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Investigation of cholinergic inhibition by donepezil-rimegepant hybrids in alzheimer's disease: An *in silico* study

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Alzheimer's disease (AD) is a neurodegenerative disorder that causes damage to brain cells, resulting in memory loss, thinking, and executive skills. AD is widely recognized as a most prevalent type of dementia that occurs in elder people. Although few medications like donepezil are available to treat AD, a new and effective drug is the need of the hour. Hence, in the present study, an *in silico* approach is used to investigate the cholinergic inhibition by donepezil-rimegepant hybrids. Inspired by the structure of donepezil and rimegepant, Donepezil-Rimegepant (DR) hybrids are generated using a hybridization-based design strategy. Their biological activity towards acetylcholinesterase, (AChE) butyrylcholinesterase (BuChE) and amyloid β (Aβ) peptides is investigated using computational techniques such as DFT and molecular docking. Our DFT calculations indicate that the DR13 is highly stable than the other hybrids considered in this study. Similarly, our molecular docking study revealed that DR13 has strong interactions with AChE, BuChE, and Aβ peptide. While considering donepezil and the rimegepant region in DR hybrid, later has strong binding with the protein targets considered in this study. This is due to the presence of two F atoms in the benzyl region of rimegepant. These halogens form strong noncovalent interactions with the amino acid residues in the protein targets. Therefore, based on our *in silico* studies DR13 is proposed as a good candidate molecule for designing a new drug against AD.

Keywords: AChE, Alzheimer's disease, Density functional theory, Donepezil, Molecular docking, Rimegepant

Alzheimer's disease (AD) is a neurodegenerative disorder that causes dementia in elderlypeople[1]. This disorder damages the central nervous system, causing mental and physiological problems[2,3]. Around 55 million people suffer from AD worldwide, for which there is now no known therapy. The USA Food and Drug Administration (FDA) has only licensed five medications for use in current pharmacotherapy forAD. In that, four medications are AChE inhibitors (tacrine, donepezil, rivastigmine, and galantamine)[4]. The fifth drug is memantine, which is a NMDA (N-methyl-D-aspartate) receptor antagonist[5]. Further, these medications are unable to treat AD, but they can slightly reduce its symptoms. Modern treatments for AD mainly focus on managing symptoms and slowing down the progression of the disease, but there is ongoing research into potential new treatments and therapies.

While the precise causes of AD are still unknown, a number of factors, including oxidative stress, tauprotein aggregation, Aβ peptide deposits, neuroinflammation, and deficiencies in acetylcholine, are thought to be important in the disease's pathophysiology[6]. Previous research has demonstrated that AChE inhibitors may increase acetylcholine (ACh) levels in AD patients by blocking AChE. AChE and BuChE are the two forms of cholinesterase (ChE) that hydrolyse ACh in the brain. However, AChE decomposes ACh more frequently than BuChE[7]. In order to increase the amount of ACh and treat AD, AChE must be inhibited[8]. AChE is a member of the carboxylesterase family and is present in a variety of conducting tissues, including sensory fibers, peripheral and central tissues, and so on. BuChE is primarily produced in the liver and secreted into the plasma; it is also found in other organs. In the cerebral cortex of humans, there are roughly two orders of magnitude with fewer BuChE-rich neurons than AChE-rich neurons. Among these two, AChE is responsible for the hydrolysis of ACh, and the role of BuChE is not known clearly. Similarly, Aβ is neurotoxic and causes quicker aggregation, contributing to the development and progression of AD[9]. The amyloid precursor protein (APP) is separated by the beta secretase enzyme, resulting in the production of Aβ. It is possible to generate Aβ with varying lengths of amino acids,

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resulting in Aβ40 and Aβ42, which are 40 and 42 amino acid residues, respectively.

Donepezil is a well-known and extremely effective FDA-approved medication among the studied AChE inhibitors[10]. It works by inhibiting the enzyme AChE. By inhibiting AChE, donepezil increases the concentration of ACh in the brain, which can improve communication between nerve cells and potentially alleviate some of the symptoms of AD. As mentioned earlier, donepezil is not able to treat AD, but it can reduce the symptoms. It is useful for the treatment of mild-to-moderate levels of AD. In addition, the currently available drugs for AD cause adverse effects such as weight loss, vomiting, nausea, diarrhoea*etc*[11]. Earlier, Morton *et al*. revealed that migraines are a significant risk factor for AD and dementia[12]. Dementia is a most common neurological disease that occurs in elder people, and headache is a neurological disorder that occurs in all age groups. Medication derivatives such as Atogepant, Rimegepant, Ubrogepant, and MK-3207 are used to treat migraines. Among them, rimegepant is used for the treatment of migraine headaches. It is a member of calcitonin gene-related peptide (CGRP) receptor antagonists. CGRP is a neuropeptide involved in the pathophysiology of migraine, and blocking its action can help alleviate migraine symptoms.

The development of hybrids ligands has attracted increasing attention in recent years for a variety of therapeutic uses, including the treatment of AD[13]. A popular method for creating novel medication hybrid is the hybridization-based design strategy, which combines the pharmacophoric properties of two medications into a single molecule[14]. Researchers have looked into hybrids that contain antioxidant action, metal chelation, or anti-inflammatory activities in addition to AChE inhibition[15]. In the last few years, enormous number of research studies on AChE inhibition by hybrids were reported[16, 17]. For instance, Chandrika *et al*. have discussed about the inhibition of both Cholinesterase and Aβ fibril assembly by chalcone-donepezil hybrids[18]. They identified chalcone-donepezil hybrids as bifunctional molecules against two hallmarks of AD. They have concluded that chalcone hybrids could be used for the treatment of AD. Similarly, Yepes *et al*. have studied the neuroprotective properties of novel donepezil-M30D hybrids for AD treatment[19]. They concluded that M30D and donepezil can be hybridized to produce biologically active compounds. These hybrids show promise as scaffolds for the development of novel, plasma-stable neuroprotective drugs to treat AD.

Although plenty of research studies on investigating AChE inhibition by hybrids were reported in the literature, the research continues to find out the most efficient medication for AD. This is due to the above-mentioned adverse effects as well as the inefficacy of current medications. Hence, the present work is focused on the investigation of new Donepezil-Rimegepant (DR) hybrids with the potential ability to interact with different targets of AD. In particular, the DR hybrids are investigated to understand their possible anti-Alzheimer activity using *in silico* techniques. DFT, and molecular docking techniques are used to investigate the cholinergic inhibition of these hybrids. DFT calculations are performed to study the structure, stability, and reactivity of the selected hybrids. The optimized hybrids are docked with AChE, BuChE, and Aβ peptides and studied for their inhibition properties. We hope this study will provide an opportunity for the design of subtype-selective enzyme inhibitors.

Computational Details

Selection of hybrids and protein targets

In the present study, donepezil and rimegepant are chosen to generate hybrids. The optimised 2d structure for donepezil and rimegepant is given in (Fig. 1). The experimental geometry of donepezil (Pubchem ID: 3152) and rimegepant (Pubchem ID: 51049968) is obtained from Pubchem database[20]. There are eighteen hypothetical hybrids of donepezil-rimegepant are generated (Fig. 2A & B) using a hybridization-based design strategy. Earlier AD studies have chosen the following protein targets for the investigation of cholinergic inhibition. They are 1EVE, 3I6M, 4EY7, 4PQE PDB IDs for AChE, 1P0I, 2Y1K, 4BDS, 4TPK PDB IDs for BuChE, and 2BEG,

