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LETTERS

Natural O-6-methylguanine-DNA methyl transferase (MGMT) gene antagonist from *Vaccinium oxycoccos*: A new hope in Alzheimer's therapeutics

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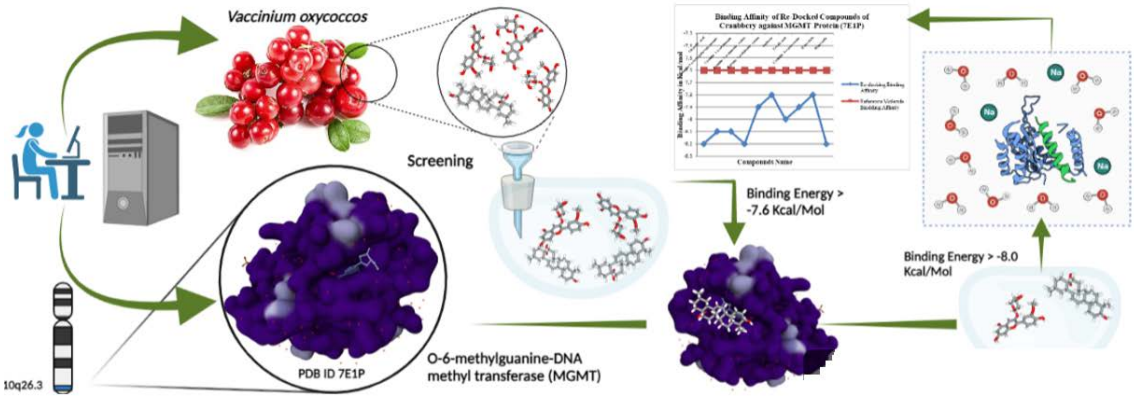
Article

ABSTRACT



Alzheimer's disease (AD) is a neurodegenerative illness with a complex pathobiology. The pathogenesis of Alzheimer's disease is majorly driven by mutation, overexpression and downregulation of β-amyloid (Aβ) gene that results in the aggregation of a β-amyloid (Aβ) protein within the neocortex. O-6-methylguanine-DNA methyl transferase (MGMT) gene coding for a DNA repair enzyme have found to play an important role in Alzheimer's disease. Some researchers have reported that Tau protein disintegration is directly tied to MGMT gene. An unavoidable, age-related increase in brain methylation of MGMT gene have shown to upregulate MGMT expression, resulting in Tau dysfunction. Due to the complex underlying pathology of Alzheimer disease (AD), treatment strategies are under extensive research as no new therapies have been approved by the US Food and Drug Administration (FDA) since 2003. Drug repositioning/molecular docking have appeared to be successful techniques to fasten the pharmacological research for AD treatment. In light to the same the study was aimed to evaluate various natural compounds from *Vaccinium oxycoccos* (cranberry) that are antagonist to MGMT gene. MGMT gene and Compounds structure was retrieved from PDB and PubChem databases and were screened for suitable interactions between them. Out of 23 compounds, four demonstrated strong binding affinity to MGMT gene and thus predicted to use as MGMT gene antagonist in developing the treatment for Alzheimer's disease (AD). These findings may be applicable to other degenerative diseases also where MGMT gene interactions have been involved.

Keywords: MGMT gene, Alzheimer's disease, β-amyloid, Cranberry compounds, Neurodegeneration

INTRODUCTION

Alzheimer's disease is a neurodegenerative illness that progresses over time and is the major cause of dementia in the elderly.[1] In total about 50 million individuals are suffering from dementia, with Alzheimer's worldwide. This accounts to 50-70 percent of Alzheimer's. Both the frequency and case of AD have found to rise with age.[2] Worldwide, the number of people aged 64 and over is predicted to grow 9.3 percent in 2020 to roughly 16.0 percent in coming 30 years. Alzheimer's disease affects around 5.8 million American people today, and the number is expected to rise. In America, the prevalence of Alzheimer's disease is roughly 3% in those between the ages of 64 and 75, 17% in those between the ages of 75 and 84, and 32% in those beyond the age of 85.[2,3] In people over the age of 60, the prevalence of Alzheimer's disease doubles every ten years.[4] Alzheimer's disease is a complex condition with several pathogenic pathways, including insulin dysregulation, neuroinflammatory process, neurodegeneration, and aggregated misfolded proteins[5], thus researching suitable compounds for pharmaceutical therapies remains a difficulty.[4] Agents that are neuroprotective, anti-inflammatory, and diabetic have prospective therapeutic possibilities. The pathogenic processes of

