to that of AChE, by an average value of ~−28.4 and ~−12.5 kJ/mol, respectively. The results suggested that the identified inhibitors can be used as the lead candidates for the development of novel inhibitors of the targeted enzymes against specific diseases, thus opening the possibility of new therapeutic strategies.

Keywords: molecular docking; acetyl-cholinesterase (AChE); butyrylcholine-esterase (BChE); active pocket.

INTRODUCTION

Alzheimer's disease (AD), a neurodegenerative disorder, is characterized by the significant decrease in the level of acetylcholine (ACh) neurotransmitter.[1-2] This neurotransmitter (ACh) plays a significant role in the normal processes of

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learning and memory by activating muscarinic and nicotinic receptors of the central nervous system.[3-4] The acetyl-cholinesterase (AChE) and butyrylcholine-esterase (BChE) are well-studied enzymes that are involved in the hydrolysis of ACh to acetate and choline in the synaptic cleft.[5-6] The major signs and symptoms of AD include dementia, confusion, memory lapses, misinterpreting spatial relationships, and decline in the ability to speak, write, think, reason, making decisions and planning. Personality and behavioral changes have also been observed including depression, anxiety, agitation, social isolation, mood swings, diurnal rhythm disturbances and delusions.[7-8] AD is characterized by various markers in the brain including large number of amyloid plaques surrounded by neurofibrillary tangles, vascular damage from plaque deposition and neuronal cell degradation. The main component of plaques is amyloid β protein, and also the major causative factor of AD. The deposition of this notorious protein leads to the development of other symptoms.[9-10] Head injuries, progression of age, sequelae of delivery, ataxic fever, paralysis, mania, apoplexy, mercury abuse, wine abuse, political upheavals, unhappy love, dietary excess, masturbation, unfulfilled love, domestic problems, poverty and fears are among the causes of AD that emerged in the last century.[11-12]

Recent research has revealed that in the brain of patients suffering from AD, the level of AChE is considerably reduced whereas that of BChE increases, thus, aggravating the toxicity of β-amyloid peptide. In such instances, it is possible that BChE may be more suitable target than AChE.[13] Both AChE and BChE share 53 % amino acid sequence similarities of their active sites.[14] Recently, the increased level of BChE has been studied in AD patients therefore resulted in increased β-amyloid peptide toxicity.[15] It is not surprising that cholinesterase inhibitors have shown better results in the treatment of AD than any other strategy explored.[16] Hence, the search for the discovery of novel cholinesterase inhibitors (ChE) is expected to continue in future since the current ChEs inhibitors are reported to have some side effects.[17] The availability of several crystal structures of both ChEs (in complexes) with different inhibitors provides the possibility to apply docking protocol to explore for protein inhibitor complexes in terms of the nature of their interactions.[18] Although there are considerable efforts being made for understanding the etiology of the neurodegenerative disorder (AD) but the development of novel inhibitor of specific target remain as an important concern in the treatment of patient. The main challenge in the development of the inhibitors of the selected targets is their potency, selectivity, and drug-ability. Therefore, there is a need of deep understanding of the structure activity relationships and functions of the selective inhibitors of selected enzymes.[19]

Over the past few years, high-throughput screening (HTS) has become a cornerstone technology of pharmaceutical research[20] but it is very expensive and technically impossible to screen a huge library of chemical compounds using these

biochemical techniques (high throughput screening). In this regard, computational methodologies have become a vital element of many drug discovery programmes, from the hit identification to the lead optimization and beyond.[21] The high throughput computational screening using pharmacophore based virtual screening, molecular docking and quantum computational studies are among the most cost-effective technique through which millions of compounds can be screened rapidly.[22] Many heterocyclic compounds have been synthesized and reported for their potential to inhibit the targeted enzymes but their molecular target was not fully defined. Among those heterocyclic derivatives, quinolones and dibenzo-azepine have been found as the most attractive scaffolds due to their broad range of biochemical activities such as angiotensin converting enzyme (ACE) inhibitor along with anti-convulsant, neuroprotective and anti-inflammatory properties.[23]

The current study is designed to relate the interest of some benzene-1,3-diol obtained from natural source as cholinesterase inhibitors but more selective as BChE inhibitors. The structures were drawn using ACD/ChemSketch 12.01, and 3D optimized.[24] The study comprises smart approach by using computational tools to find value added product in short time without wasting of chemicals. The crystal structure of both enzymes co-crystalized with their inhibitors were obtained from protein data bank.[25] The selected compounds were further explored along with novel inhibitors to determine the possible binding interactions of different amino acids within the active site of both enzymes, respectively using Autodock 4.2 software.[26] Moreover, the ADMET studies were also performed using ADMET LAB 2.0.[27] The deep understanding of the structure activity relationships and functions of the identified inhibitors/drug like molecules provide a great hope for the development of future novel drugs.

EXPERIMENTAL

In order to gain insight of the binding interactions, molecular docking studies of the selected compounds were performed using AutoDock 4.2.[26] The crystal structure of the human AChE (PDB ID 4BDT) bound to standard inhibitor huprine W and human BChE (PDB ID 4BDS) bound to standard inhibitor tacrine, Figure 1, were downloaded from RCSB Protein Data Bank and used for docking studies.[25] The visual inspection for binding pattern was done using the Discovery Studio Visualizer software, Version 17.2.[28]

Docking procedure

Ligand preparation

The selected compound structures were downloaded in Spatial Data File (SDF) format from PubChem.[29] The structures of the compounds were drawn using ACD/ChemSketch 12.01, and 3D optimized.[24] The 3D structures were converted to PDB format which were further processed by Autodock 4.2. The IUPAC (International Union of Pure and Applied Chemistry) name and InChIKey of the selected compounds are mentioned in table I and their respective structures are given in suplementary file.

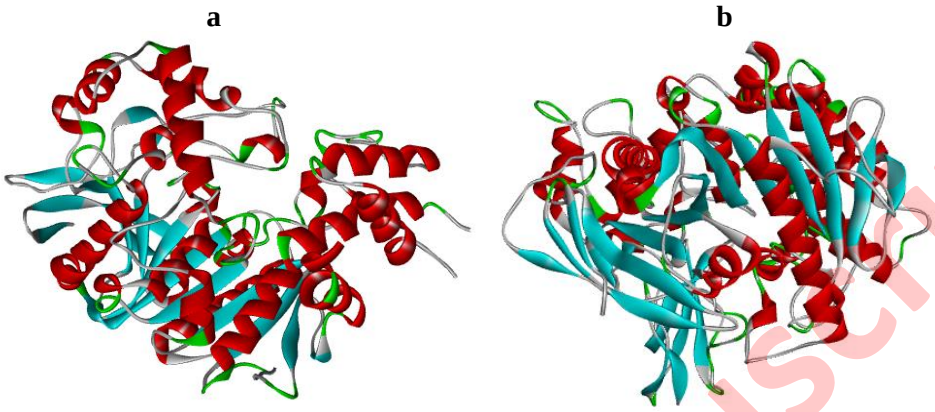

Figure 1. Crystal structure of (a) human AChE (PDB ID 4BDT) and (b) human BChE (PDB ID 4BDS) from Protein Data Bank (https://www.rcsb.org/search)[25]