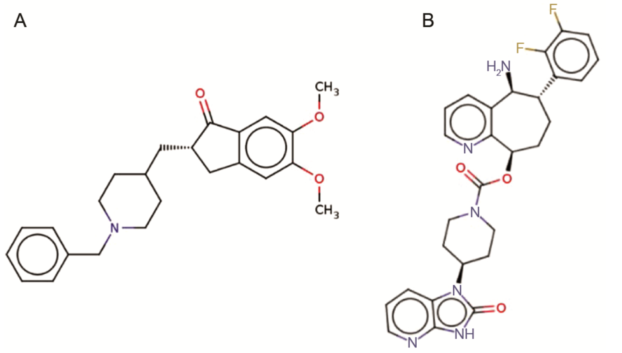


Fig. 1 — Optimized molecular (2d) structure of (A) Donepezil; and (B) Rimegepant

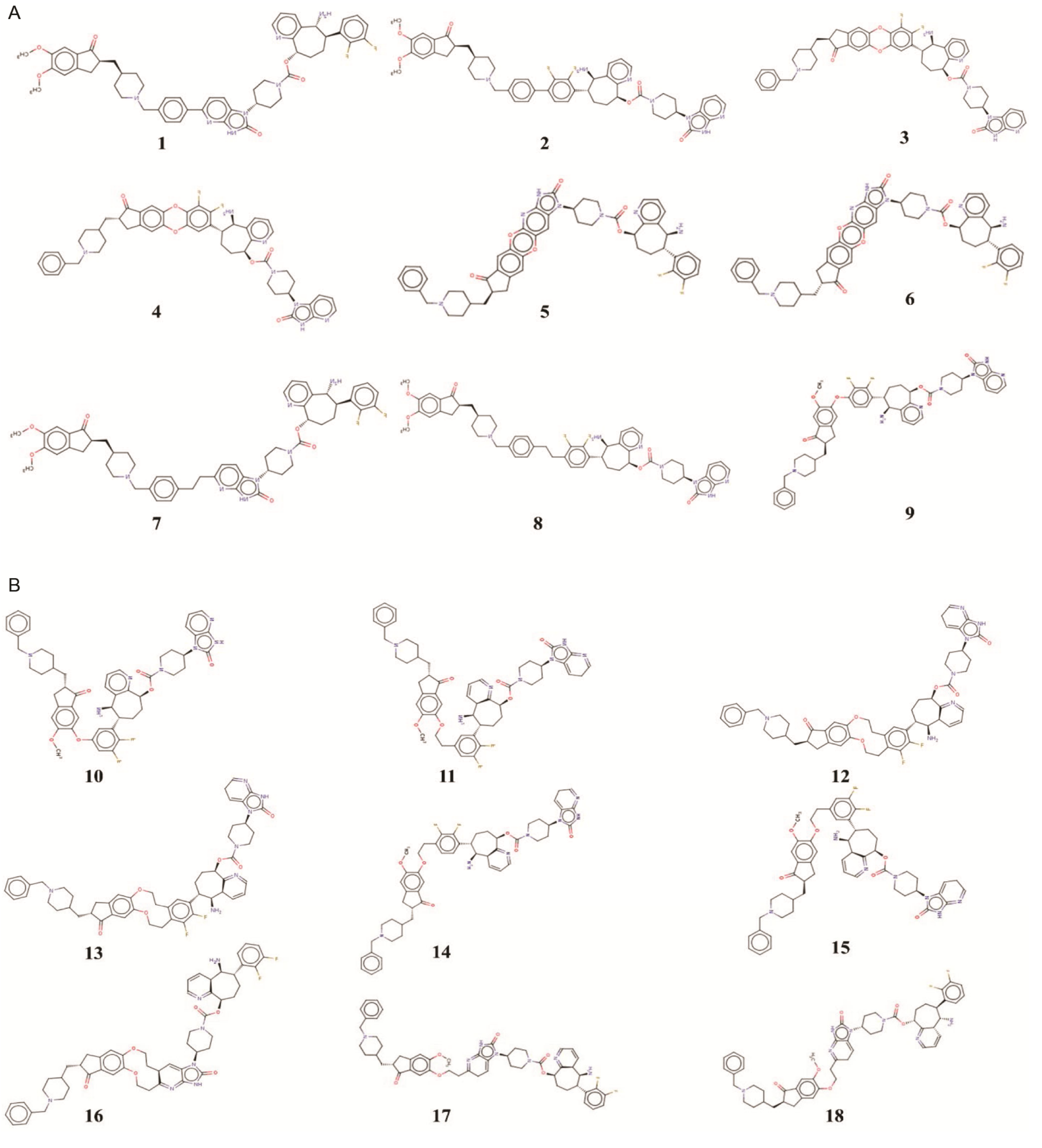


Fig. 2 — Optimized molecular (2d) structure of Donepezil-Rimegepant (DR) hybrids (A) (1-9); and (B) (11-18)

1IYT for Aβ peptide. Hence, we chose the above mentioned PDB IDs, and their crystal structures were obtained from the protein data bank for our docking study[21]. The 3D crystal structure of selected PDB IDs is given in (Fig. 3).

DFT calculations

All the selected DR hybrids, along with donepezil and Rimegepant, are investigated using the DFT method. In particular, the molecular geometries of all generated hybrids, donepezil and Rimegepant, are

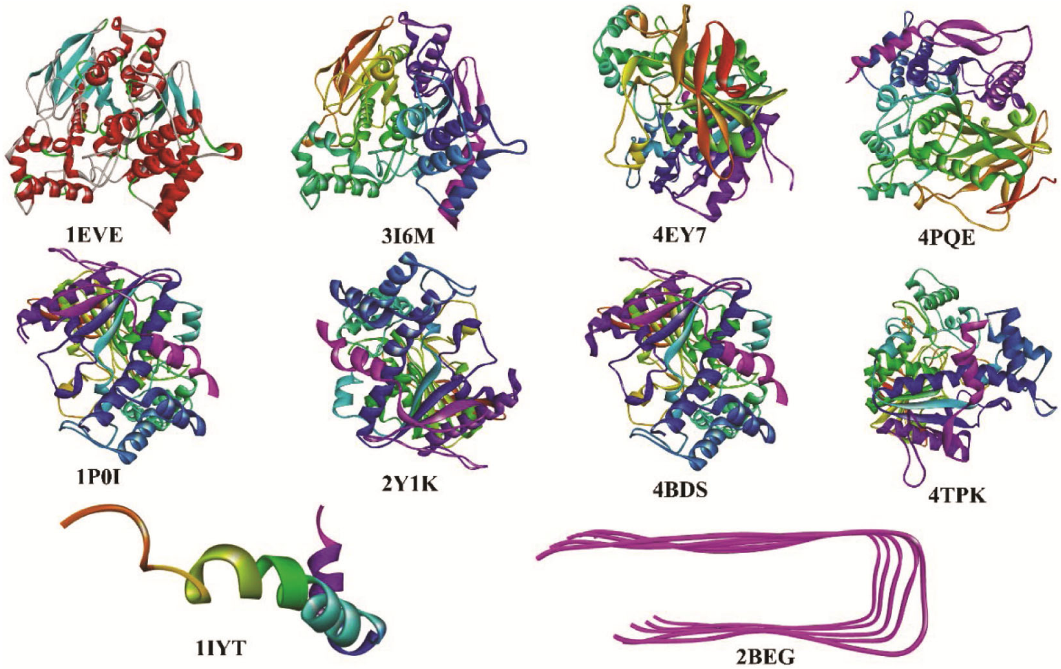


Fig. 3 — 3D Crystal structure of Acetylcholinesterase (1EVE, 3I6M, 4EY7, 4PQE), butyrylcholinesterase (1P0I, 2Y1K, 4BDS, 4TPK), and Amyloid beta (1IYT, 2BEG) peptide

optimized using B3LYP functional with def2-TZVPP basis set[22]. Earlier studies have mentioned that B3LYP functional with def2-TZVPP shows better results on drug related systems[23, 24]. The chemical hardness (η), chemical potential (μ) and electrophilicity index (ω) are calculated using the highest occupied molecular orbital (E_{HOMO}) and the lowest unoccupied molecular orbital (E_{LUMO}) energies. They are,

$$\eta = \frac{I-A}{2} \quad ...(1)$$

$$\mu = -\frac{I+A}{2} \quad ...(2)$$

$$\omega = \frac{\mu^2}{2\eta} \quad ...(3)$$

In Eq. (1 & 2), where $I = -E_{HOMO}$ and $A = -E_{LUMO}$, where I and A are the ionization potential and electron affinity of the molecules, respectively. In Eq. (3), where μ and η are the chemical hardness and chemical potential of the molecule, respectively, all the DFT calculations are performed using ORCA 5.0 program[25].

Molecular Docking

The molecular docking study is performed for all the selected DR hybrids, donepezil and Rimegepant, towards AChE (1EVE, 3I6M, 4EY7, 4PQE), BuChE (1P0I, 2Y1K, 4BDS, 4TPK) and Aβ (1IYT, 2BEG). Standard cleaning procedures have been done before the docking. The proteins are prepared using Biovia Discovery Studio by subtracting cofactors, water molecules, and metal ions and adding charges and polar hydrogen atoms[26]. The prepared proteins are loaded into PyRx and converted into macromolecules. All the selected hybrids are converted into PDB style for input to AutoDock Vina in PyRx. Finally, the proteins and the hybrids are converted into PDBQT and ready for docking. All the selected DR hybrids are docked with AChE, BuChE and Aβ using PyRx program[27].