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A-beta and tau in development of AD are being discovered as structural biology experimental techniques.[5] Thus, understanding the pathophysiology of Alzheimer's disease allows us to discover new therapeutic agents for its treatment.[6] Drug repositioning/Molecular docking might prove to be a successful technique,[7] to fasten the pharmacological research for AD treatment. There are several advantages to drug repositioning. Firstly, repurposing existing medications for new therapeutic use is cost effective than inventing a new compound for treatment. Second, repurposed medications' ADME and toxicity have already been studied.[8] Hence, it is simpler for these medications to reach advanced clinical stages in order to evaluate their therapeutic efficacy in Alzheimer's disease. Some possible medications, such as Hyperoside, Oleanolic acid, Peonidin 3-arabinoside, and Peonidin-3-o-beta-galactopyranoside, have been proposed in this manner. The difficulties mainly lay in selecting current medications, which is dependent on our understanding of AD pathology. Despite the significant and long-term impacts of Alzheimer's disease, existing therapies have failed to generate sufficient therapeutic results to halt AD progression. Till today, only five medications are authorized by the FDA for the treatment of Alzheimer's disease. These include rivastigmine, tacrine, galantamine, memantine, and donepezil[9]. The first four medications are acetylcholinesterase inhibitors (AChEIs), while the fifth is an N-methyl-D-aspartate receptor (NMDAR) antagonist.[9] AChEIs are recommended as first-line pharmacotherapies for mild to moderate Alzheimer's disease in both American and European standards.[10] However, AChEIs have a small effect on cognitive impairments with a non-significant effect on functional ability in mild to severe Alzheimer's disease. Memantine has relatively little effectiveness in treating cognitive symptoms without improving functional outcomes.[10] Therefore, finding new therapies for Alzheimer's disease has become critical. As, the O-6-methylguanine-DNA methyl transferase (MGMT) gene have found to play an important role in Alzheimer's disease, the present research is aimed to find novel agents against AD from the plant *Vaccinium oxycoccos* (cranberry). Four natural compounds have been found that have strong binding affinity against the new gene MGMT (-8.2 Kcal/mol energy). These compounds are predicted to be effective in treating Alzheimer's disease.

RESULTS AND DISCUSSION

Structure-based virtual screening

All the 23 chosen compounds[11] were examined using Structure-based virtual screening (SBVS) against the native ligands active pocket, and these 23 flavonoids were shown to have antiviral, antibacterial, and Alzheimer's disease-absorbing properties. These compounds' binding scores ranged from -5.2 to -8.2 Kcal/mol, which is a significant amount of energy for the ligand acting as an antagonist. Based on their high energies, the top 10 compounds are further analyzed for redocking and intra- and intermolecular interactions between proteins and ligands, and they are compared to reference ligand (2~{R},3~{S},5~{R})-5-(2-azanyl-6-methoxy-purin-9-yl)-2-(hydroxymethyl) oxolan-3-ol.

Table1: Selected 23 compounds from cranberry screened against MGMT-protein PDBID; 7E1P shows their binding affinity at zero RMSD and RMSF value.

