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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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Table S-I. Structures of the selected compounds for study

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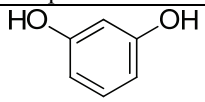
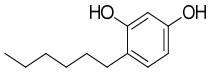
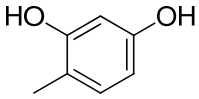
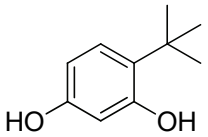
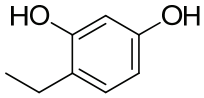
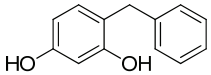
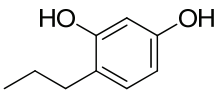
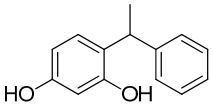
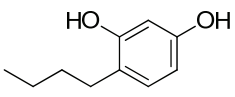
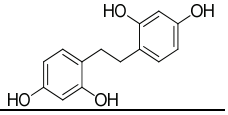
5054	1a		3610	1f	
10333	1b		3014087	1g	
17927	1c		75294	1h	
87874	1d		11171903	1i	
205912	1e		24849532	1j	

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AChE docking studies

Bonding and non-bonding interaction of the amino acid residues of AChE enzyme with benzene-1,3-diol derivatives are shown in Fig. S-1. The amino acid residues which were involved in bonding and non-bonding interactions with, the compound **1a** were SER26, ARG24, LYS32, GLU49, LEU76 and LYS51. It was notable that the 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 the benzene ring. It is evident that the OH group has strong electronegativity difference which can excert 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 the OH group present at 3rd position of the benzene ring. The LYS32 was also involved in making π-sigma bond with the benzene ring of resorcinol. Moreover, LYS51 was involved in strong π-cation interaction with the core benzene ring of interacting compound. Docking score of the 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 the interacting compound exhibited binding energy of -12.60 kJ/mol, which was due to the presence of methyl group at 4th position of benzene ring. It is well known that methyl group imparts the positive mesomeric effect, by donating electrons to the core of the benzene ring. It was also observed that OH groups present at 1st and 3rd position of the benzene ring were involved in making a strong hydrogen bond with LYS32 and ARG24 of activation loop. Furthermore, it was notable that the 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 the interacting compound.

The 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, (Fig. 4). It was notable that the substituted ethyl group had the electron donating tendency, which was imparting the positive mesomeric effect (+*M*). The substituted ethyl group was also involved in the formation of strong π-alkyl bond with LYS32 and LEU76 residues of active site. Furthermore, it was observed that the OH groups played the vital role in forming strong inhibiting interactions with amino acid residues of the active site. It was found that the carboxylate end of LYS32 and GLU49 was forming a hydrogen bond with the hydrogen atom of hydroxyl

group, which is present at position 1st and 3rd, respectively. Moreover, SER26 and ARG24 was found to be involved in the 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 a strong π-cation bond between benzene ring and LYS51. This π-cation was stabilizing the electrostatic interaction between a cation and a polarizable electronic cloud of the aromatic ring. Moreover, the aromatic ring was also involved in the π-sigma bonding with LYS32. Other amino acid residues which were involved in the 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 the 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 the positive mesomeric effect (+*M*). Another significance of propyl group included the strong π-alkyl interaction with LEU76 residue of the active site. It was obvious that hydroxyl groups played the vital role in the determination of the 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 the hydroxyl group, whereas other two formed hydrogen bond with the positive hydrogen atom. The presence of core aromatic ring also played a significant role in the 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 the substitution of butyl group at 4th position of aromatic ring. It was surprising to be seen that the butyl group was theo nly exposed to Van der Waals interactions, with few amino acid residues *i.e.*, ALA343, GLY345, PHE346 and VAL340. Whereas, the hydroxyl group was involved in making hydrogen bond with ARG24 and LYS32. Previously, it was seen that SER26 and GLU49 were also involved in the hydrogen bond formation, with the both hydroxyl groups, but in present case it was not observed. Moreover, LYS32 was again involved in forming π-sigma bond with the core aromatic ring. This π-sigma bonding significantly stabilizes the protein-ligand complex. The binding score for the 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. The last compound showed the significant docking score, -20.59 kJ/mol, and it can be suggested that it might be due to presence of long chain hexyl substituent.