Table I. List of the Selected Compounds for Study

PubChem CID	Codes	IUPAC name	InChIKey
5054	**1a**	benzene-1,3-diol	GHMLBKRAJCXXBS-UHFFFAOYSA-N
10333	**1b**	4-methylbenzene-1,3-diol	FNYDIAAMUCQQDE-UHFFFAOYSA-N
17927	**1c**	4-ethylbenzene-1,3-diol	VGMJYYDKPUPTID-UHFFFAOYSA-N
87874	**1d**	4-propylbenzene-1,3-diol	DJDHQJFHXLBJNF-UHFFFAOYSA-N
205912	**1e**	4-butylbenzene-1,3-diol	CSHZYWUPJWVTMQ-UHFFFAOYSA-N
3610	**1f**	4-hexylbenzene-1,3-diol	WFJIVOKAWHGMBH-UHFFFAOYSA-N
3014087	**1g**	4-Tert-butylbenzene-1,3-diol	YBKODUYVZRLSOK-UHFFFAOYSA-N
75294	**1h**	4-benzylbenzene-1,3-diol	QVFIWTNWKHFVEH-UHFFFAOYSA-N
11171903	**1i**	4-(1-phenylethyl)benzene-1,3-diol	PQSXNIMHIHYFEE-UHFFFAOYSA-N
24849532	**1j**	4-[2-(2,4-dihydroxyphenyl)ethyl]-benzene-1,3-diol	WKIFTWPZTZUMRN-UHFFFAOYSA-N

Preparation of enzyme (receptor)

Before docking, the protein structure was prepared and refined using Autodock 4.2.[26] The standard preparation steps included; removal of co-crystallized ligands and water molecules followed by the addition of hydrogen and gasteiger partial charges to the protein structure. The protein was set to be rigid and ligands were allowed to dock within the activation loop of selected protein. Active site of a protein was determined by selecting a dimension grid of 60×60×60 Å around the co-crystallized ligands *i.e.*, huprine W in case of AChE and tacrine in case of BChE.

Molecular docking

After preparation of ligand and protein files, the Autogrid and Autodock utility of Autodock 4.2 programme were used for docking protocols. The software used the in-house default forcefield and the Lamarckian Genetic Algorithm (LGA) as a search parameter. LGA is a type of Random or Stochastic docking Algorithm, which actually deals with the calculation of random changes in flexible parts of the ligand and further determines its interaction with the amino acid residues of active site pocket. The Autodock 4.2 software

calculates the different energy parameter and stores them, accordingly. The number of poses were set to 100 and population size was set upto 300. High number of poses are good practice to increase the accuracy of the result. After docking, top ten docked conformation with best ligand-protein interaction and high binding energy were selected for comparison with co-crystal standard ligand.

Visual inspection

The structures of each selected compound against AChE and BChE were visualized and inspected for the best fit orientation within the active pocket of the enzyme, respectively. This was done using Discovery Studio Visualizer software, version 17.2.

Drug likeness evaluation and calculated ADME properties

The ADME (Absorption, Distribution, Metabolism, and Excretion) properties for all the tested compounds were calculated using online integrated tool ADMET LAB 2.0.[27] All synthesized compounds showed moderate ADME properties as shown in table III.

RESULTS AND DISCUSSION

Potential binding site in receptors

Commercially available Molecular Operating Environment 2015.10 (MOE) software[30] was used for the prediction of most potential active site where the selected ligand can bind and interact within the activation loop of targeted proteins *i.e*., both AChE and BchE (Figure 2).

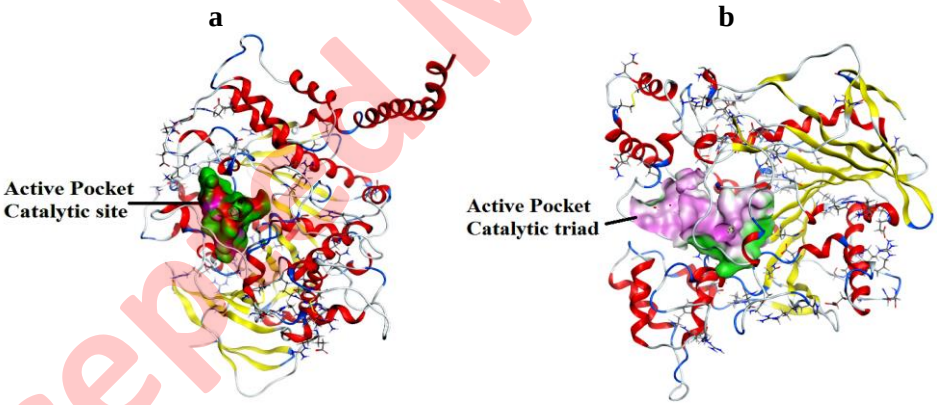

Figure 2. Binding site prediction of (a) Human AChE (PDB ID 4BDT) and (b) Human BChE (PDB ID 4BDS) using MOE 2015.10

The active pocket of AChE contained total 36 amino acid residues including; GLN71, TYR72, VAL73, ASP74, GLY82, THR83, TRP86, ASN87, PRO88, TYR119, GLY120, GLY121 GLY122, TYR124, SER125, GLY126, ALA127, LEU130, TYR133, GLU202, SER203, ALA204, TRP236, PHE295, PHE297, SER336, TYR337, PHE338, TYR341, LEU437, TRP439, PRO446, HIS447, GLY448, TYR449 and ILE451. Similarly the active pocket of BChE contained total 45 amino acid residues and includes; GLN67, ASN68, ILE69, ASP70,

GLN71, SER72, GLY78, SER79, TRP82, ASN83, PRO84, TYR114, GLY115, GLY116, GLY117, GLN119, THR120, GLY121, THR122, LEU125, TYR128, GLU197, SER198, TRP231, GLU276, ALA277, VAL280, TYR282, GLY283, THR284, PRO285, LEU286, SER287, VAL288, ASN289, ALA328, PHE329, TYR332, PHE398, TRP430, MET437, HIS431, GLY439, TYR440 and ILE442. The selected ligands formed hydrogen bonding with the amino acid residues of the active pockets. In comparison to AChE, the ligands formed maximum interactions with amino acid residues of BChE thus ultimately resulted in the improved binding energies.

Docking analysis studies

In-silico study was conducted using Autodock 4.2 and visualization of docked conformations were carried out using Discovery Studio visualizer 17.2. The selected derivatives of benzene 1,3 diol were docked within activation loop of AChE and BChE enzymes. The most possible 2D and 3D binding interactions of docked conformations were obtained using Discovery Studio visualizer 17.2. All selected ligands showed comparable interactions and docking scores with both enzymes, when compared to the standard Donepezil. The interactions are given in the Figures 3 and 4 and docking scores are tabulated in Table II.

Table II. Docking score of selected compounds by considering bound and unbound states of the ligand.

No.	Code	Acetylcholinesterase (AChE)		Butyrylcholinesterase (BChE)		Selectivity for BchE[a]
		Docking score, kJ/mol	Predicted inhibition constant, µM	Docking score, kJ/mol	Predicted inhibition constant, µM	
1.	**1a**	-6.69	2300	-17.58	836.67	2.8
2.	**1b**	-12.60	989.03	-20.68	472.10	2.0
3.	**1c**	-17.04	527.89	-20.89	309.80	1.7
4.	**1d**	-17.20	818.97	-23.19	171.02	4.8
5.	**1e**	-18.50	572.90	-24.82	174.55	3.3
6.	**1f**	-20.59	247.72	-25.03	81.68	3.0
7.	**1g**	-20.05	306.32	-23.19	87.16	3.5
8.	**1h**	-21.68	95.91	-25.07	40.86	2.3
9.	**1i**	-22.02	43.04	-26.92	27.24	1.5
10.	**1j**	-22.60	109.61	-29.14	9.30	12.1
11	**Hup**	-29.01	8.38	-----	-------	----
12	**Tac**	-----	------	-28.84	8.8	------
Ctrl.	**Don**	-30.14	2.67	-32.65	0.035	89

[a]Selectivity index defined as *IC*[AChE]/*IC*[BChE]; **Hup** - Huprine, **Tac** - Tacrine, **Don** - Donepezil

Visual inspection

The structure of each selected compound against AChE and BChE was visualized for best fit orientation within the active pocket of the enzyme, respectively. Particularly compound **1h**, **1i** and **1j** formed stable protein-ligand complex with both enzymes. The results are given in Figure 3 and 4.