Results and Discussion

Structure and reactivity of ligands

DFT calculations are performed to obtain more detailed molecular-level information in particular, the structural stability and reactivity parameters of the selected DR hybrids. The drug molecules such as donepezil and rimegepant are optimized using B3LYP functional with def2-TZVPP basis set. The optimized molecular structures in 2D of donepezil and rimegepant are given in (Fig. 1). Similarly, all the selected DR hybrids are optimized using B3LYP functional with def2-TZVPP basis set. The optimized structures in 2D of the selected DR hybrids are given in (Fig. 2A & B). Donepezil consists of indanone, piperidine, and benzyl regions, along with carboxylic groups (Fig. 1A). Similarly, Rimegepant consists of indanone, piperidine, cyclohexanopyridine, and

Table 1 — Calculated dipole moment (μ_M in D), energy gap (E_g in eV), Ionization potential (I in eV), electron affinity (A in eV), chemical hardness (η in eV), chemical potential (μ in eV) and Electrophilicity index (ω in eV)

hybrids	μ_M	E_{HOMO}	E_{LUMO}	E_g	I	A	η	μ	ω
DR1	7.04	-5.6971	-1.6051	4.09	5.70	1.61	2.05	-3.65	3.26
DR2	2.46	-6.0250	-1.5875	4.44	6.03	1.59	2.22	-3.81	3.26
DR3	6.67	-5.9923	-1.8162	4.18	5.99	1.82	2.09	-3.90	3.65
DR4	8.96	-6.0034	-1.8161	4.19	6.00	1.82	2.09	-3.91	3.65
DR5	6.46	-5.6081	-1.7689	3.84	5.61	1.77	1.92	-3.69	3.54
DR6	2.97	-5.6087	-1.7731	3.84	5.61	1.77	1.92	-3.69	3.55
DR7	7.58	-5.9171	-1.5666	4.35	5.92	1.57	2.18	-3.74	3.22
DR8	4.55	-5.9834	-1.5831	4.40	5.98	1.58	2.20	-3.78	3.25
DR9	4.18	-6.0045	-1.7834	4.22	6.00	1.78	2.11	-3.89	3.59
DR10	10.63	-6.0136	-1.6015	4.41	6.01	1.60	2.21	-3.81	3.29
DR11	8.65	-5.9879	-1.5633	4.42	5.99	1.56	2.21	-3.78	3.22
DR12	8.22	-5.9740	-1.4280	4.55	5.97	1.43	2.27	-3.70	3.01
DR13	11.46	-5.9150	-1.3730	4.54	5.92	1.37	2.27	-3.64	2.92
DR14	9.17	-5.9900	-1.6151	4.37	5.99	1.62	2.19	-3.80	3.31
DR15	6.01	-6.0030	-1.6062	4.40	6.00	1.61	2.20	-3.80	3.29
DR16	8.89	-5.7846	-1.4540	4.33	5.78	1.45	2.17	-3.62	3.02
DR17	3.28	-5.9550	-1.4390	4.52	5.96	1.44	2.26	-3.70	3.03
DR18	2.68	-5.9516	-1.5216	4.43	5.95	1.52	2.22	-3.74	3.15
Donepezil	3.75	-5.7223	-1.2984	4.42	5.72	1.30	2.21	-3.51	2.79
Rimegepant	6.29	-6.0897	-1.1512	4.94	6.09	1.15	2.47	-3.62	2.65

benzyl regions, along with amino and carboxylic groups (Fig. 1B). In addition, it also consists of two F atoms bonded with a benzene ring. These functional groups/rings exist in the selected hybrids as well as. These groups are favourable sites for the formation of noncovalent interactions with the specific region in the enzyme. Noncovalent interactions such as hydrogen bonding, vander Waals, halogen bonding and π–π interactions can exist when these ligands dock with enzymes.

In general, long-range electrostatic forces have a significant impact on the properties of biomolecules. These forces arise due to the presence of permanent electric dipole moments in the system. In the case of proteins, the particular arrangements of atoms will induce a strong electric field. This is the reason for the secondary structures such as α-helix and β-sheet possess large macro-dipoles. The calculated dipole moments of the selected hybrids are given in (Table 1) and illustrated in (Fig. 4). From Figure 4 it can be seen that the DR13 has a higher dipole moment than the other selected hybrids (11.46 D) and standard drugs. The large dipole moment indicates that this hybrid is highly polar in nature. DR10 has the second highest dipole moment (10.63 D). The dipole moment of the selected hybrids varies from 2.46 D (DR2) to 11.46 D (DR13) (Table 1). The First tenDR hybrids based on dipole moment values are 13 > 10 > 14 > 4 > 16 > 11 > 12 > 7 > 1 > 3. The hybrids with a higher dipole moment indicate that those hybrids are highly stable in the liquid state. Further, the calculated dipole moments of donepezil and rimegepant are 3.75 and 6.29 D, respectively. Most of the selected hybrids have a higher dipole moment than these standard drugs. That is, 4 hybrids have a lesser dipole moment than donepezil, and 7 hybrids have a lesser dipole moment than rimegepant. Earlier, Junaid *et al.* have calculated dipole moment of the metal-based donepezil analogues. They found that the metal containing donepezil analogues have a higher dipole moment (> 10 D)[28].

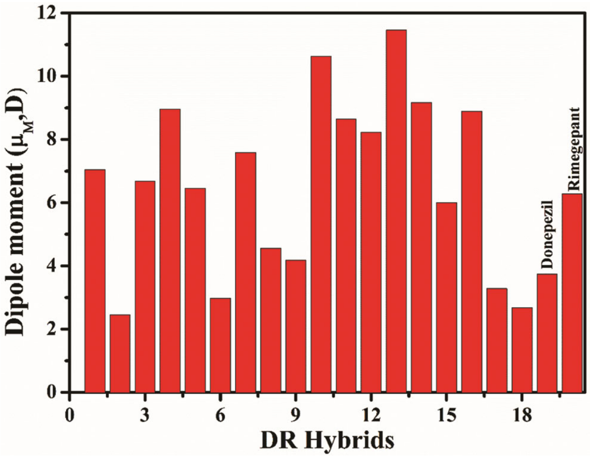


Fig. 4 — Calculated dipole moment values of eighteen donepezil-rimegepant (DR) hybrids

The frontier molecular orbital analysis is performed to obtain the reactivity parameters of the selected DR hybrids. The calculated reactivity parameters such as

HOMO and LUMO energies, energy gap, ionization potential, electron affinity, chemical hardness, chemical potential, and electrophilicity index are given in (Table 1). The energy gap and chemical hardness values of all hybrids are plotted and illustrated in (Fig. 5). From Table 1, it can be seen that the calculated energy gap value varies from 3.5 to 4.25 eV for all selected hybrids. This indicates that all the selected hybrids are highly stable. In particular, DR12 and DR13 have higher energy gap values of 4.55 and 4.54 eV, respectively. The order of stability of 10 most stable hybrids is 12 > 13 > 17 > 2 > 18 > 11 > 10 > 8 > 15 > 14. According to the maximum hardness principle, the molecule with a higher chemical hardness value is highly stable[29]. A similar trend is noted between energy gap and chemical hardness values (Fig. 5) in the present study. That is same order of stability is noted between the energy gap and chemical hardness. In general, (HOMO) shows the electron donor nature, and the (LUMO) shows the electron receptor nature of the molecule. The energy gap between HOMO and LUMO represents the chemical stability, hardness, and softness of the molecule[30]. The HOMO and LUMOs of donepezil, Rimegepant, and DR13 are given in (Fig. 6). The blue color indicates HOMO, and the green color indicates LUMO, respectively. In donepezil, HOMOs are localized in the piperidine ring, and LUMOs are localized in the indanone ring. In rimegepant, HOMOs are localized in the indanone ring, and LUMOs are localized in the cyclohexanopyridine ring. During the formation of hybrids, large transfers take place between the functional groups as well as rings. This leads to HOMO and LUMO electrons being localized in the donepezil region (electron donor nature) only (Fig. 6A). In particular, HOMO electrons are

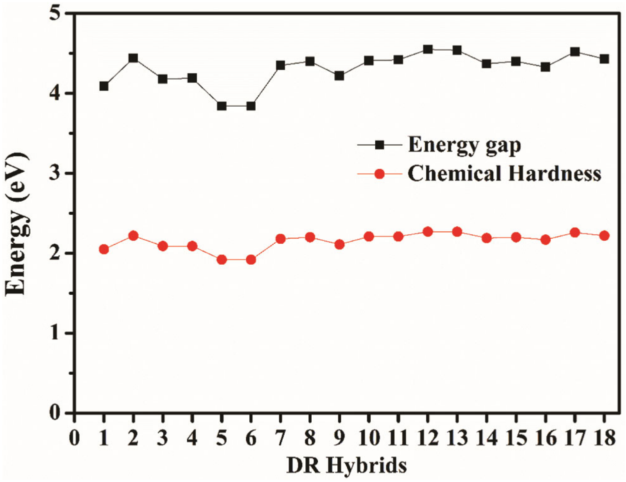


Fig. 5 — Calculated energy gap and chemical hardness values of eighteen donepezil-rimegepant (DR) hybrids