Ligand CID	Compound names	Binding Affinity	rmsd/ub	rmsd/lb
10494	Oleanolic acid	-8.2	0	0
12137510	Peonidin 3-arabinoside cation	-8.2	0	0
5281643	Hyperoside	-8.2	0	0
11454027	Peonidin-3-o-beta-galactopyranoside	-8.1	0	0
12137509	Cyanidin 3-o-arabinoside cation	-8.1	0	0
64945	Ursolic acid	-8	0	0
12137511	Malvidin 3-arabinoside cation	-7.9	0	0
441699	Cyanidin 3-o-galactoside	-7.9	0	0
124221768	Sophorin	-7.8	0	0
5280459	Quercitrin	-7.8	0	0
5280804	Isoquercitrin	-7.8	0	0
12311099	Myricetin-3-o-hexoside	-7.7	0	0
9064	Cianidanol	-7.7	0	0
22841567	Myricetin 3-o-glucoside	-7.5	0	0
21477996	Myricetin-3-o-pentoside	-7.3	0	0
441667	Kuromanin	-7.3	0	0
64971	Betulinic acid	-7.3	0	0
1794427	Chlorogenic acid	-7.2	0	0
5878729	Reynoutrin	-7	0	0
445858	Ferulic acid	-5.5	0	0
637542	4-hydroxycinnamic acid	-5.5	0	0
689043	Caffeic acid	-5.4	0	0
157010309	P-coumaroyl hexose	-5.2	0	0

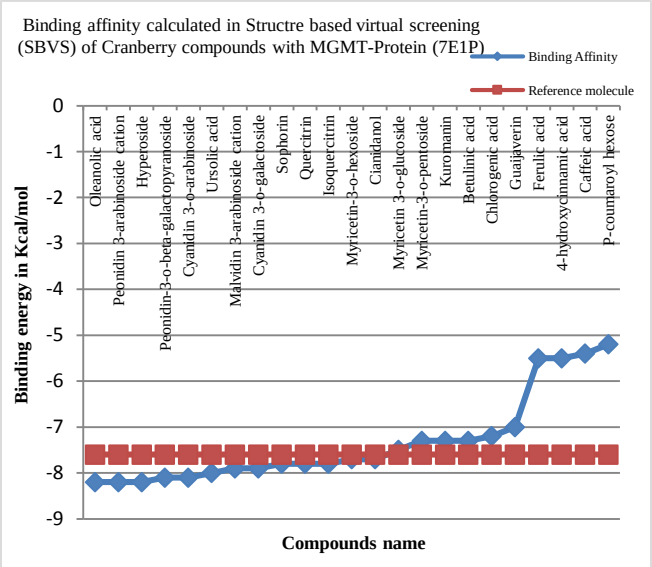
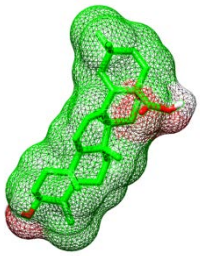
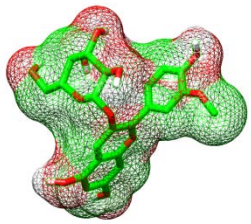
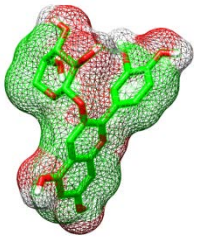
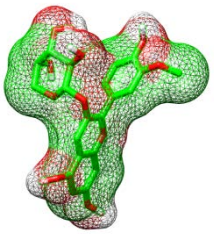
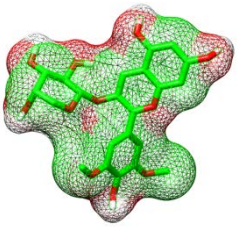
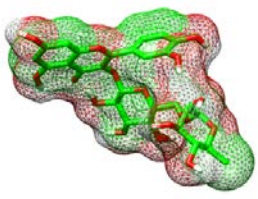
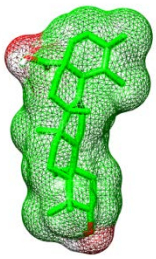
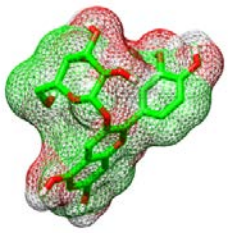
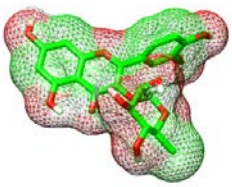
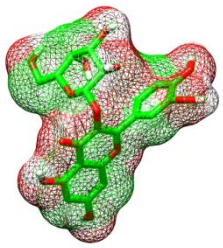


Figure 1: Screening of compounds shows good binding affinity as compared to reference molecule.

Table2: Selected top ten compounds for Re-docking in UCSF Chimera tool filtered from SBVS.