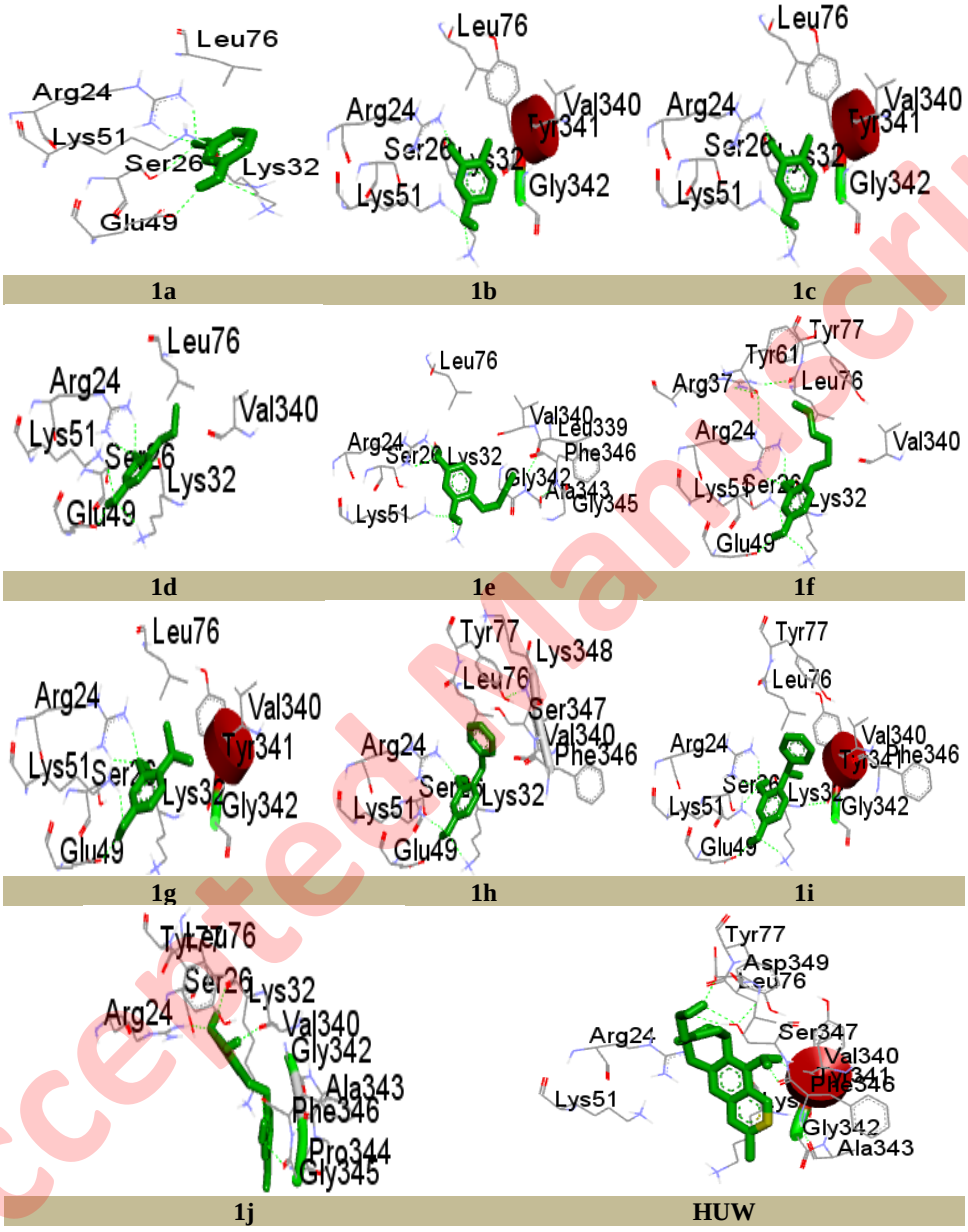

Figure 3. Protein-ligand complex formed by docked structures of AChE inhibitors

AChE docking studies

In terms of detailed docking interaction studies, only three potent compounds **1h**, **1i** and **1j** are being discussed here. All the detailed discussion of other compounds are provided in Supplementary Material.

The docked conformation of compound **1h**, **1i** and **1j** showed potent inhibitory potential of AChE enzyme (Figure 3). Docking scores of these three compounds were found to be best among all other compounds which were -21.68, -22.02 and -22.60 kJ/mol respectively. It was seen that compound **1h** contain aromatic ring as substituent at 4th position of core aromatic ring. It was notable that presence of aromatic ring has significantly increased the docking energy. It might be due to the resonance effect of aromatic ring. Moreover, substituted aromatic ring was also involved in alkyl interaction with LEU76 and VAL340. Similarly, compound **1i** contain phenyl ethyl as substituent. This substitution has significantly improved the binding energy which might be due to positive inductive electron donating effect of ethyl group and interaction of phenyl ring with LEU76 of active site. Moreover, the resonating π-electrons of benzene ring also involved in binding interactions with amino acid residues which further improved its docking energy. According to free binding energy score, compound **1j** was found to be the most potent derivative among all compounds. It was found that amino acid residues which were involved in bonding and non bonding interactions with compound **1j** were LYS32, ARG24, VAL340, PHE346, PRO344, LEU76, SER26, TYR77, ALA343 and GLY342. It was observed that parent compound was substituted with ethyl phenyl ring having two hydroxyl groups at ortho position. Previously it was observed that hydroxyl groups was responsible for establishing strong hydrogen bonding with amino acids of active site. Whereas, benzene ring was itself involved in strong π-cation and π-sigma bonding with amino acid residues of active site. Similarly, in present compound two benzene rings, one ethyl group and 4 hydroxyl groups have significantly contributed to most potent inhibitory potential of the compound. It can be seen that hydroxyl groups of both rings were involved in hydrogen bonding with LYS32, ARG24, VAL340 and PHE346 respectively. Whereas, aromatic rings were involved in π-alkyl and π-cation interactions. These factors corresponded to highest inhibiting potential of the compound. The detailed 2D interactions of AChE enzyme with all compounds are shown Supplementary Material.

Co-crystal ligand of AChE enzyme showed binding energy of -29.01 kJ/mol. It was notable that the standard compound also possessed aromatic moiety in its structure which was responsible for formation of π-alkyl and π-cation interactions with amino acids residues. Whereas, prescence of single hydroxyl group was contributing toward formation of hydrogen bonding with amino acid residue of active site. So, it is well understood that presence of hydroxyl group, aromatic ring and electron donating alkyl groups are important determinants of anti-cholinesterase activity of the compound (see Supplementary Material).

BChE docking studies

In terms of detailed docking interaction studies, only three potent compounds **1h**, **1i** and **1j** are being discussed here. All the detailed discussion of other compounds are provided in separate Supplementary Material.