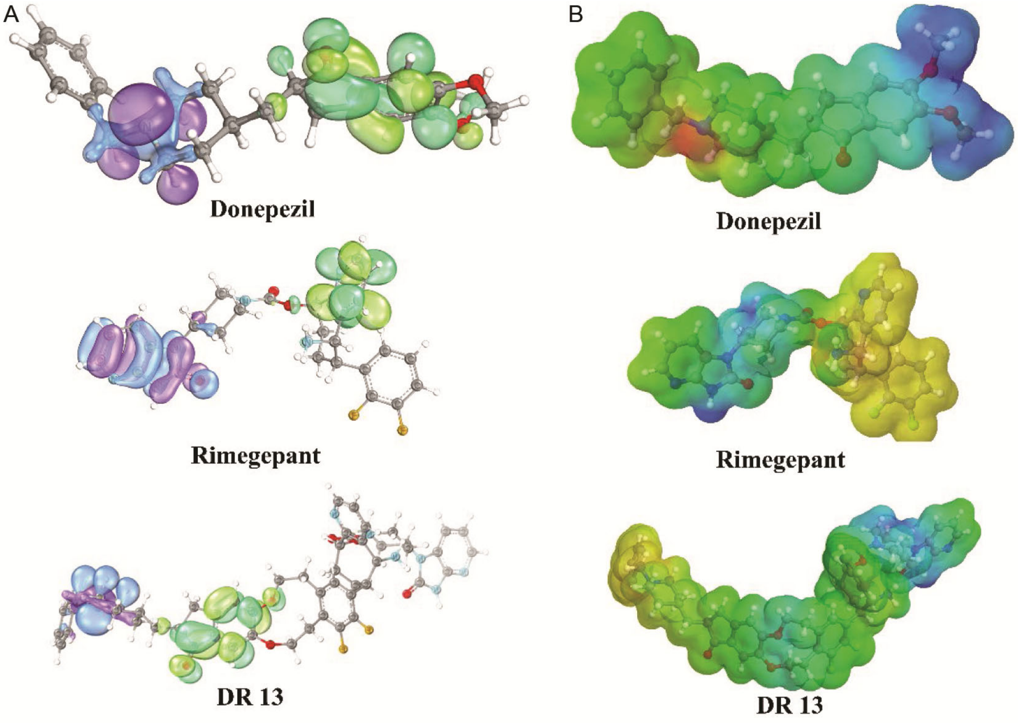


Fig. 6 — (A) HOMO and LUMO; and (B) MEP plot for Donepezil, Rimegepant and DR13 hybrid

localized at the indanone ring with an energy of -5.9150 eV. LUMO electrons are localized in the piperidine as well as the benzene rings with an energy of -1.3730 eV. This indicates that these regions are favourable for the formation of noncovalent interactions with the AChE, BuChE and Aβ peptides considered in this study. No HOMO and LUMO electron localization is noted in the rimegepant region of DR13. The positive HOMO and LUMO energy values are connected to the ionization potential and electron affinity of the system, respectively. It must be noted that organic molecules have a higher ionization potential[31]. A similar trend is observed in the present study as well. That is, the ionization potential value of the selected hybrids varies from 5.61 to 6.03 eV. The calculated ionization potentials of donepezil and rimegepant are 5.72 and 6.09 eV, respectively. Similarly, the lower electron affinity (~ 2 eV) noted for the studied hybrids. The ability of the hybrids to absorb electrons is measured by the electrophilicity index, which is based on chemical potential and chemical hardness. Among the selected hybrids, the electrophilicity index value is low (2.92 eV) for DR13 only. This indicates, the DR13 is more stable as well as bioactive than the other studied hybrids. Earlier, Zhou *et al.* mentioned that compounds with a lower global electrophilicity index are highly stable[32]. This trend coincides well with our calculated energy gap and chemical hardness values. All other hybrids have an electrophilicity index value is greater than 3 eV. Further, the energy gap, ionization potential, electron affinity, and chemical hardness values of DR13 lies between the values of donepezil and rimegepant. However, chemical potential and electrophilicity index values are greater than those of donepezil and rimegepant drugs.

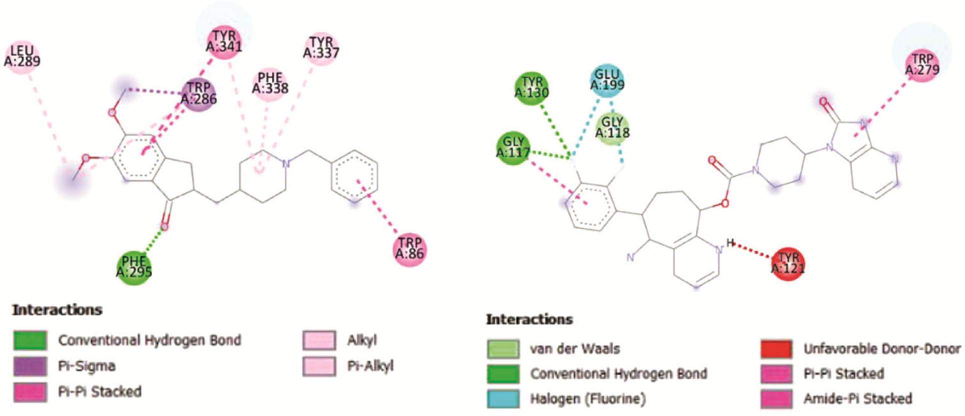
The molecular electrostatic potential (MEP) map can be used to study the relationship between the molecular structure and its physiochemical properties[33]. That is, MEP is used to understand the electrostatic effects produced by the charge distribution in the systems. MEP is related to electron density, and it will be useful to predict molecular active sites, recognition, and hydrogen bonds. Because, the accurate prediction of molecular recognition and the interaction of two molecules are important in the field of drug design. The MEP surface of the donepezil, Rimegepant, and DR13 is shown in (Fig. 6B). The electrophilic and nucleophilic regions are shown in green color and blue color, respectively. The light blue color indicates zero potential. From the (Fig. 6B), it can be seen that all the oxygen atom sites represent the most negative potential region (dark red), whereas fluorine and nitrogen are blue or green color. This indicates these sites are less negative regions. In general, these sites must be more negative because they are electronegative elements. Large charge delocalization turns these sites to be less negative. Similarly, hydrogen atom sites are represented by light blue or dark blue in color, *i.e.* positive potential regions. In the DR13, green color is predominant in the entire DR region; this indicates that these sites are electrophilic in nature. Further, the red color in piperidine ring in donepezil indicates that, it is electron rich site and favourable for non-covalent interactions than the other sites. This trend coincides well with the HOMO and LUMO electron localizations in DR hybrids. Both HOMO and LUMO electrons are localized in the donepezil region only (Fig. 6A).

Molecular Docking analysis

The molecular docking study is performed to investigate the allosteric binding mode of the ligand with an appropriate biological effect on the enzyme[34]. In the present study, it is performed to understand the best binding mode (active region) in the selected hybrids as well as the best ligand for cholinergic inhibition. AChE is a member of the hydrolase class that has 531 amino acid residues in a single chain (A). Proteins have 9% helices, 49% beta-sheets, 41% coils, and 21% twists in their three-dimensional structure. Similarly, BuChE is a member of the hydrolase class that has 529 amino acid residues in a single chain (A). In the case of Aβ peptide, either 40 or 42 amino acid residues are present. The molecular docking study is performed for all the DR hybrids towardsAChE (1EVE, 3I6M, 4EY7, 4PQE), BuChE (1P0I, 2Y1K, 4BDS, 4TPK) and Aβ peptide (1IYT, 2BEG). Similarly, the docking study is performed on the standard drugs donepezil and rimegepant towards AChE, BuChE and Aβ peptide for the comparison purpose. The selected protein targets of AChE, BuChE and Aβ peptide and theirbinding sites are given in (Table 2). Previously reported binding sites of the selected protein targets are considered for the molecular docking study. Earlier studies have mentioned that AChE has two distinct binding sites, they are peripheral anionic site (PAS) and the catalytic active site (CAS). It is well known that PAS acts as a favourable site for the substrate acetylcholine. Tryptophan (TRP86) is an important

Table 2 — Protein targets and their active sites

cholinesterase /amyloid	PDB IDs	Active sites
AChE	1EVE	Trp279, Arg289, Tyr121, Glu129, Trp84, His440, Phe331, Arg289
	3I6M	Tyr 70, Asp 72, Ty121, Trp 279, Tyr 334, Tyr341, Phe295
	4EY7	Tyr72, Trp86, Trp286, Ser293, Phe95, Tyr337, Phe338, Tyr 341
	4PQE	Tyr 121, Trp 279, Tyr 70, Asp 72, Tyr 334, Trp 84, Tyr 130, Phe 330
BuChE	1P0I	Asn241, Asn245, Pro281, Phe278, Tyr282, Arg240, Tyr237
	2Y1K	Glu197, Gly115, Tyr332, Tyr128, Ser198
	4BDS	Trp82, Trp430, Ala328, His438, Glu197, Ser19
	4TPK	Asp70, Trp82, Trp231, Leu286, Val288, Ser198, His438
Aβ	1IYT	Asp1, Ala2, Arg5, His6
	2BEG	Leu17, Val18, Phe19, Phe20, Ala21, Val36 and Val 40



Donepezil(4EY7) **Rimegepant (3I6M)**

Fig. 7 — Best pose of 2d interaction of Donepezil and Rimegepant with the proteins considered in this study

amino acid residue that forms an intermolecular interaction with the substrate ACh.