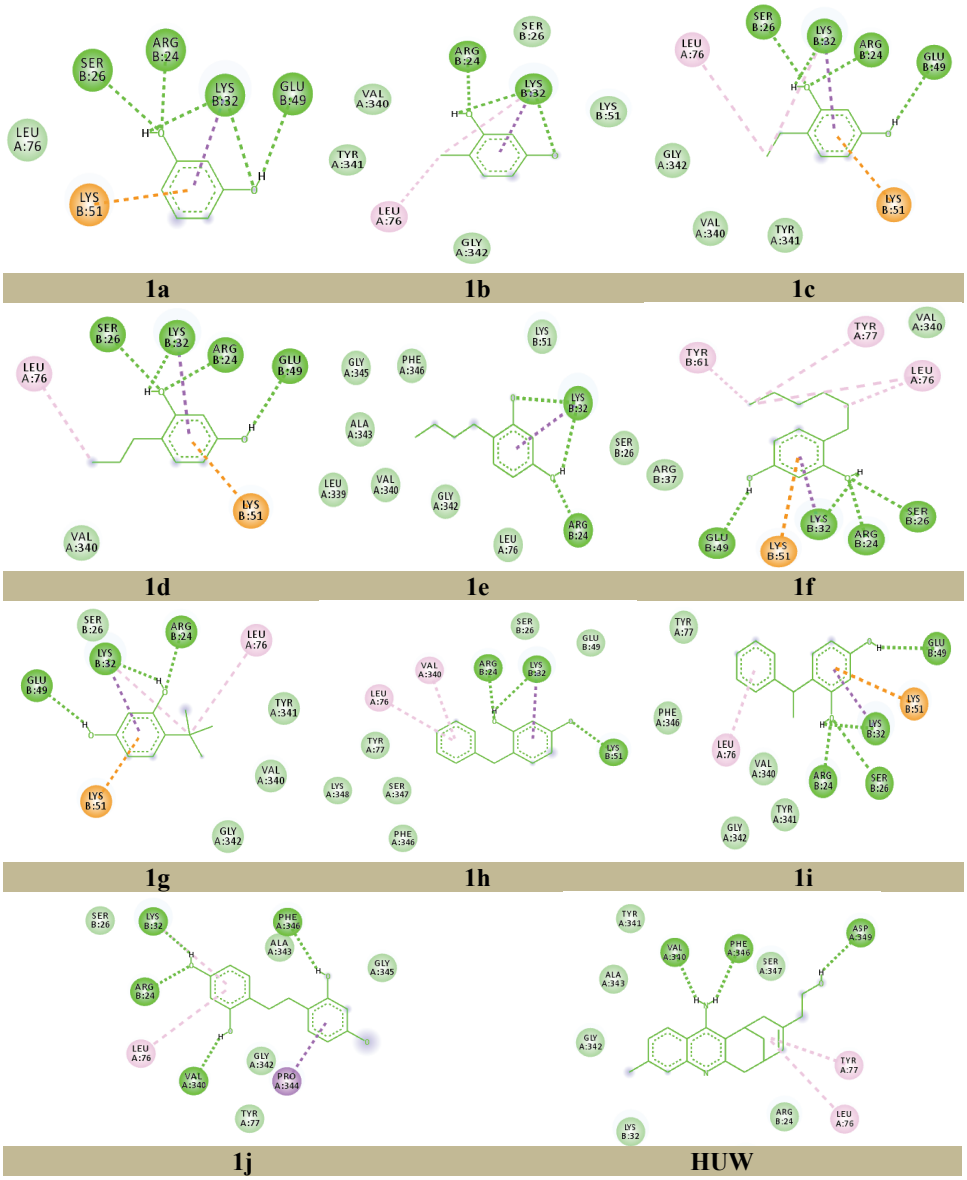

Fig. S-1. 2D interactions of benzene 1,3 diol derivatives with in active site of AChE enzyme.