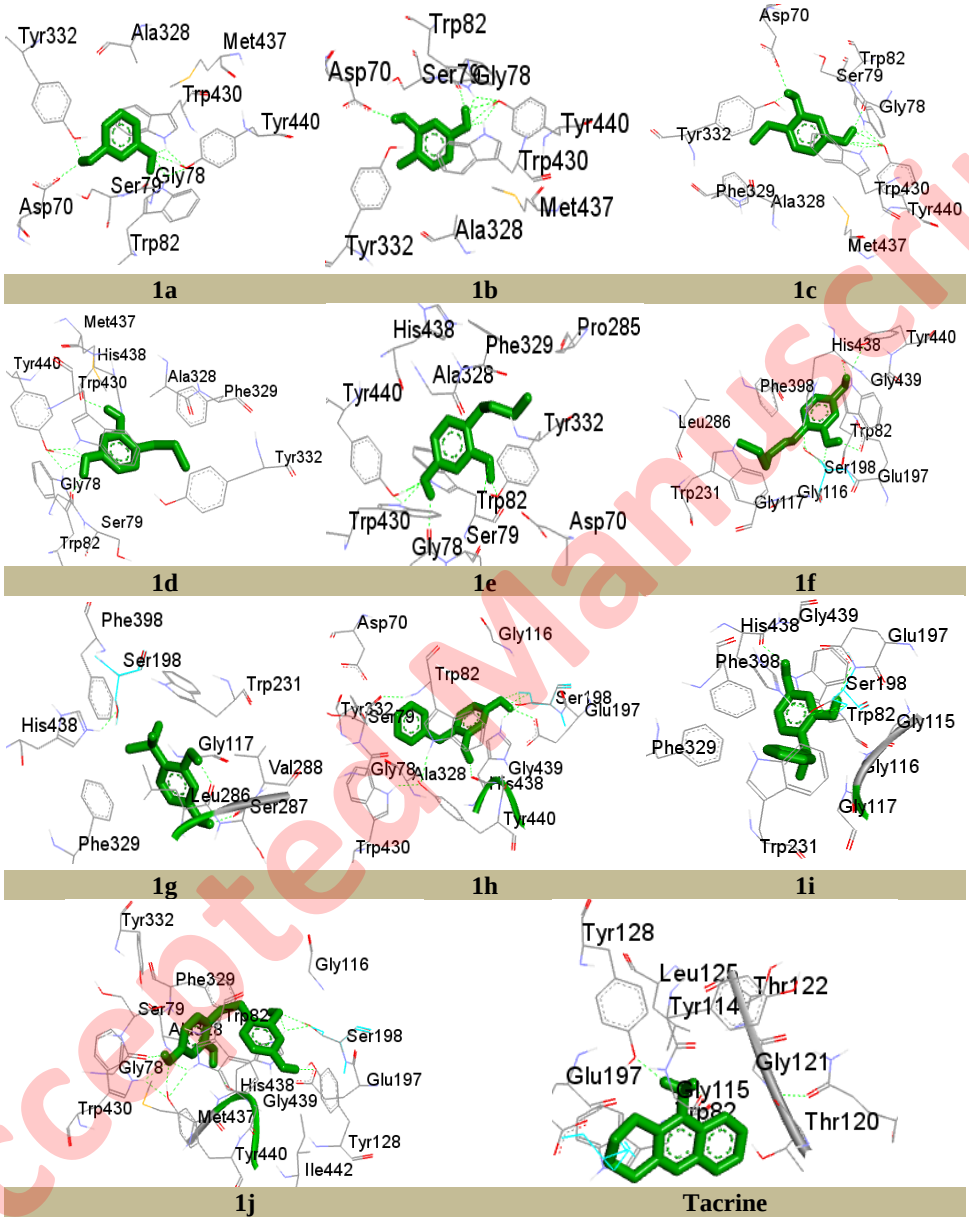

Figure 4. Protein-ligand complex formed by docked structures of BChE inhibitors

The docked conformation of compound **1h**, **1i** and **1j** showed potent inhibitory potential against BChE enzyme (Figure 4). The inhibitory potential of these derivatives were most potent against BChE enzyme. Docking scores of these three compounds were found to be best among all other compounds which

were -25.07, -26.92 and -29.14 kJ/mol respectively. These scores were found to be higher than docking scores obtained with AChE enzyme which further strengthen the testimoney that these derivatives are more selective and potent toward BChE enzyme. Compound **1h** contain aromatic ring as a substituent. The substituted aromatic ring was involved in alkyl interaction with GLY115. Similarly, the compound **1i** contained phenyl ethyl as a substituent. This substitution has significantly improved the binding energy which might be due to positive inductive electron donating effect of ethyl group and interaction of phenyl ring with ALA328 of active site. Moreover, resonating π-electrons of benzene ring was also involved in binding interactions with amino acid residues which further improved its docking energy.

According to free binding energy score, compound **1j** was found to be most potent derivative among all compounds which has docking score of -29.14 kJ/mol with predicted inhibitory constant value of 9.30 µM against BChE enzyme. It was found that the amino acid residues which were involved in bonding and non bonding interactions with compound **1j** were as follows GLU197, GLY116, GLY117, LEU286, HIS438, TRP231, GLY439, TRP82 and VAL288. It can be observed that parent compound was substituted with ethyl phenyl ring having two hydroxyl groups at ortho position. Previously, we have observed that, hydroxyl groups was majorily resposnible for establishing strong hydrogen bonding with amino acids of active site. Whereas, benzene ring was itself involved in strong π-cation and π-sigma bonding with amino acid residues. Similarly, in present compound, two benzene rings, one ethyl group and 4 hydroxyl groups significantly contributed to most potent inhibitory potential of the derivative. It can be seen that hydroxyl groups of both rings were involved in hydrogen bonding GLU197, GLY117, GLY116 and LEU286, respectively. Whereas, aromatic rings were involved in π-alkyl and π-cation interactions. These factors corresponded to highest inhibiting potential of **1j** compound. The detailed 2D interactions of BChE enzyme with all compounds are shown in supplementary data file.

Co-crystal ligand Tacrine of BChE enzyme showed binding energy of -28.84 kJ/mol. It was notable that compound **1j** showed much better binding conformation than standard tacrine. It was evident that standard and potent derivatives possessed aromatic moiety in their structures which was responsible for formation of stablizing hydrophobic interactions *i.e.*, π-alkyl and π-cation interactions with amino acids residues. Whereas, prescence of hydroxyl group was contributing toward formation of hydrogen bonding with amino acid residue of active site. It was observed that potent 1,3 diol derivatives possessed more number of hydroxyl group which was responsible for more hydrogen bondings with amino acid residues of active site. So it is concluded that presence of hydroxyl group, aromatic ring and electron donating alkyl group

were important determinants for anti-cholinesterase activity of the compounds (see Supplementary Material).

Drug likeness evaluation and calculated ADME properties

During the drug discovery process, determination of ADME properties of drug like molecule is very important step. These properties were calculated by using online tool ADMET LAB 2.0. The octanol–water distribution coefficients (S + logP and MlogP), the pH dependent octanol–water distribution coefficient (S + logD), number of hydrogen bond donors; HBDH, hydrogen bond acceptor (sum of nitrogen and oxygen atoms); MNO and topological polar surface area; TPSA were determined for each molecule.

Among all the properties, the TPSA is a valuable molecular descriptor which is used for the calculation of drug absorption properties. The TPSA value of less than 60 $Å^2$ gives prediction that the molecule has sufficient bioavailability properties but if the value exceeds 140 $Å^2$, the molecule is considered to be undesirable. Similarly the compounds with the molecular weight <500, HBDH<10, MNO <5 and logP <5 are considered to be orally bio-available with a favorable ADME profile.All the selected compounds exhibited promising ADME properties within the limits of Lipinski's rule of 5. The properties of the selected compounds are enlisted in Table III.