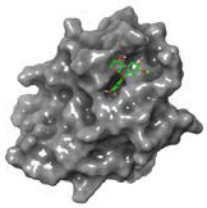
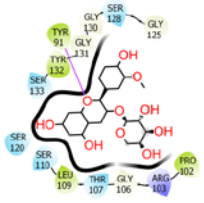
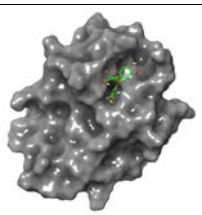
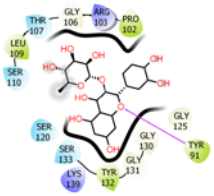
PubChem CID	Common name	IUPAC	Canonical smiles	3D ligand
10494	Oleanolic acid	(4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropicene-4a-carboxylic acid	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)C(=O)O)C	
11454027	Peonidin-3-o-beta-galactopyranoside	(2S,3R,4S,5R,6R)-2-[5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)chromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	COC1=C(C=CC(=C1)C2=[O+]C3=CC(=CC(=C3C=C2OC4C(C(C(O4)CO)O)O)O)O)O	
12137509	Cyanidin 3-o-arabinoside	(2S,3R,4S,5S)-2-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxyoxane-3,4,5-triol	C1C(C(C(O1)OC2=CC3=C(C=C(C=C3[O+]=C2C4=CC(=C(C=C4)O)O)O)O)O)O	
12137510	Peonidin 3-arabinoside cation	(2S,3R,4S,5S)-2-[5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)chromenylium-3-yl]oxyoxane-3,4,5-triol	COC1=C(C=CC(=C1)C2=[O+]C3=CC(=CC(=C3C=C2OC4C(C(C(O4)O)O)O)O)O)O	
12137511	Malvidin 3-arabinoside cation	(2S,3R,4S,5S)-2-[5,7-dihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)chromenylium-3-yl]oxyoxane-3,4,5-triol	COC1=CC(=CC(=C1O)OC)C2=[O+]C3=CC(=CC(=C3C=C2OC4C(C(C(CO4)O)O)O)O)O)O	
12422768	Sophorin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	CC1C(C(C(C(O1)OCC2C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O	
64945	Ursolic acid	(1S,2R,4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid	CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1C)C)C(=O)O	
4416991	Cyanidin 3-o-galactoside	(2S,3R,4S,5R,6R)-2-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	C1=CC(=C(C=C1C2=[O+]C3=CC(=CC(=C3C=C2OC4C(C(C(O4)CO)O)O)O)O)O)O	
5280459	Quercitrin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxychromen-4-one	CC1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)O)C4=CC(=C(C=C4)O)O)O)O)O	
5281643	Hyperoside	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C(C(C(C(O4)CO)O)O)O)O)O	

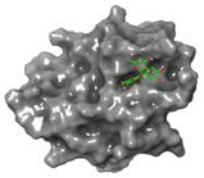
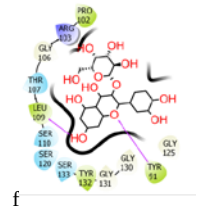
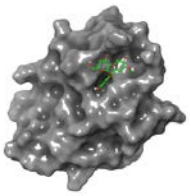
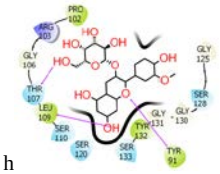
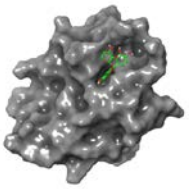
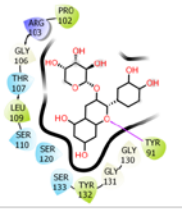
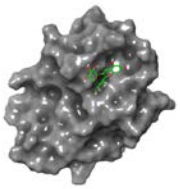
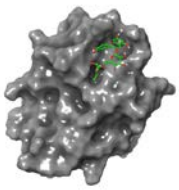
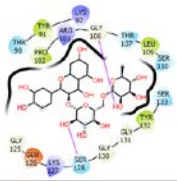
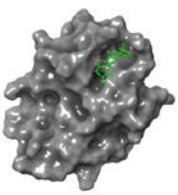
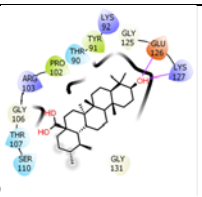
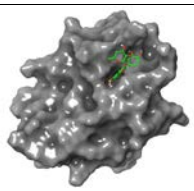
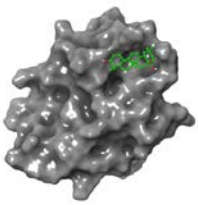
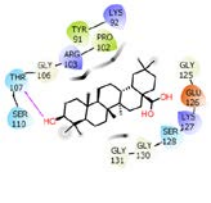
Redocking of screened compounds