It is well known that alkyl groups showes the inductive electron donating effect in all medium. The 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 the hexyl substituent was involved in the formation of π-alkyl bonding with TYR77, TYR61 and LEU76. Furthermore, the hydroxyl group of compound **1f** formed strong hydrogen bond with SER26, ARG24, GLU49 and LYS32. Moreover, the 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 included Van der Waals interaction with VAL340. The compound **1g** was substituted with the tertiary butyl substituent, which didn't show any significant difference with **1f**. It was observed that the branched chain butyl group substituent had no significant effect on the interaction with amino acid residues, in fact the the current substitution decreased the free binding energy to -20.05 kJ/mol. The 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 are shown in Fig. S-2. It was found that the 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 the highly electronegative oxygen atom of OH group present at 1st 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 1st position of benzene ring. It is evident that the 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 3rd position of benzene ring. It was noticed that TYR332 was involved in a 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 an interaction of π-electronic cloud of benzene ring and alkyl group of amino acid residue. The 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 the interacting compound exhibited the binding energy of -20.68 kJ/mol with the value of 472 μM, which was due to presence of methyl group at 4th position of benzene ring. It is well known that the methyl group imparts the positive mesomeric effect by donating electrons to the core benzene ring. Furthermore, the 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 a strong hydrogen bond with GLY78 and ASP70 of the activation loop. Furthermore, it was notable that the parent benzene ring also showed major contribution toward the interaction of the amino acid residues through the formation of strong π-π stacked and π-alkyl bond with TYR332 and ALA328 residues, respectively. The other interacting amino acid residues like HIS438, TRP82 and SER79 were involved in the formation of Van der Waals interactions with that compound.

The docked conformation of compound **1c** with BChE enzyme showed that the 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 the substituted ethyl group has approximately the same electron donating tendency as that of the methyl group, due to which it was imparting positive mesomeric effect (+*M*). The docking score of the compound **1c** *i.e.*, -20.89 kJ/mol didn't show any significant difference with the compound **1b**. Moreover**,** the substituted ethyl group was also involved in the 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 the strong inhibiting interactions with the amino acid residues of the active site. It was found that the carboxylate end of ASP70 and GLY78 formed the hydrogen bond with the 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 the oxygen atoms of hydroxyl group. Another major interaction was the formation of strong π-π stacked bonding between the benzene ring and TYR332. Moreover, the aromatic ring was also involved in π-alkyl bonding with ALA328. The 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 the present compound, the 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 the predicted inhibitory constant value of 171 μM. The

substituted propyl group has the 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 the active site. It was obvious that the 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. The presence of core aromatic ring was also playing a significant role in the 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 the 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 the substitution of butyl group at 4th position of aromatic ring. It was observed that the substituted butyl group was making strong π-sigma with TRP231 and π-alkyl bonding with HIS438, PHE239, PHE398 and ALA199 of BChE enzyme, but the 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, the hydroxyl groups were involved in forming hydrogen bond with SER287 and LEU286. Moreover, LEU286 also formed π-alkyl bond with the 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. The present compound showed significant docking score that was –25.03 kJ/mol. It might be due to the presence of long chain hexyl substituent. It is well known that alkyl groups show the inductive electron donating effect in every medium. The electron donation causes the shielding effect due to which carbon and hydrogen of benzene ring resonate at higher frequency. It was observed that the hexyl substituent was involved in the formation of π-sigma, π-alkyl and π-π T-shaped interactions with TRP231, PHE298, HIS438 and TRP82, respectively. Furthermore, the hydroxyl group of compound **1f** formed a strong hydrogen bond with SER198, GLU197 and HIS438. Moreover, the parent aromatic ring formed the π-cation interaction with HIS438. The π-cation interaction was stabilizing the electrostatic interaction between a cation and the polarizable cloud of π-electrons. The other interactions were included Van der Waals interaction with GLY116 and GLY117. The compound **1g** was substituted with the tertiary butyl group which didn't show significant difference with compound **1e**, which was substituted with *n*-butyl chain. It was observed that the branched chain butyl

substituent had no significant effect on the interacting amino acid residues, in fact the current substitution decreased the free binding energy to -23.19 kJ/mol, therefore it was concluded that tertiary butyl had the negative impact on docking energy**.** The overall binding interactions of compound **1f** was similar to compound **1e**.