Table III. Calculated ADME properties of the selected compounds

Compound	MWt	S+logP	S+logD	MlogP	HBDH	MNO	TPSA
1a	110.11	0.751	0.736	0.893	2	2	40.46
1b	124.14	1.109	1.098	1.246	2	2	40.46
1c	138.16	1.595	1.588	1.58	2	2	40.46
1d	152.19	2.109	2.103	1.897	2	2	40.46
1e	166.22	2.664	2.66	2.2	2	2	40.46
1f	194.27	3.791	3.788	2.773	2	2	40.46
1g	166.22	2.647	2.644	2.2	2	2	40.46
1h	200.23	2.897	2.891	2.786	2	2	40.46
1i	214.26l	3.201	3.197	3.05	2	2	40.46
1j	246.26	1.882	1.871	1.942	4	4	80.92

CONCLUSION

Structure based virtual screening was employed to study the protein-ligand interactions for the identification of new BChE inhibitors that could be a starting point for a promising lead candidate in the treatment of AD. Pubchem database was filtered, treated, and subsequently screened against both AChE and BChE protein. Moreover, their predicted inhibition constant values were also in correlation with their binding energy values. Among the different derivatives, 4-(1-phenylethyl)benzene-1,3-diol (**1i**) and 4-[2-(2,4- dihydroxyphenyl) ethyl]benzene-1,3-diol (**1j**) showed strong inhibition and strong interactions with BChE. Thus,

these compounds could be the starting point for the future development of novel inhibitors of BChE.

SUPPLEMENTARY MATERIAL

Supplementary Material are available electronically from https://www.shd-pub.org.rs/index.php/JSCS/article/view/10672 or from the corresponding author on request.

И З В О Д

ДЕРИВАТИ БЕНЗЕН-1,3-ДИОЛА КАО ИНХИБИТОРИ БУТИРИЛХОЛИНЕСТЕРАЗЕ: НОВА МЕТА У АЛЦХАЈМЕРОВОЈ БОЛЕСТИ

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Молекулско моделовање (Molecular docking) снажан и значајан приступ у идентификовању водећих молекула (lead molecules) на основу виртуелног скрининга. На овај начин, велики број једињења може да буде испитан, и на основу добијених резултата једињења могу да буду рангирана и може се претпоставити како одабрана једињења могу инхибирати циљани протеин. Имајући у виду важност постизања селективне инхибиције холинестераза, у овом истраживању фокусирали смо се на одређивање механизма везивних интеракција неколико деривата бензен-1,3-диола у активном месту ацетилхолинестеразе (AChE) и бутирилхолинестеразе (BChE). Показано је да сви одабрани лиганди имају већи афинитет за везивање са бутирилхолинестеразом (BChE) у поређењу са цетилхолинестеразе (AChE), са просечним вредностима ~−28.4 and ~−12.5 kJ/mol, редом. Резултати нашег истраживања указују да идентификовани инхибитори могу бити узети као водећи кандидати за развој нових инхибитора циљаних ензима у третману специфичних болести, и на тај начин се отвара могућност за нове терапеутске стратегије.

(Примљено 16. априја; ревидирано 21. августа; прихваћено 23. августа 2021)

REFERENCES

1. K. H. Chen, E. A. Reese, H. W. Kim, S. I. Rapoport, J. S. Rao, *J. Alzheimers Dis* **26** (2011) 755 (https://dx.doi.org/10.3233/JAD-2011-110002)
2. N. R. Jabir, F. R. Khan, S. Tabrez, *CNS Neurosci. Ther*. **24** (2018) 753 (https://dx.doi.org/10.1111/cns.12971)
3. J. L. Yakel, *Pflug Arch. Eur. J. Phys.* **465** (2013) 441 (https://dx.doi.org/10.1007/s00424-012-1200-1)
4. B. H. Rasch, J. Born, S. Gais, *J. Cogn. Neurosci.* **18** (2006) 793 (https://dx.doi.org/10.1162/jocn.2006.18.5.793)
5. A. C. Halliday, S. A. Greenfield, *Protein Pept. Lett.* **19** (2012) 165 (https://dx.doi.org/10.2174/092986612799080149)
6. Z. Mokhtari, T. Baluchnejadmojarad, F. Nikbakht, M. Mansouri, M. Roghani, *Biomed. Pharmacother*. **87** (2017) 135 (https://doi.org/10.1016/j.biopha.2016.12.067)

7. K. L. Lanctôt, J. Amatniek, S. Ancoli-Israel, S. E. Arnold, C. Ballard, J. Cohen-Mansfield, Z. Ismail, C. Lyketsos, D. S. Miller, E. Musiek, R. S. Osorio, *Alzheimers Dement (NY)* **3** (2017) 440 (https://dx.doi.org/10.1016/j.trci.2017.07.001)
8. S. Karantzoulis, J. E. Galvin, *Expert Rev. Neurother*. **11** (2011) 1579 (https://dx.doi.org/10.1586/ern.11.155)
9. L. Mucke, *Nature* **461** (2009) 895 (https://dx.doi.org/10.1038/461895a)
10. K. Iman, M. U. Mirza, N. Mazhar, M. Vanmeert, I. Irshad, M. A. Kamal, *CNS Neurol. Disord- Drug Targets* **17** (2018) 54 (https://dx.doi.org/10.2174/1871 52731 7666180115162422)
11. F. Boller, K. Bick, C. Duyckaerts, *Cortex* **43** (2007) 565 (https://dx.doi.org/10.1016/S0010-9452(08)70251-X)
12. M. R. Zarrindast, F. Khakpai, *Brain Res.* **1710** (2019) 92 (https://doi.org/10.1016/j.brainres.2018.12.002)
13. N. H. Greig, T. Utsuki, D. K. Ingram, Y. Wang, G. Pepeu, C. Scali, Q. S. Yu, J. Mamczarz, H. W. Holloway, T. Giordano, D. Chen, *Proc. Natl. Acad. Sci.* **102** (2005) 17213 (https://doi.org/10.1073/pnas.0508575102)
14. A. Saxena, A. M. Redman, X. Jiang, O. Lockridge, B. P. Doctor, *Biochem*. **36** (1997) 14642 (https://doi.org/10.1021/bi971425+)
15. Z. Yu, H. Ji, J. Shen, R. Kan, W. Zhao, J. Li, L. Ding, J. Liu, *Food Funct.* **11** (2020) 6643 (https://doi.org/10.1039/D0FO00971G)
16. Q. Li, H. Yang, Y. Chen, H. Sun, *Eur. J. Med. Chem*. **132** (2017) 294 (https://doi.org/10.1016/j.ejmech.2017.03.062)
17. F. L. Ansari, S. Kalsoom, Z. Ali, F. Jabeen, *Med. Chem. Res*. **21** (2012) 23 (https://doi.org/10.1007/s00044-011-9754-6)
18. Z. Ul-Haq, W. Khan, S. Kalsoom, F. L. Ansari, *Theor. Biol. Medical Model* **7** (2010) 22 (https://doi.org/10.1186/1742-4682-7-22)
19. R. Yan, *Transl. Neurodegener*. **5** (2016) 13 (https://doi.org/10.1186/s40035-016-0061-5)
20. R. Macarron, M. N. Banks, D. Bojanic, D. J. Burns, D. A. Cirovic, T. Garyantes, D. V. Green, R. P. Hertzberg, W. P. Janzen, J. W. Paslay, U. Schopfer, *Nat. Rev. Drug Discov*. **10** (2011) 188 (https://doi.org/10.1038/nrd3368)
21. X. Fradera, K. Babaoglu, *Curr. Protoc. Chem. Biol*. **9** (2017) 196 (https://doi.org/10.1002/cpch.27)
22. A. J. Banegas-Luna, J. P. Cerón-Carrasco, H. Pérez-Sánchez, *Future Med. Chem*. **10** (2018) 2641 (https://doi.org/ 10.4155/fmc-2018-0076)
23. E. Vitaku, D. T. Smith, J. T. Njardarson, *J. Med. Chem*. **57** (2014) (https://doi.org/10.1021/jm501100b)
24. *ACD/ChemSketch*, version 12.01, Advanced Chemistry Development Inc, Toronto, ON, Canada, www.acdlabs.com, 2013
25. https://www.rcsb.org/search
26. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, *J. Comput. Chem*. **30** (2009) 2785 (https://doi.org/10.1002/jcc.21256)
27. G. Xiong, Z. Wu, J. Yi, L. Fu, Z. Yang, C. Hsieh, M. Yin, X. Zeng, C. Wu, A. Lu, X. Chen, *Nucleic. Acids. Res.* **49** (2021) 5 (https://doi.org/10.1093/nar/gkab255)
28. D. S. Biovia. *Discovery Studio Modeling Environment, Release 2017*, San Diego: Dassault Systèmes. Version 17.2
29. www.pubchem.ncbi.nlm.nih.gov
30. *MOE (The Molecular Operating Environment)* Version 2010.10, Chemical Computing group Inc. (http://www.chemcomp.com).