Molecular docking of donepezil and rimegepant towards AChE, BuChE and Aβ peptide

The molecular docking study is performed on donepezil and rimegepant towards AChE, BuChE, and Aβ. The best docking poses of these drugs are given in Figure 7 and the binding energy values are given in (Table 3). It must be noted that plenty of molecular docking studies were performed on donepezil with AChE and BuChE[35] as well as various drug candidates with AChE and BuChE. Similarly, the molecular docking study of rimegepant on various proteins is also reported in the literature[36]. However, no docking study of rimegepant with AChE or BuChE or Aβ is reported in the literature. Among the selected protein targets, donepezil shows strong interaction with 4EY7 (-11.9 kcal/mol) in AChE, 4BDS (-10.2 kcal/mol) in BuChE, and 2BEG (-6.5 kcal/mol) in Aβ (Table 3). Similarly, the donepezil shows better inhibition activity towards AChE than BuChE and Aβ peptide protein targets considered in this study. This trend coincides well with previous experimental studies.Earlier, Bolea *et al*. studied inhibition activity of donepezil against AChE and BuChE using IC_{50}value. They found that BuChE has a higher IC_{50} value than AChE[37]. Rimegepant shows strong interaction with 3I6M (-13.1 kcal/mol) in AChE and 4TPK (-12.6 kcal/mol) in BuChE, and 2BEG (-7.3 kcal/mol) in Aβ. While comparing donepezil and rimegepant, the later exhibits a strong interaction with most of the protein targets considered in this study except 4EY7. In this case, donepezil exhibits strong interaction. The reason for the strong interaction of rimegepant with protein targets considered in this study is due to the presence of two F atoms in the benzene ring of rimegepant. These F atoms form a halogen bond with the amino acid residues, which makes the rimegepant region have a strong interaction with the amino acid residues of the protein targets. Earlier studies have mentioned that the indanone ring in donepezil has a direct π–π interaction with the AChE. Similarly, the benzyl region forms π–π

Table 3 — Calculated binding energy (in kcal/mol) donepezil-rimegepant hybrids and drug molecules towards AChE, BuChE and Aβ peptide

hybrids	AChE				BuChE				Aβ	
	1EVE	3I6M	4EY7	4PQE	1P0I	2Y1K	4BDS	4TPK	1IYT	2BEG
1	-12.6	-12.8	-10.8	-11.1	-13.0	-12.3	-12.4	-12.2	-8.6	-7.9
2	-12.1	-13.0	-12.0	-10.2	-12.7	-12.0	-11.9	-11.9	-8.1	-7.3
3	-14.4	-13.7	-15.6	-11.2	-13.4	-14.2	-13.6	-13.0	-8.6	-8.6
4	-14.3	-14.2	-14.6	-10.3	-12.7	-13.7	-12.8	-12.5	-8.2	-8.5
5	-14.0	-13.8	-15.7	-12.8	-13.2	-13.2	-13.5	-13.0	-8.2	-7.9
6	-14.0	-14.9	-15.6	-12.2	-11.6	-13.3	-13.3	-12.3	-8.5	-8.2
7	-11.1	-12.2	-11.2	-9.5	-12.9	-11.7	-12.9	-11.3	-8.6	-7.2
8	-10.2	-12.4	-11.2	-10.7	-10.8	-10.6	-11.9	-11.3	-7.6	-7.7
9	-12.5	-12.4	-13.3	-10.8	-12.4	-12.4	-12.9	-13.3	-8.3	-7.4
10	-13.5	-12.9	-14.2	-10.4	-11.5	-11.9	-12.1	-12.3	-7.1	-7.7
11	-13.2	-11.8	-14.1	-10.1	-12.2	-12.6	-12.7	-12.4	-6.3	-6.8
12	-14.7	-14.0	-14.4	-11.5	-13.6	-13.4	-14.0	-12.9	-8.2	-7.5
13	-15.5	-15.1	-14.6	-12.9	-14.0	-13.5	-14.1	-13.4	-8.7	-8.0
14	-13.1	-12.3	-13.2	-9.2	-11.2	-11.9	-11.4	-12.0	-6.9	-7.1
15	-10.5	-12.6	-12.9	-10.4	-12.4	-12.3	-12.9	-12.5	-6.5	-7.9
16	-14.4	-14.0	-14.0	-10.7	-12.5	-13.0	-12.1	-13.1	-8.8	-7.4
17	-12.2	-13.1	-12.8	-11.2	-10.4	-11.3	-11.3	-11.6	-6.4	-8.4
18	-12.3	-12.5	-13.5	-11.3	-11.3	-11.6	-12.3	-11.2	-7.7	-6.9
Donepezil	-11.1	-10.7	-11.9	-8.5	-9.9	-9.2	-10.2	-9.5	-6.1	-6.5
Rimegepant	-12.6	-13.1	-10.8	-10.3	-11.8	-11.7	-12.3	-12.6	-6.8	-7.3

interaction with the indole ring of Trp86 in AChE. Our study coincides well with the earlier molecular docking study. In the case of donepezil, π–π interaction takes place between the indanone ring and TYR341, TRP289, and TRP86 with the benzyl ring. Apart from these two, there are conventional hydrogen bond interactions that takes place between indanone and PHE295, π-alkyl interactions between piperidine and PHE338, TYR337. As mentioned above, rimegepant exhibits a strong interaction with 3I6M with a binding energy of -13.1 kcal/mol. The reason is the formation of halogen bond with GLU199, hydrogen bonds with TYR130 and GLY117, van der Waals interaction with GLY118, unfavourable donor-donor interactions with piperidine, π–π interactions takes place between indanone and TRP279.

Molecular docking of selected DR hybrids towards AChE, BuChE and Aβ peptide

All generated DR hybrids are docked in the binding sites of the selected protein targets, and their binding energy values are given in (Table 3). The best pose of hybrid interaction with the protein is determined by the lowest binding energy. The best pose of DR13 docked with the selected protein targets is given in (Fig. 8). From Table 3, it can be seen that DR13 has good and significant inhibitory activity towards AChE and BuChE protein targets except 4EY7. In this case, DR5 has a stronger interaction with 4EY7 (-15.6 kcal/mol) than the other hybrids considered in this study. This is due to multiple hydrogen bond interactions that take place between amino acid residues (PHE295 with CO group in the donepezil region, HIS287 with the CO group, and ASN283 with the amino group in the rimegepant region) in 4EY7 with DR5. Multiple π–π stacked interactions take place between TRP286 and the indanone ring in donepezil and rimegepant regions. In addition to this, when compared with DR5, less number of noncovalent interactions (conventional hydrogen bond, carbon hydrogen bond, π–π interaction, π-alkyl interaction) takes place between DR13 and amino acid residues in 4EY7. Further, it is interesting to see that hybrid such as DR5 and 13binds with both PAS (TRP286) and CAS (TRP86) active sites in 4EY7. As mentioned earlier, DR13 consists of Indanone, piperidine, and benzyl rings in donepezil as well as in rimegepant regions. These regions form noncovalent interactions such as conventional hydrogen bonds, halogen bonds, π-π interactions *etc* with amino acid residues in the protein targets considered in this study. Earlier Cheung *et al*. have mentioned that the indanone moiety is most favourable for the formation of a noncovalent bond in AChE[38]. A similar trend is noted in our study. That is, multiple interactions take place between the indanone ring in the donepezil region and the rimegepant region with amino acid residues of the selected protein targets are in (Fig. 8 & Suppl. Fig. S1). In particular, conventional hydrogen bonds and π-π interactions take place between