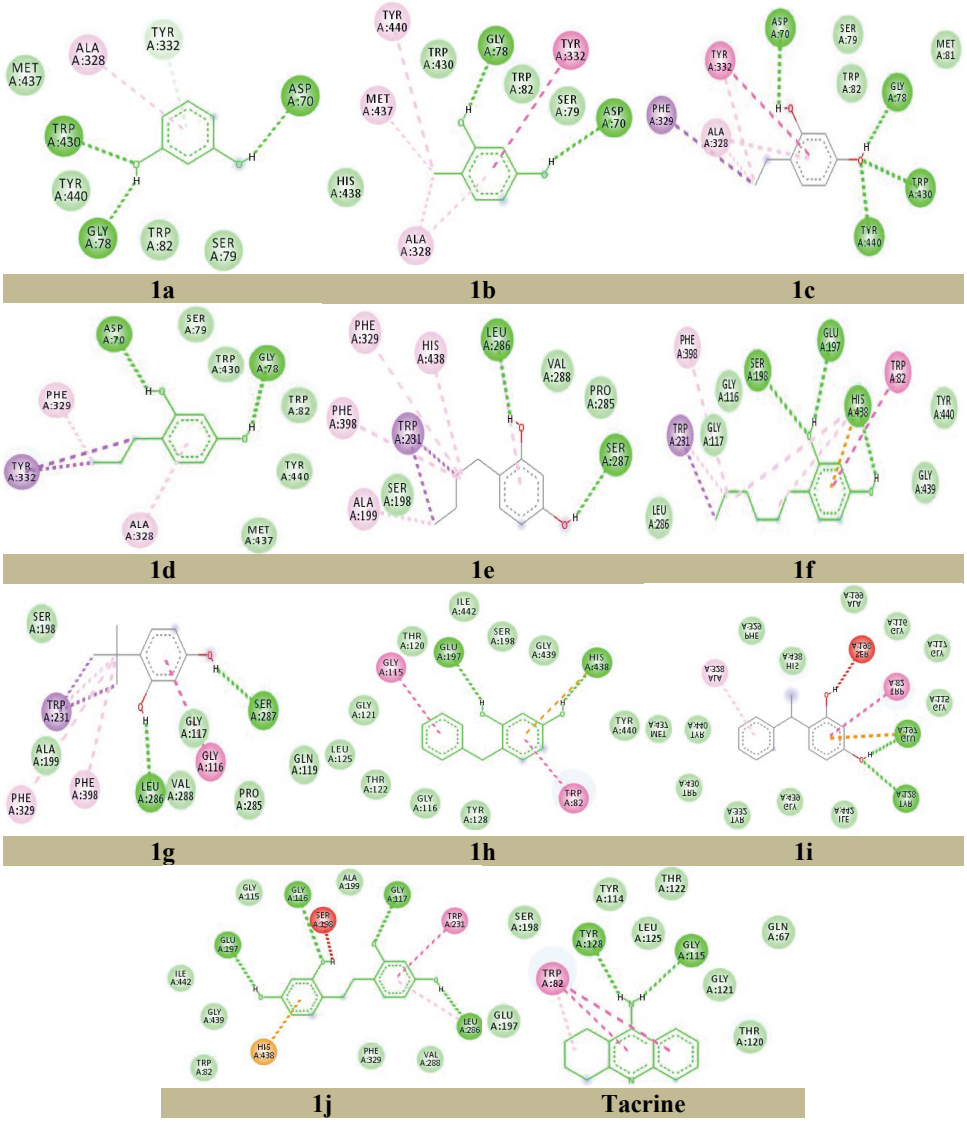

Fig. S-2. 2D interactions of benzene1,3 diol derivatives with in active site of BChE enzyme.