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SUPPLEMENTARY MATERIAL TO

Benzene-1,3-diol derivatives as the inhibitors of butyrylcholinesterase: An emergent target of Alzheimer's disease

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INTRODUCTION

S-Table I: Structures of the Selected Compounds for Study

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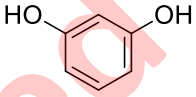
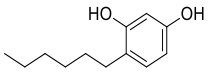
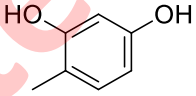
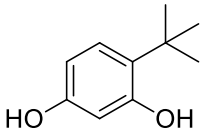
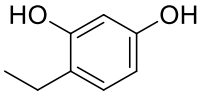
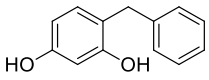
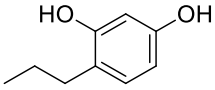
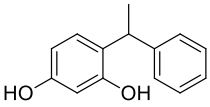
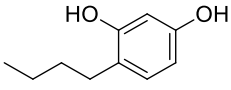
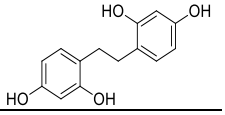
5054	1a		3610	1f	
10333	1b		3014087	1g	
17927	1c		75294	1h	
87874	1d		11171903	1i	
205912	1e		24849532	1j	

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RESULTS AND DISCUSSIONS

AChE docking studies

Bonding and non-bonding interaction of amino acid residues of AChE enzyme with benzene 1,3 diol derivatives (Figure S-1). The amino acid residues which were involved in bonding and non-bonding interactions with compound **1a** were SER26, ARG24, LYS32, GLU49, LEU76 and LYS51. It was notable that highly electronegative oxygen atom of OH group present at 1st position of benzene ring was making strong hydrogen bond with SER26 and ARG24 amino acid residues of AChE. Whereas LYS32 was making hydrogen bond with hydrogen atom of OH group at position 1st of benzene ring. It is evident that OH group have strong electronegativity difference which can exert strong electrostatic force of attraction. Moreover, it was also involved in imparting positive inductive effect (+*I*). Similarly, LYS32 and GLU49 were involved in making hydrogen bond with OH group present at 3rd position of benzene ring. The LYS32 was also involved in making π-sigma bond with benzene ring of resorcinol. Moreover, LYS51 was involved in strong π-cation interaction with core benzene ring of interacting compound. Docking score of current conformation was found to be -6.69 kJ/mol.

The amino acid residues which were involved in bonding and non-bonding interactions with the compound **1b** were ARG24, LYS32, LEU76, VAL340, TYR341, LYS51, GLY342 and SER26. It was observed that interacting compound exhibited binding energy of -12.60 kJ/mol, which was due to presence of methyl group at 4th position of benzene ring. It is well known that methyl group imparts positive mesomeric effect by donating electrons to core benzene ring. It was also observed that OH groups present at 1st and 3rd position of benzene ring was involved in making strong hydrogen bond with LYS32 and ARG24 of activation loop. Furthermore, it was notable that parent benzene ring had also showed major contribution toward interacting amino acid residues through the formation of strong π-sigma and π-alkyl bond with LYS32 and LEU76 residues, respectively. Other interacting amino acid residues like VAL340, TYR341 and SER26 were involved in the formation of Van der Waals interactions with interacting compound.

Docked conformations of compound **1c** with AChE enzyme showed that SER26, LYS32, ARG24, GLU49, LYS51, LEU76, GLY342 and TYR341 amino acid residues were involved in bonding and non-bonding interactions, (Figure 4). It was notable that substituted ethyl group had electron donating tendency which was imparting positive mesomeric effect (+*M*). The substituted ethyl group was also involved in formation of strong π-alkyl bond with LYS32 and LEU76 residues of active site. Furthermore, it was observed that OH groups played vital role in forming strong inhibiting interactions with amino acid residues of active site. It was found that carboxylate end of LYS32 and GLU49 was forming

hydrogen bond with hydrogen atom of hydroxyl group present at position 1st and 3rd, respectively. Moreover, SER26 and ARG24 was found to be involved in hydrogen bond with oxygen atoms of both hydroxyl group. In terms of binding energies, it was calculated as -17.04 kJ/mol. Another major interaction was strong π-cation bond between benzene ring and LYS51. This π-cation was stabilizing electrostatic interaction between a cation and polarizable electronic cloud of aromatic ring. Moreover, aromatic ring was also involved in π-sigma bonding with LYS32. Other amino acid residues which were involved in Van der Waals interactions were GLY342 and TYR341.

The amino acid residues which were involved in bonding and non-bonding interactions with compound **1d** were ARG24, SER26, LYS32, GLU49, LYS51, LEU76 and VAL340. It can be seen that propyl group was substituted at 4th position of benzene ring due to which docking score was slightly better than compound **1c**. The docking score was appeared to be -17.20 kJ/mol. The substituted propyl group had the ability to donate electrons and imparted positive mesomeric effect (*+M*). Another significance of propyl group included the strong π-alkyl interaction with LEU76 residue of active site. It was obvious that hydroxyl groups played vital role in determining inhibiting potential of benzene 1,3 diol derivatives. Both OH groups were involved in making strong hydrogen bond with SER26, ARG24, GLU49 and LYS32. Most particularly, SER32 and ARG24 predominantly formed hydrogen bond with the negative end of hydroxyl group whereas other two formed hydrogen bond with positive hydrogen atom. Presence of core aromatic ring also played significant role in inhibitory potential of these derivatives as aromatic ring was itself involved in two major interactions *i.e.*, π-sigma and π-cation with LYS32 and LYS51, respectively. Amino acid residues like VAL340 and TYR341 were involved in Van der Waals interactions.

The amino acid residues which were involved in bonding interactions with **1e** were PHE346, GLY345, ALA343, LEU339, VAL340, LYS32, ARG24, GLU49, SER26, LYS51 and LEU76 (Figure 4). The compound **1e** had substitution of butyl group at 4th position of aromatic ring. It was surprisingly seen that butyl group was only exposed for Van der Waals interactions with few amino acid residues *i.e.*, ALA343, GLY345, PHE346 and VAL340. Whereas, hydroxyl group was involved in making hydrogen bond with ARG24 and LYS32. Previously, we have seen that SER26 and GLU49 were also involved in hydrogen bond formation with both hydroxyl groups but in present case, it was not observed. Moreover, LYS32 was again involved in forming π-sigma bond with core aromatic ring. This π-sigma bonding significantly stabilizes the protein-ligand complex. Binding score for compound **1e** was found to be -18.50 kJ/mol.

The amino acid residues which were involved in bonding and non-bonding interactions with compound **1f** were TYR77, TYR61, LEU76, VAL340, SER26, LYS32, ARG24, GLU49, LYS51 and ARG37. This compound showed

significant docking score -20.59 kJ/mol and it can be suggested that it might be due to presence of long chain hexyl substituent.