Molecular docking techniques have been widely employed for bioactive chemicals or drug repurposing against various drug targetable proteins involved in illnesses and infections. Protective potential of natural compounds from cranberry against Alzheimer's disease was predicted by its MGMT protein inhibition abilities. This work also used the molecular docking technique for the chosen natural compounds mentioned in table 2. In the active pocket of Alzheimer's disease MGMT, all docked Compounds and O (6)-Methyl-2'-Deoxyguanosine inhibitor shown considerable docking confirmation with binding affinity energies > -7.6 kcal/mol at least RMSD in comparison to O (6)-Methyl-2'-Deoxyguanosine native inhibitor. The docked Alzheimer's disease MGMT- Hyperoside complex had a significant docking score of -8.2 kcal/mol and formed two hydrogen bonds with the MGMT through active residues Tyr91, and Leu109, other interactions, including hydrophobic (Tyr91, Pro102, Leu109, and Tyr132), polar (Thr107, Ser110, Ser120, and Ser133), Positive (Arg103), and Glycine (Gly106, Gly125, Gly130, and Gly131). While single hydrogen bonds were formed

with the active residue Thr107 in the Alzheimer's-MGMT docked complex with Oleanolic acid, which had a docking energy of -8.2 kcal/mol. Additionally, interactions between the residues of the Alzheimer's-MGMT and Oleanolic acid included hydrophobic interactions (Tyr9, and Pro102), polar interactions ((Thr107, Ser110, and Ser128), positive interactions (Lys92, and Arg103), negative interactions (Glu126), and glycine interactions (Gly106, Gly125, Gly13, and Gly131). Like this, the Alzheimer's-MGMT- Peonidin 3-arabinoside cation complex had an important docking score of -8.2 kcal/mol coupled with the formation of single hydrogen bonds at crucial Alzheimer's-MGMT residues Tyr91. There were also polar interactions (Thr107, Ser110, Ser120, Ser128, and Ser133), hydrophobic interactions (Tyr91, Pro102, Leu109, and Tyr132), positive interactions (Arg103), and glycine interactions in the corresponding complex (Gly106, Gly125, Gly130, and Gly131). The Alzheimer's-MGMT -Peonidin-3-o-beta-galactopyranoside complex, on the other hand, displayed a significant binding energy of around -8.1 kcal/mol, which was followed by the creation of three hydrogen bonds with the residues Tyr91, Pro102, and Leu109 in the active pocket of MGMT-protein. For the Alzheimer's-MGMT -Peonidin-3-o-beta-galactopyranoside complex, additional intermolecular interactions, including hydrophobic interactions (Tyr91, Pro102, Leu109, and Tyr132), polar interactions (Thr107, Ser110, Ser120, Ser128 and Ser133), positive interactions (Arg103), and glycine interactions (Gly106, Gly125, Gly130, and Gly131). Interaction of Alzheimer's-MGMT to other natural compounds mentioned in the table2 shows good binding energy with active site residues mentioned in the table3.

Table 3: The orientation of the high binding energy and bond interactions of bioactive compounds from *Vaccinium oxycoccos* in 3D and 2D pose in the active pocket of MGMT with its active pocket amino acid Tyr91. CID of ligands are mentioned in the table3 with protein ID 7E1P-

CID	3D Structure	2D Structure
12137510	a 	 b
5280459	 c	d 

5281643	 e	 f
11454027	 g	 h
12137509	 i	 j
12137511	 k	 l
124221768	 m	 n
64945	 o	 p
441699	 q	 r
10494	 s	 t

Refer to table's No. 1 and 2 for the names of the compounds.

