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Virtual Screening, Docking and DFT Study of PRMT5

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Abstract

Protein Arginine Methyltransferase 5 (PRMT5), a significant member of the PRMT family, is a promising anticancer target. In this study, novel small compounds that act against the PRMT5 target are found by combining virtual screening with ChEMBL database medicines and Density Functional Theory. The ChEMBL database compounds were screened to retrieve the hit molecules, which further subjected for DFT analysis. Finally we have evaluated that ChEMBL- approved drugs such as Lifitegrast, Abiraterone acetate and Solifenacin may be potential inhibitors for PRMT5.

Keywords: PRMT5, Virtual Screening, DFT

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1. Introduction

Protein arginine methyltransferase 5 (PRMT5) is one of the predominant epigenetic regulators and plays an important role in a number of multiple cancer regulatory pathways that involve progression and therapy response[1]. Remarkably, PRMT5 is the only PRMT that functions with an obligate co-factor, methylosome protein 50 (MEP50). Protein methylation on arginine residues was primarily reported in the late 1960s and early 1970s and PRMT1 was the leading member of the protein arginine methyltransferase (PRMT) family[2]. PRMT5 was first recognized in 1996, Soon after, nine others, including PRMT5, were discovered. Methylated arginine proteins take part in a numeral of important cellular progressions that are needed aimed at tissue homeostasis and identifying disease phenotype in cancer, PRMT5, PRMT1, and CARM1 are the three PRMT family members that are most highly expressed, and this extended expression is associated with a poor prognosis for patients with a variety of cancer forms[3]. Since PRMT5 is well-known to regulate the expression of genes involved in cooperation with tumor promotion and suppression, it plays a complex role in the development of cancer. By repressing miRNAs that target genesthat promote tumor growth, increased PRMT5 expression in human cancers is linked to tumor elevation[4]. In view of the importance of PRMT5 in tumor progression, it is essential to develop pioneering inhibitors for targeting PRMT5.

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Development of novel inhibitors against PRMT5 target, several in silico studies proposed lead inhibitors earlier based on the binding scoresand best fit of small molecules in the active site conformation of PRMT5. There are two binding sites are present in PRMT5 namely, SAM binding and substrate binding pocket[5]. The catalytic active site residues E435 and E444 of PRMT5 protein. In this study, Molecular docking based on virtual screening was performed to identify new novel inhibitors targeting SAM-binding pocket of PRMT5 using ChEMBL database. The final lead molecules were evaluated by DFT method.

2. Materials and methods

2.1 Protein Structure Preparation

In this study,the crystal structure of human PRMT5:MEP50 complex were chosen (PDB: 4GQB) from Protein data bank[6]. The water molecules, co-crystal ligand and the complex MEP50 were removed bypymol tool. The complex structure were optimized by adding polar hydrogen atoms, kollman charges and compute Gasteiger charges using autodock tool parameters. Further, protein was converted into PDBQT format to perform docking in Autodock vina.

2.2 Ligand Database Preparation

Small molecules retrieved from ChEMBL database were chosen for virtual screening. The SDF format of small molecules are taken from Pubchem database. Further the SDF format small molecules were converted into PDB format by Openbabel software. The small molecules are further optimized in Autodock tool and through detecting the torsion root, the torsion angles, allocatingcharges and saved as pdbqt format.

2.3 Molecular Docking

Docking is an *in-silico* method which is used to analyse the interacting region between target protein and the small molecules. The docking is accomplished by autodock vina. PyRx virtual screening open source software is used to perform autodock vina in this study[7]. The grid box is set as 120*120*120 and results can be viewed under 'analyse results' tab. Subsequently top ten small molecules are refined based on the binding affinity.