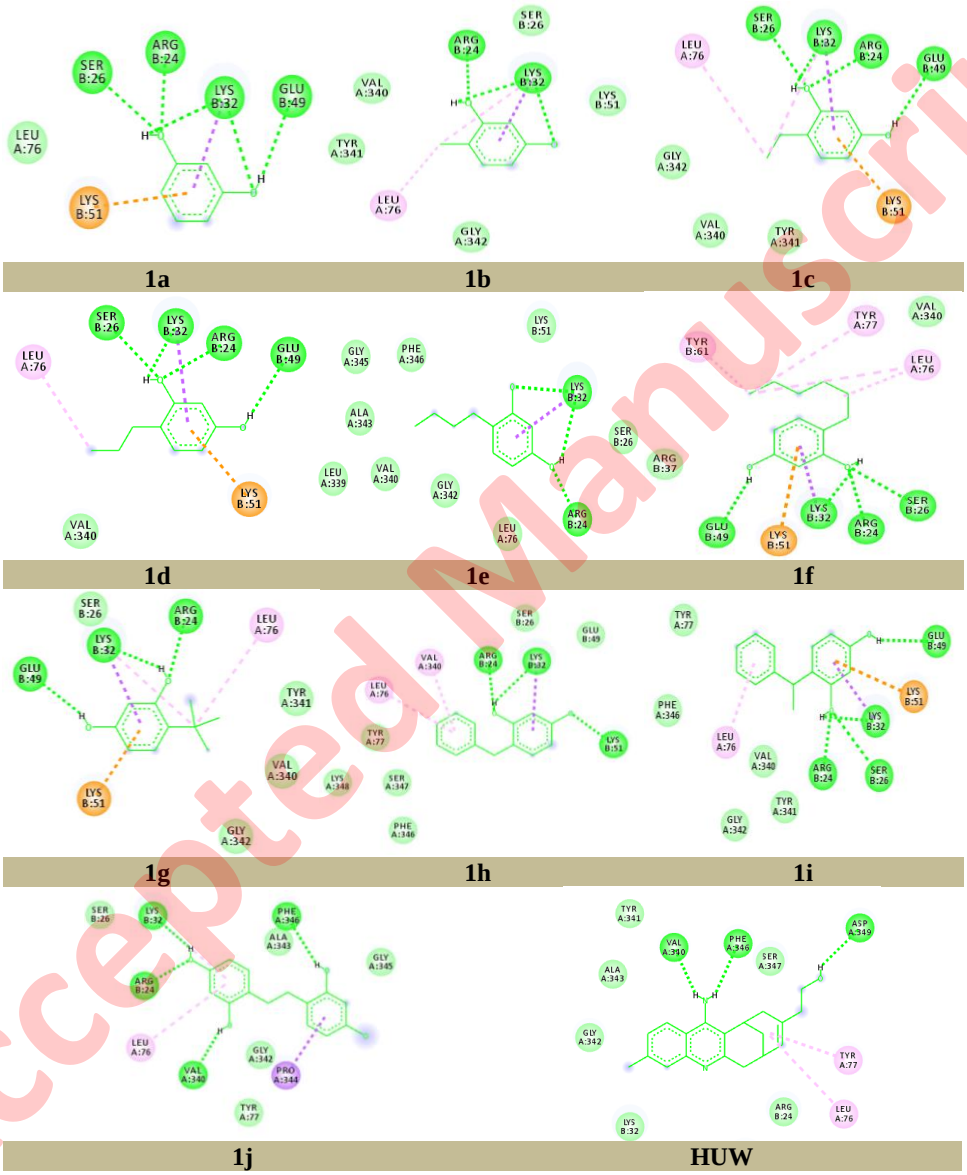

Figure S-1. 2D interactions of benzene 1,3 diol derivatives with in active site of AChE enzyme

It is well known that alkyl group showed inductive electron donating effect in all medium. Electron donation causes the shielding effect due to which carbon and

hydrogen atoms of the benzene ring resonate at higher frequency. It was observed that hexyl substituent was involved in the formation of π-alkyl bonding with TYR77, TYR61 and LEU76. Furthermore, hydroxyl group of compound **1f** formed strong hydrogen bond with SER26, ARG24, GLU49 and LYS32. Moreover, parent aromatic ring formed π-sigma interaction with LYS32 which stabilized the protein-ligand complex. In addition to π-sigma bonding, aromatic ring was involved in strong π-cation interaction with LYS51. The π-cation interaction was involved in stable electrostatic interaction between a cation and polarizable cloud of π electrons. Other interaction was included Van der Waals interaction with VAL340. Compound **1g** was substituted with tertiary butyl substituent which didn't show any significant difference with **1f**. It was observed that branched chain butyl group substituent had no significant effect on interacting amino acid residues, in fact the current substitution decreased the free binding energy to -20.05 kJ/mol. Overall binding interactions were similar to the compound **1f**.

BChE docking studies

Bonding and non-bonding interaction of amino acid residues of BChE enzyme with benzene 1,3 diol derivatives (S-Figure 2). It was found that docked conformation of compound **1a** showed reasonable bonding and non-bonding interactions with BChE than AChE. The amino acid residues which were involved in bonding and non-bonding interactions were as follows: ASP70, GLY78, TRP430, ALA328, TYR440, MET437, and SER78. It was notable that highly electronegative oxygen atom of OH group present at 1^{st} position of benzene ring was involved in making strong hydrogen bond with TRP430 residue. Whereas, GLY78 was involved in making hydrogen bond with electropositive hydrogen atom of OH group at 1^{st} position of benzene ring. It is evident that OH group have strong electronegativity difference which can exert strong electrostatic force of attraction. Moreover, it was also involved in imparting positive inductive effect *(+I)*. Similarly, ASP70 was involved in hydrogen bond formation with OH group present at 3^{rd} position of benzene ring. It was noticed that TYR332 was involved in π-donor hydrogen bond with benzene ring of resorcinol. Moreover, ALA328 was involved in strong π-alkyl bonding with core benzene ring of interacting compound. π-alkyl bonding is significant as it was involved in interaction of π-electronic cloud of benzene ring and alkyl group of amino acid residue. Docking score of current conformation was found to be -17.58 kJ/mol.

The amino acid residues which were involved in bonding and non-bonding interactions with compound **1b** were as follows; ASP70, GLY78, TYR440, MET437, ALA328, TYR332, TRP430 and SER79 (Figure 6). It was observed that interacting compound exhibited binding energy of -20.68 kJ/mol with possessed value of 472 µM which was due to presence of methyl group at 4^{th} position of benzene ring. It is well known that methyl group imparts the positive mesomeric

effect by donating electrons to core benzene ring. Furthermore, substituted methyl group had strong π-alkyl and alkyl interactions with π-electronic cloud of TYR440, MET437 and ALA328. Moreover, it was also observed that OH groups present at 1st and 3rd position of benzene ring were involved in strong hydrogen bond with GLY78 and ASP70 of activation loop. Furthermore, it was notable that parent benzene ring also showed major contribution toward interacting amino acid residues through the formation of strong π-π stacked and π-alkyl bond with TYR332 and ALA328 residues, respectively. Other interacting amino acid residues like HIS438, TRP82 and SER79 were involved in formation of Van der Waals interactions with that compound.