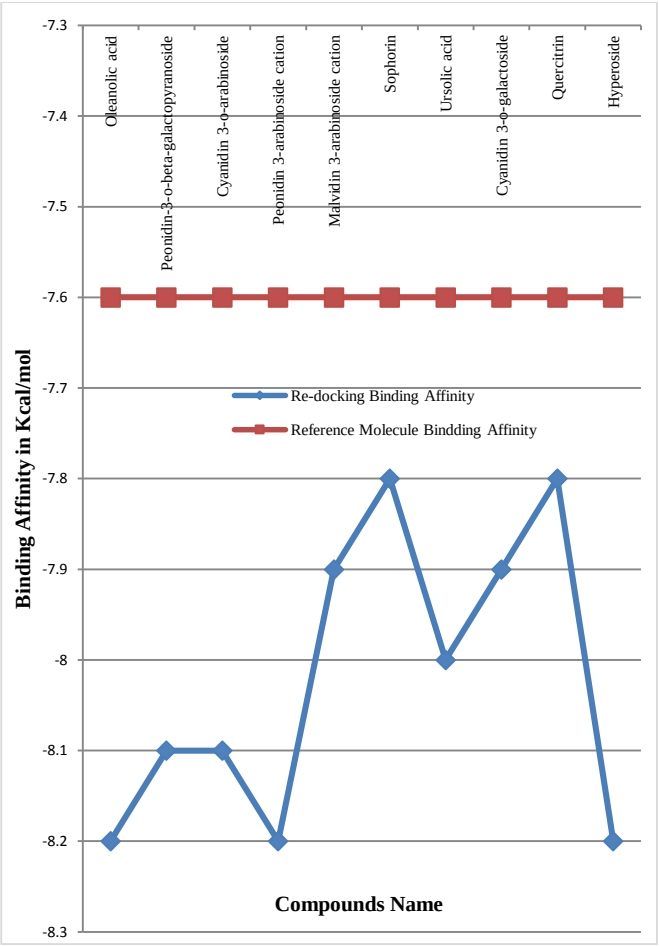
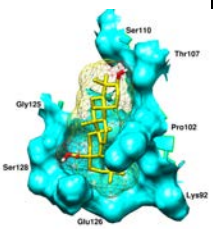
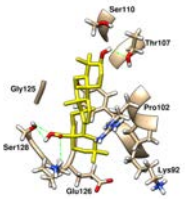
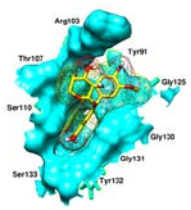
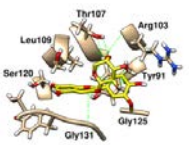
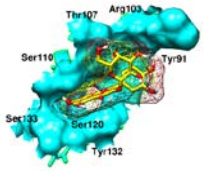
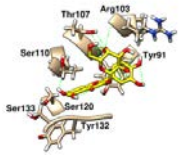
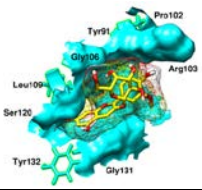
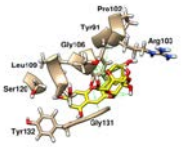
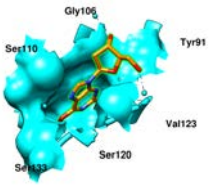
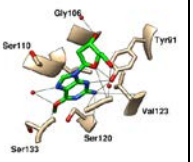


Figure 2: Binding Affinity of Re-Docked Compounds of Cranbbery against MGMT-Protein (7E1P): Comparison of binding energy of top ten complexes as compared to complex with reference molecule.