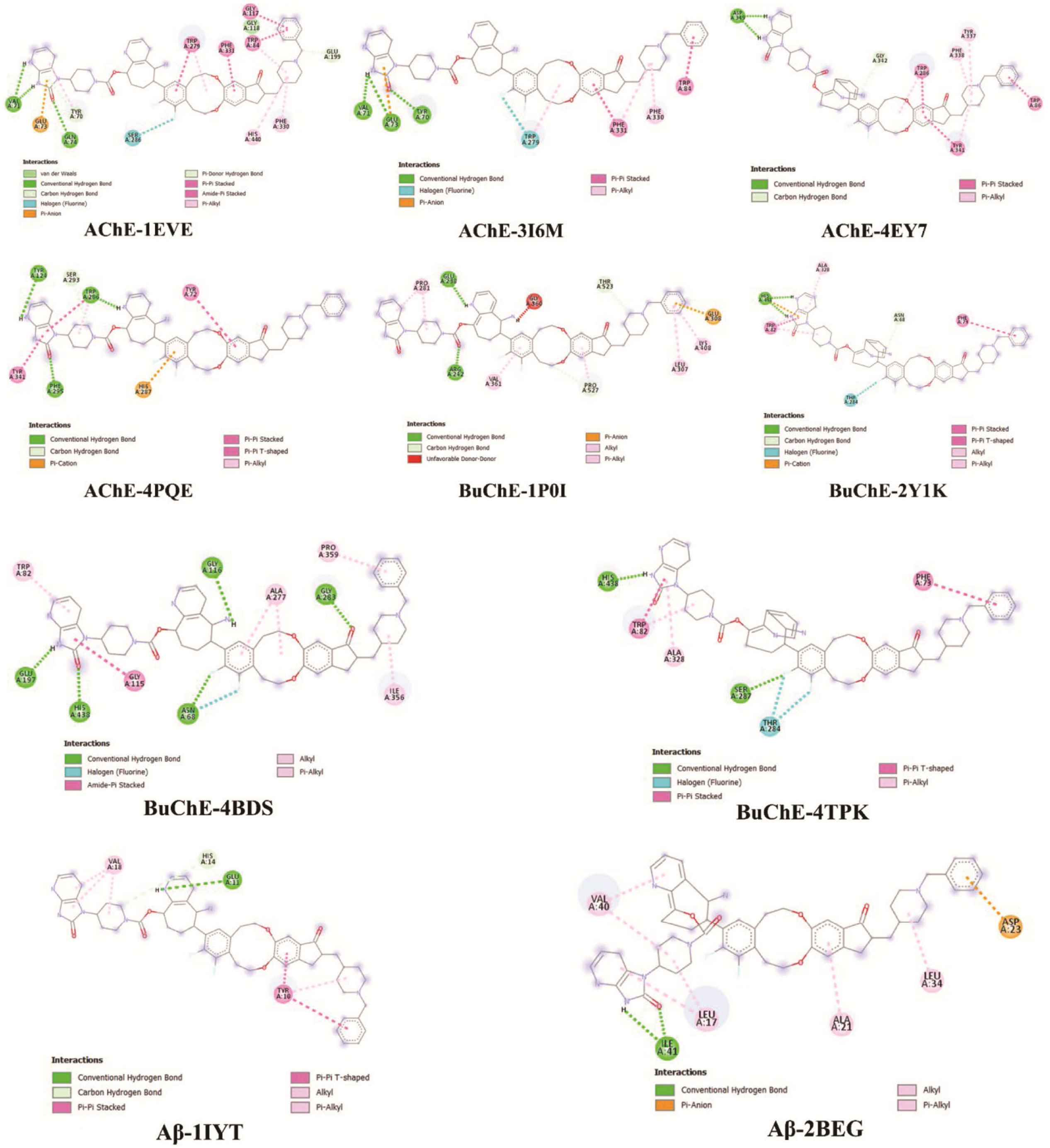


Fig. 8 — 2d interaction of best docking pose of DR13 with all protein targets considered in this study

donepezil as well as the rimegepant region with amino acid residues in most of the cases considered in this study. The best poses of other DR hybrids are given in (Suppl. Fig. S1).

In the case of BuChE, DR13 exhibits a strong interaction with all the BuChE proteins considered in this study, except 2Y1K. In this case, DR3 shows good inhibition activity (-14.2 kcal/mol) towards 2Y1K. The reason is, DR3 has more number (16) of noncovalent interactions with 2Y1K than DR13 (7). Further, multiple halogen bond interactions take place between DR3 and 2Y1K, whereas only one halogen bond interaction takes place in DR13. Similarly, in the case of the Aβ peptide, DR16 best docked with 1IYT and DR3 best docked with 2BEG (Fig. S1). This indicates that, these hybrids (DR16 and DR3) have good inhibition activity towards Aβ peptide than DR13. DR3 shows good inhibition activity (-8.6 kcal/mol) towards both Aβ peptides considered in this study. This is due to the greater number of noncovalent interactions take place between these hybrids and Aβ peptides. However, the interaction energy is less compared with AChE and BuChE. That is, the hybrids considered in this study show weaker interactions with Aβ peptide than AChE and BuChE. This is due to the absence of halogen bond formation between the rimegepant region and amino acid residues in the Aβ peptide. In most of the protein targets, the formation of a halogen bond with the rimegepant region yields strong interaction with the protein targets considered in this study are given in (Fig. 8). Overall, DR13 shows strong binding with most of the AChE, and BuChE and DR3 show strong interaction with Aβ peptide. In summary, our molecular docking study revealed that, rimegepant region interacts strongly with the amino acid residues of the protein targets considered in this study. This is due to the presence of halogen atoms in rimegepant, which makes strong halogen bonds with the amino acid residues. Although we are demonstrating DR13 has good inhibition activity towards AChE and BuChE, experimental validation is required to confirm this conclusion.

In order to identify the best hybrid towards each protein target considered in this study, the binding energies of selected DR hybrids towards each protein target are given in (Fig. 9). The PDB ID 1EVE is three-dimensional structure of AChE complexed with the Aricept drug (donepezil). In our study, DR13 has a strong interaction (-15.5 kcal/mol) with 1EVE. Earlier, Sahoo *et al*. have performed a molecular docking study on modified donepezil with 1EVE[39]. They have replaced the piperidine ring in donepezil with a natural product. They mentioned that the best interaction energy of the modified donepezil with 1EVE is -13.2 kcal/mol. The PDB ID 3I6M is 3D Structure of Torpedo californica AChE complexed with N-piperidinopropyl-galanthamine. DR13 showed strong interaction (-15.1 kcal/mol) with 3I6M in our study. Recently, Malik *et al*. have performed molecular docking study on Chalcone scaffolds with 3I6M[40]. Their calculated binding energy is -8.55 kcal/mol. Our calculated binding energy is comparably greater than their study. However, they have used auto dock software for their docking studies. The PDB ID 4EY7 is the crystal structure of recombinant human AChE, which is complexed with donepezil. As mentioned earlier, DR5 shows a strong interaction (-15.6 kcal/mol) with 4EY7. Recently, Pradeep *et al*. have performed *in silico* investigation of Anticholinesterase activity of Areca Catechu[41]. They found that deserpidine has strong interaction (-10.7 kcal/mol) with 4EY7. Our DFT calculations indicate that DR5 has lesser dipole moment (6.46 D) and energy gap (3.84 eV) values than DR13 (Table 1). Hence, DR13 is a more preferred choice than DR5 in this case. The PDB ID 4PQE is the crystal structure of human AChE. As mentioned earlier, DR13 has a strong interaction (-12.9 kcal/mol) with 4PQE. However, their binding energy is comparably less than the other AChEs considered in this study. The reason is halogen bond formation takes place in 4EVE and 3I6M, which is missing in 4PQE. Recently Furquan *et al*. have studied the interaction between cannabis constituents and AChE[42]. They found that the Tetrahydrocannabinol has a strong interaction (-9.3 kal/mol) with 4PQE, which is comparably less than our predicted energy.