Docked conformation of compound **1c** with BChE enzyme showed that following amino acid residues were involved in bonding and non-bonding interactions; ASP70, GLY78, TYR440, TRP430, TYR332, PHE329, ALA328 and SER79. It was notable that substituted ethyl group has approximately same electron donating tendency as that of methyl group due to which it was imparting positive mesomeric effect (+*M*). Docking score of compound **1c** *i.e.*, -20.89 kJ/mol didn't show any significant difference with compound **1b**. Moreover**,** substituted ethyl group was also involved in formation of strong π-alkyl, alkyl and π-sigma bonding with ALA328, TYR332 and PHE329 respectively. Furthermore, it was observed that OH groups played vital role in forming strong inhibiting interactions with amino acid residues of active site. It was found that carboxylate end of ASP70 and GLY78 formed hydrogen bond with electropositive hydrogen atom of hydroxyl group present at 1st and 3rd position of benzene ring, respectively. Moreover, TRP430 and TYR440 was found to be involved in hydrogen bond formation with oxygen atoms of hydroxyl group. Another major interaction was formation of strong π-π stacked bonding between benzene ring and TYR332. Moreover, aromatic ring was also involved in π-alkyl bonding with ALA328. Other amino acid residues which were involved in Van der Waals interactions with compound **1c** were MET437 and MET81.

The amino acid residues which were involved in bonding and non-bonding interactions with compound **1d** were as follows; GLY78, ASP70, ALA328, PHE329, TYR332, SER79 and MET437. In present compound, propyl group was substituted at 4th position of benzene ring due to which docking score was slightly better than compound **1c**. The docking score of **1d** was calculated as -23.19 kJ/mol with predicted inhibitory constant value of 171 µM. The substituted propyl group has ability to donate electrons and impart the positive mesomeric effect (+*M*). Another significance of propyl group was the formation of strong π-sigma and π-alkyl interaction with TYR332 and PHE329 residues of active site. It was obvious that hydroxyl groups played vital role in determining the inhibiting potential of benzene 1,3 diol derivatives. It was found that both OH groups formed strong hydrogen bond with ASP70 and GLY78. Presence of core

aromatic ring was also playing significant role in inhibitory potential of these derivatives as it was involved in π-alkyl interaction with ALA328. SER79 and TRP 430 were involved in Van der Waals interaction.

The docked conformation of another 1,3 diol derivative *i.e.*, **1e** was observed to show strong bonding and non-bonding interactions with amino acid residues of active site of enzyme. The following amino acid residues were involved; SER287, LEU286, HIS438, PHE329, TRP231, PHE398, ALA199, GLY116 and GLY117. Compound **1e** had substitution of butyl group at 4th position of aromatic ring. It was observed that substituted butyl group was making strong π-sigma with TRP231 and π-alkyl bonding with HIS438, PHE239, PHE398 and ALA199 of BChE enzyme but same compound lacked these important interactions with AChE enzyme which suggested that these compounds had better inhibitory potential against BChE enzyme with docking score of -24.82 kJ/mol. Furthermore, hydroxyl groups were involved in forming hydrogen bond with SER287 and LEU286. Moreover, LEU286 also formed π-alkyl bond with core aromatic ring. This π-alkyl bonding significantly stabilized the protein- ligand interaction.

The amino acid residues which were involved in bonding and non-bonding interactions with compound **1f** were as follows; HIS438, GLU197, SER198, TRP82, PHE398, TRP231, GLY116, GLY117 and GLY439. Present compound showed significant docking score that was -25.03 kJ/mol. It might be due to presence of long chain hexyl substituent. It is well known that alkyl group show inductive electron donating effect in all medium. Electron donation causes the shielding effect due to which carbon and hydrogen of benzene ring resonate at higher frequency. It was observed that hexyl substituent was involved in formation of π-sigma, π-alkyl and π-π T-shaped interactions with TRP231, PHE298, HIS438 and TRP82, respectively. Furthermore, hydroxyl group of compound **1f** formed strong hydrogen bond with SER198, GLU197 and HIS438. Moreover, parent aromatic ring formed π-cation interaction with HIS438. The π-cation interaction was stabilizing the electrostatic interaction between a cation and polarizable cloud of π-electrons. Other interactions were included Van der Waals interaction with GLY116 and GLY117. The compound **1g** was substituted with tertiary butyl substituent which didn't show significant difference with compound **1e** which was substituted with n-butyl chain. It was observed that branched chain butyl substituent had no significant effect on the interacting amino acid residues, in fact current substitution decreased the free binding energy to -23.19 kJ/mol so it was concluded that tertiary butyl had negative impact on docking energy**.** Overall binding interactions of compound **1f** was similar to compound **1e**.

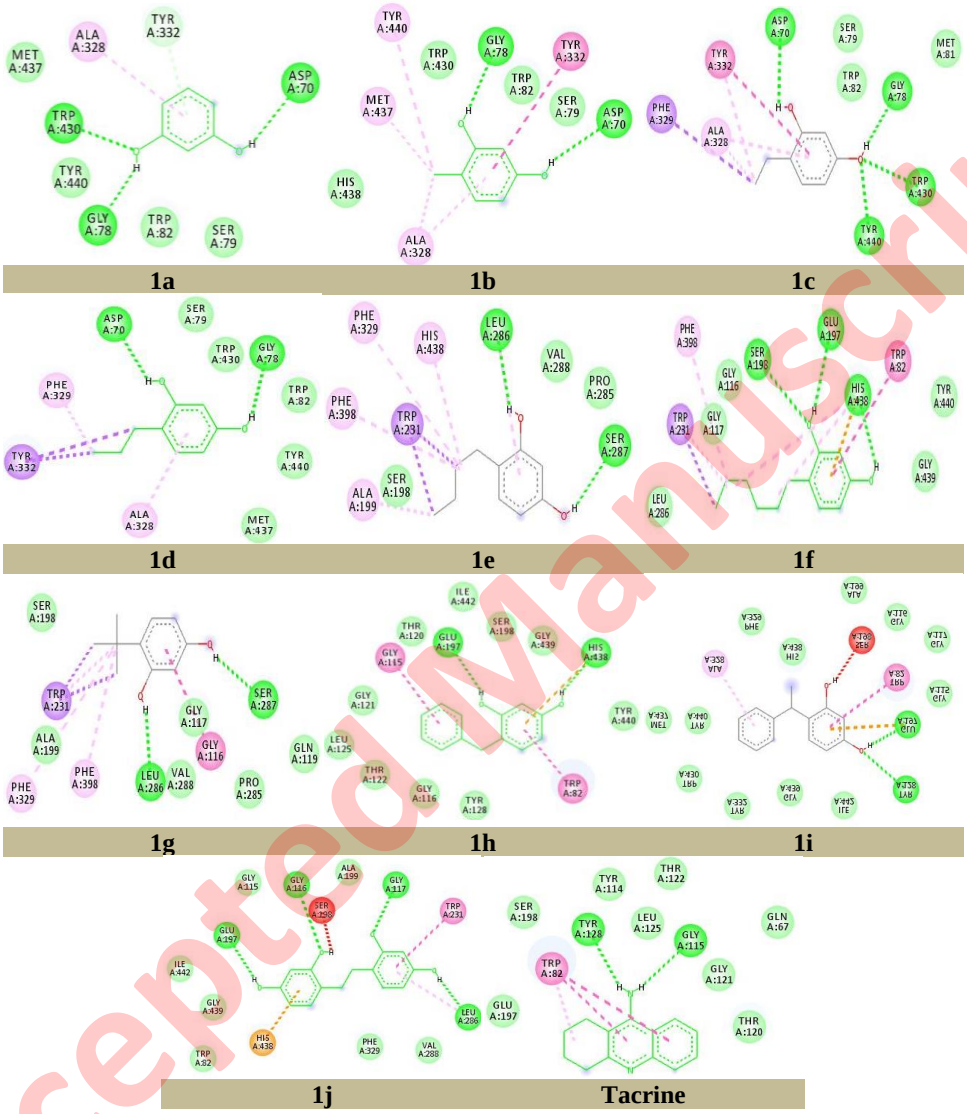

Figure S-2. 2D interactions of benzene1,3 diol derivatives with in active site of BChE enzyme.