Table4: Final Four lead-compounds with high binding energy at zero RMSD and RMSF in a mess structure at its active pocket with active pocket amino acid residue and fifth is control molecule in the native active pocket.[12]

Complex of MGMT with ligands	**3D Structure of complex with amino acid residue at radius of 3.5** Å	**2D Structure of complex with its active pocket at radius of 3.5** Å
Oleanolic acid	i 	ii 
Peonidin 3-arabinoside cation	iii 	iv 
Hyperoside	v 	vi 
Peonidin-3-o-beta-galactopyranoside	vii 	viii 
(2~{R},3~{S},5~{R})-5-(2-azanyl-6-methoxy-purin-9-yl)-2-(hydroxymethyl)oxolan-3-ol **(control molecule)**	ix 	x 

METHODOLOGY

Data retrieval and priming

Studies have found that the MGMT gene on chromosome 10q26.3 [4,13]is a novel target for therapy, and cranberry has antiviral and anti-bacterial effects that can cure Alzheimer's disease [14]. To find the novel compounds from the plant *Vaccinium oxycoccos* (cranberry) against MGMT gene, crystal structure of MGMT protein was retrieved from protein data bank with 1.80 Å resolutions and PDB ID is 7E1P of Sequence length 156 and co-crystaled with (2~{R},3~{S},5~{R})-5-(2-azanyl-6-methoxy-purin-9-yl)-2-(hydroxymethyl)oxolan-3-ol (MF: C11H15N5O4 ; MW: 281.27g/mol) including the cranberry compounds (Table 1) from PubChem database in 3D.sdf format[15]. The MGMT gene-retrieved protein goes through a priming stage in which native ions, solvents, and ligands are eliminated. The incorporation of polar hydrogen and charge inside chains is also included. During the preparation of the ligand for screening, hydrogen is added to it[16].

Screening of Cranberry compounds

Using the PyRx program[17], compounds were screened in order to identify a more appropriate complex (protein-ligand). All the default parameters, such as PDB file conversion to PDBQT, minimization, and grid creation, are performed with the aid of openbable, Autodock vina, and wxPython utilities combined with PyRx. The grid size and center for screening are set to 16.5865X21.1138X20.4327 Å and -12.9415X18.0300X0.6418 Å, respectively. The energy of cranberry compounds (ten poses for each component) in Kcal/mol is shown in table1, and all the poses with the lowest RMSD and RMSF values were chosen. Selected are then tested for effectiveness in UCSF chimaeras via docking.[16]

Re-Docking of screened compounds

In order to find the bond interactions between the targeted protein and ligand, we used the UCSF Chimera plugin with Auto-dock Vina[7,16]. To achieve this, we minimized the chimera's structure and first prepared the protein and ligands by removing solvent ions and the native ligand from the protein and adding polar hydrogen and charges to the targeted protein, while also adding hydrogen atoms and charges to the ligands. Then, in order to allow the cranberry ligands to interact inside the pocket, we execute molecular docking using the Auto-dock Vina plugin into the chimera in the native ligand pocket with a grid size of 16.5865 X 21.1138 X 20.4327 Å and center for all active site residues -12.9415 X 18.0300 X 0.6418 Å for selected cranberry compounds and native ligand (as reference compound). Each protein-cranberry ligand complex had ten conformers available as a consequence of the docking process, and the conformer with the lowest docking energy and RMSD (root mean square deviation) was chosen for further investigation. We search for non-covalent interactions in this study, such as π-π interactions, salt bridges, hydrogen bonds, π-cation interactions, hydrophobic interactions, and interactions between positive and negative amino acids. Images from the table-3 chimera in 2D and 3D depict these interactions.

CONCLUSION

MGMT gene plays a significant role in the progression of Alzheimer's disease. The bioactive compounds from *Vaccinium oxycoccos* were docked to find out their potential activity against Alzheimer's disease by evaluating their binding affinity against the MGMT gene. Four potent flavonoids were identified through virtual screening followed by evaluation for ADME. These potent molecules showed good binding energy and multiple bond interactions with the MGMT gene. Also, molecular docking simulation confirmed the valid bond interaction between protein and ligand (Complex) from different orientations. Due to the formation of multiple intermolecular interactions, these complexes can be considered stable.

Thus, based on the overall analysis of the drug-likeness, toxicity, binding energy, and protein-ligand interaction profiles, it is concluded that the bioflavonoids Oleanolic acid; Peonidin 3-arabinoside cation; Hyperoside; and Peonidin-3-o-beta-galactopyranoside may have potential therapeutic agents against O-6-methylguanine-DNA methyl transferase of Alzheimer's disease.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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