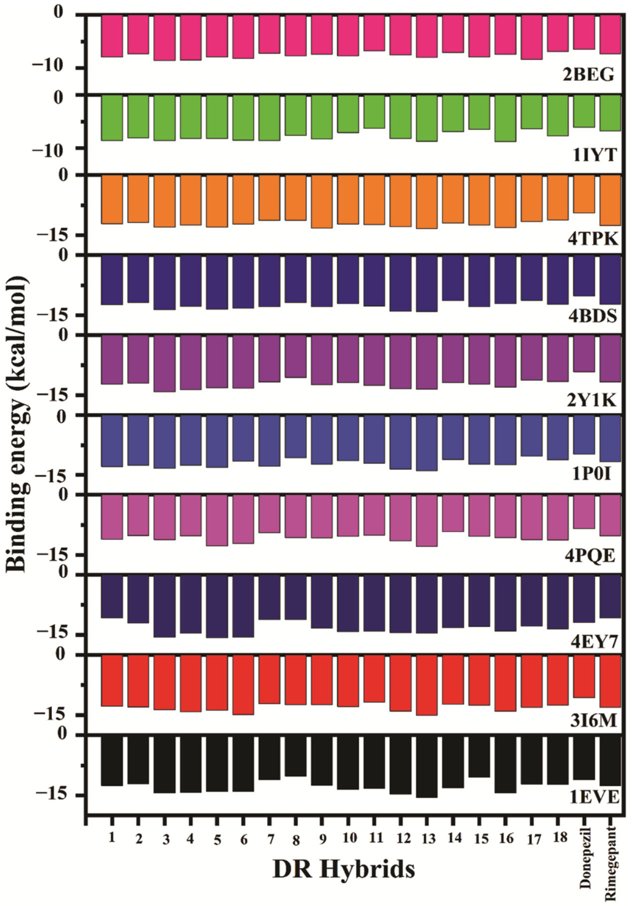


Fig. 9 — Binding energy interaction of all DR hybrids

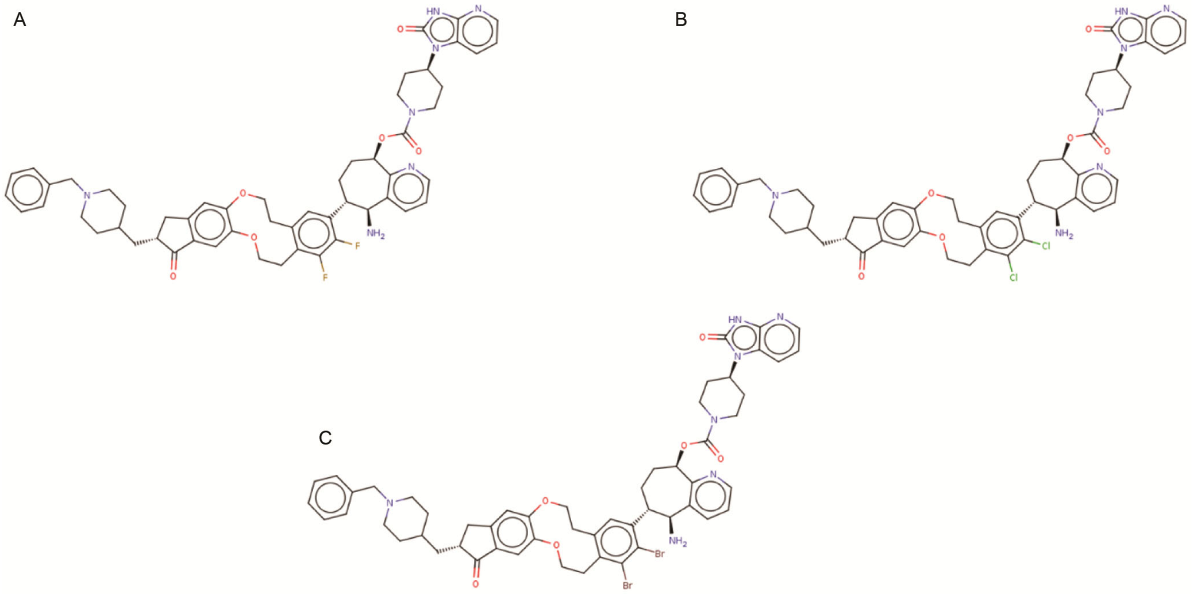


Fig. 10 — Optimized molecular (2d) structure of Halogen (A) F; (B) Cl; and (C) Br substituted DR hybrid

In the case of BuChE, the PDB ID 1P0I is the crystal structure of human BuChE. DR13 has a strong interaction (-14.0 kcal/mol) with 1P0I. Earlier, Khare *et al*. studied different flavonoids and alkaloids interaction with Bauhinia variegate[43]. They found that Dihydroquercetin has a strong interaction (-10.67 kcal/mol) with 1P0I. Their interaction energy is comparably less than our predicted energy. The PDB ID 2Y1K is the crystal structure of human BuChE inhibited by CBDP. Our docking study revealed that 2Y1K has a strong interaction with DR3. However, our DFT calculations suggested that DR3 has less dipole moment (6.67 D) and energy gap (4.18 eV) than DR13. This indicates that DR13 is more preferred than DR3 in this case. The PDB ID 4BDS is the crystal structure of human BuChE in complex with tacrine. The PDB ID 4TPK is the crystal structure of Human BuChE in complex with a naphthamide derivative. In both these cases, DR13 shows strong interaction with these BuChE protein targets, -14.1 kcal/mol for 4BDS, -13.5 kcal/mol for 4TPK, respectively. These values are comparably greater than the previous studies. In the case Aβ peptide, the PDB ID 1IYT represents solution structure of the amyloid beta-peptide, and 2BEG is the 3d structure of Aβ fibrils. As mentioned earlier, DR3 shows a strong interaction with both the protein targets. However, its dipole moment and energy gap are comparably smaller than DR13. This shows DR13 is the most favourable hybrid for binding with these protein targets. Although plenty of molecular docking studies are available in the literature, we have included very few previous studies in the above discussion. Similarly, the variation of binding energy values in the above discussion is due to the interaction that takes place between different amino acid residues and functional groups in the selected ligands. Further, one must understand that the protein targets considered in this study have different roles in AD. The aim of the present study is to demonstrate how donepezil-rimegepant hybrids bind to these protein targets. Then to identify the functional groups in the hybrids that are involved in binding with these protein targets. From the knowledge gained from our study, one can design a new potential candidate for the AD drug.

Halogenated DR13

A key approach for lead optimization in drug development is halogenation. There have been plenty of halogen containing drugs approved by the FDA in recent years. The drugs prepared with F or Cl are useful for the treatment of various diseases such as cancer, migraine, cardiovascular disease, *etc*. However, there is no proper theoretical algorithm for understanding the interaction between the ligand's halogen and the protein available. In this context, we focus on the noncovalent interactions of halogens from ligand to protein targets from theoretical viewpoints. From our docking study, one can realize that the presence of electron withdrawing group F in rimegepant enhances the interaction between the ligand and the protein target considered in this study. Figure 10 shows the Optimized molecular 2d structure

Table 4 — Calculated dipole moment (μ_M in D), energy gap (E_g in eV), Ionization potential (I in eV), electron affinity (A in eV), chemical hardness (η in eV), chemical potential (μ in eV) and Electrophilicity index (ω) for halogen (F, Cl, Br) substituted in DR13

Halogens	μ_M	E_{HOMO}	E_{LUMO}	E_g	I	A	η	μ	ω
F	11.46	-5.915	-1.373	4.54	5.92	1.37	2.27	-3.64	2.92
Cl	11.50	-5.912	-1.375	4.54	5.91	1.38	2.27	-3.64	2.93
Br	11.47	-5.916	-1.388	4.53	5.92	1.39	2.26	-3.65	2.95

Table 5 — Calculated binding energy (in kcal/mol) for halogens substituted DR13 towards AChE, BuChE and Aβ peptide

	AChE				BuChE				Aβ	
Halogen	1EVE	3I6M	4EY7	4PQE	1P0I	2Y1K	4BDS	4TPK	1IYT	2BEG
F	-15.5	-15.1	-14.6	-12.9	-14	-13.5	-14.1	-13.4	-8.7	-8
Cl	-14.6	-13.9	-12.2	-10.6	-13.1	-12.4	-13.3	-13.5	-8.6	-7.1
Br	-14.7	-13.9	-13.1	-11.3	-14.1	-12.4	-12.9	-13.5	-8.5	-6.5

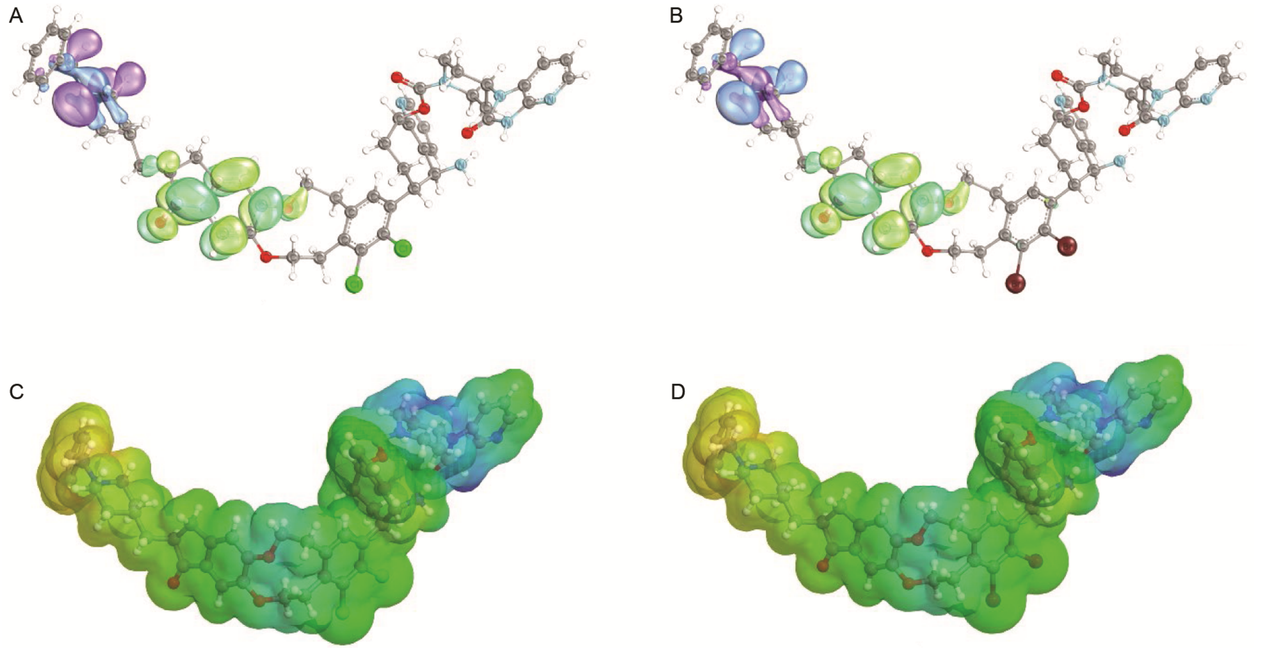


Fig. 11 — (A) HOMO and LUMO of Cl substituted DR13; (B) HOMO and LUMO of Br substituted DR13; (C) MEP plot of Cl substituted DR13; and (D) MEP plot of Br substituted DR13

of Halogen (a) F, (b) Cl and (c) Br substituted DR hybrid. Hence, in order to geta better understanding of the influence of halogen on cholinergic inhibition, the DFT and docking studies is performed by replacing F with Cl or Br. The calculated DFT results are given in (Table 4) and docking results are given in (Table 5). The 2D structure of halogen (F, Cl, and Br) substituted hybrids is given in (Fig. 10), and molecular docking for Cl and Br substituted (best pose) interactions are given in (Suppl. Fig. S2). Our DFT calculations indicate that there is no significant change in stability or reactivity, *i.e.*, all three halogen substituted hybrids produce similar results in (Table 4). The HOMO and LUMO electrons are localized in the donepezil region only for Cl, Br (Fig. 11A & B) and are similar in DR13. Substitution of halogens does not make any major changes in the HOMO, LUMO, and MEP plots (Fig. 11C & D). This indicates that the indanone, piperidine, and benzyl regions, as well as halogens, readily form noncovalent interactions with the AChE, BuChE, and Aβ peptides considered in this study. Further, docking study also performed for halogen substituted hybrids towards AChE, BuChE, and Aβ peptides at the same binding sites as mentioned in (Table 3). Their calculated binding energy values are given in (Table 6). From Table 5, we can clearly see that F has lowest binding energy for all proteins. It can be concluded that the substitution of halogens does not influence the binding energy of the system. But F has the strong binding energy compared to Cl and Br. This is due to the higher electronegativity of

Table 6 — Calculated ADME property for standard drugs and DR13 hybrid

Parameters	Donepezil	Rimegepant	DR13 Hybrid
Molecular weight (g/mol)	379.49	534.56	940.09
No. of Hydrogen bond acceptors	4	8	12
No. of Hydrogen bond donors	0	2	2
Fraction Csp	0.46	0.36	0.46
Molar refractivity	115.31	143.39	275.92
TPSA (A^2)	38.77	119.13	132.12
ESOL	-4.81	-4.67	-7.05
ALI	-4.81	-4.43	-5.18
SILICOS	-6.90	-7.14	-11.39
iLOGP	3.92	3.34	0
XLOGP3	4.28	2.29	2.75
WLOGP	3.83	4.30	3.59
MLOGP	3.06	2.59	4.26
SILICOS-IT	4.91	2.98	4.78
Lipinski	Yes;0 violation	Yes; 1 violation: MW>500	No; 3 violations: MW>500, MLOGP>4.15, NorO>10
Veber	Yes	No; 2 violations: MW>480, MR>130	No; 3 violations: MW>480, MR>130, #atoms>70
Ghose	Yes	Yes	Yes
Egan	Yes	Yes	No; 1 violation: TPSA>131.6
Bioavailability Score	0.55	0.55	0.17
Synthetic accessibility	3.36	5.20	9.01

F than Cl or Br. The results presented here could be useful for the design of halogenated ligands as inhibitors and drugs for AD.

The standard drugs donepezil and rimegepant has some side effects such as weight loss, vomiting, nausea, diarrhoea *etc*. Similarly, the proposed Dr hybrids also have some side effects. ADME study is done for standard drugs and DR13 hybrid. Among all DR hybrids DR13 shows best dipole moment, so we use DR13 for all calculations. DR13 hybrid has good reactive parameters, stability and efficacy but DR13 hybrid has some limitation in ADME property. Future, experimental studies are done for DR13 hybrid to reduce the limitations. The DR13 hybrid and standard drugs ADME are given in (Table 6).

Bioactivity of selected DR hybrids

In order to understand the biological activity of the selected hybrids, bioactivity scores are calculated. The biological activity of the selected DR hybrids is investigated using the molinspiration web tool[44]. The obtained results are given in the supporting information file (Suppl. Table S1). As anticipated, our studies indicate that the selected DR hybrids have moderate bioactivity. Earlier studies have mentioned that if the bioactivity score is greater than 0, then the compound is bioactive. If the bioactivity score lies between 0 to -5, then the compound is moderately active. If the score is less than -5 then the compound is bio inactive. The bioactive score of all the selected DR hybrids lies between 0 to -5, which indicates the hybrids considered in this study are moderately bioactive. Similarly, the selected DR hybrids do not possess a drug nature due to the violation of the Lipinski rule. This clearly indicates that a suitable modification is required in the chemical structure to enhance the bioactivity score as well as drug likeliness of the selected hybrids. Our molecular docking study reveals that multiple noncovalent interactions takes place between benzyl region attached with piperidine ring in donepezil with protein targets considered in this study. In the rimegepant, multiple noncovalent interactions take place between indanone ring, and F containing the benzylregion with amino acid residues of the protein targets considered in this study. Hence, it is proposed that donepezil like rimegepant hybrids can be designed from these functional groups. Figure 12 shows one example of the donepezil like rimegepant hybrid that could be used as a potential drug candidate for AD. Because it satisfies the Lipinski Rule (Molecular Weight= 445.23 dalton, nHA = 3, nHD = 2, logP = 5.097). However, additional *in vitro* and *in vivo* studies are necessary to validate our conclusion. Similarly, *in silico* studies are going on in our laboratory on modified donepezil like gepant hybrids to explore their potential application as an inhibitor candidate for AD.

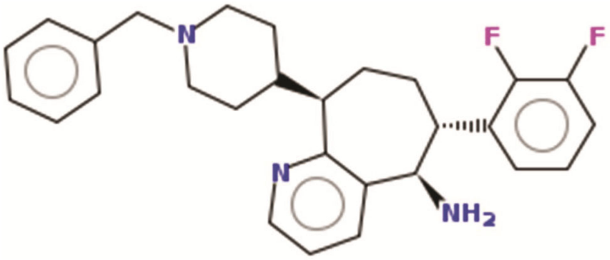


Fig. 12 — Molecular structure of the donepezil like rimegepant hybrid

Conclusion

In this study, the donepezil-rimegepant (DR) hybrids are investigated to explore their potential anti-Alzheimer activity using *in silico* techniques. There are 18 hypothetical DR hybrids generated using a hybridization-based design strategy and we studied their stability and reactivity using DFT. The binding affinity of selected hybrids towards AChE, BuChE, and Aβ is evaluated using molecular docking techniques. The reactive sites, such as electrophilic and nucleophilic sites, of the selected 18 hybrids are identified using DFT. Further, our DFT calculations indicate that the DR13 is highly stable than the other hybrids considered in this study. Our docking study revealed that, among the 18 hybrids, DR13hasa strong interaction with AD protein targets considered in this study. The rimegepant region in the DR hybrid has strong binding with the active sites in the protein target. The presence of F in rimegepant attributed the strong halogen bond interaction with the amino acid residues in the proteins. Among AChE, BuChE, and Aβ, DR13exhibits good inhibition in the order AChE > BuChE > Aβ. Hence, it is concluded that DR13 is a good inhibitor for cholinergic inhibition. Therefore, based on our *in silico* studies, DR13 is proposed as a good candidate molecule for designing a new drug against AD disease. However, additional *in vitro* and *in vivo* studies are necessary to validate our conclusion. The findings of the current study may aid medical and pharmaceutical chemists in developing and synthesizing more potent medication candidates for anti-Alzheimer drugs.

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Conflict of interest

Both the authors declare no conflicts of interest.

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