2.4 Density Functional Theory (DFT)

Is a successful and promising approach adopted by quantum chemists in the quantum mechanical simulation of periodic system. There is substantial evidence that DFT provides an accurate description of the electronic and structural properties of small molecules by computing the electronic structure of matter. There are about ten different descriptors are calculated, which includes HOMO, LUMO, energy gap, global softness, absolute hardness, molecular dipole moment, electronegativity, electrophilicity index and chemical potential[8].

3. Results and discussion

3.1 Binding Site Characterization

The PRMT5 protein possess two binding site co factor SAM and substrate arginine. Previously different in-silico studies were carried out on focusing both the binding site

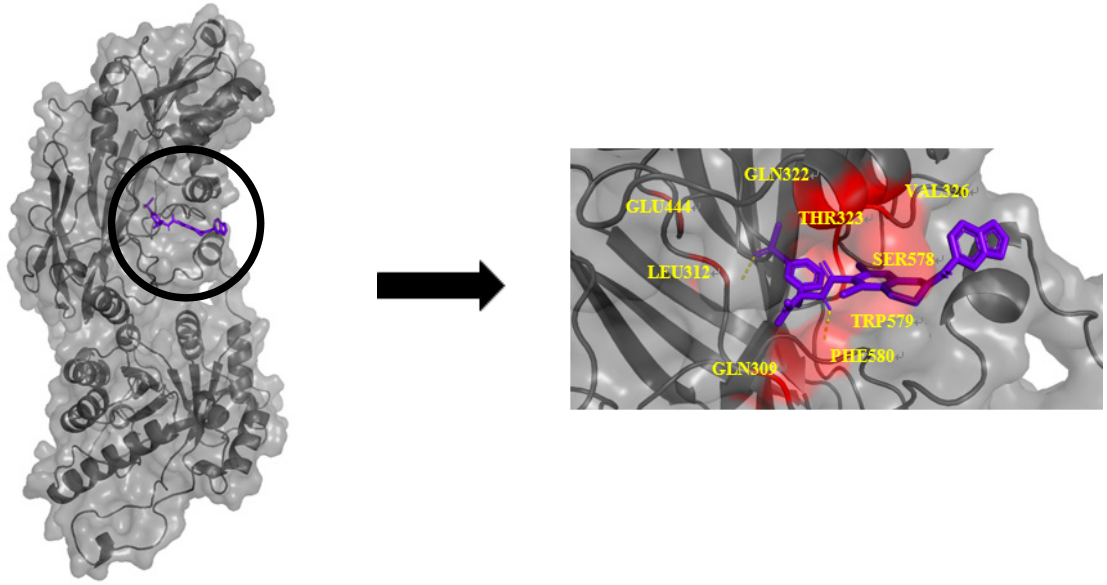
and hence to attain more PRMT5 small molecules inhibitors, a selective method that focused on SAM binding site was performed. The catalytic active site residues E435 and E444 of PRMT5 protein. The residues obtained from molecular docking based on virtual screening are almost matched the ones suggested by literature[9]. In this study, residues to be a part of binding site are Gln309, Leu312, Glu444, Gln322, Val326, Thr323, Ser578, Trp579 and Phe580respectively.

3.2 Molecular Docking

Docking is a method which analyses the conformation of small molecules into the binding site of a target protein. The ligands are docked against PRMT5 protein to recognize potential small molecules inhibitors. All the ligands were docked in the targeted site that was proposed. Lifitegrast, Venetoclax, Avapritinib, Alectinib, Atovaquone, Abiraterone acetate, Norethynodrel, Capmatimib, Stanzolol and Solifenacin displayed better binding affinity with high docking scores compared to the rest of the small molecules in ChEMBL database. Subsequently Venetoclax, Avapritinib, Alectinib binds in allosteric site of PRMT5 protein. One conformation from each ligands were selected and the ligands formed hydrogen bonding with residues Gln309, Leu412, Gly438, Ser439, Ser578, Leu312, Pro311, Thr323, Ser310. The hydrogen and hydrophobic interaction are tabulated in Table 1. The hydrophobic residues Gln309, Leu312, Gln322, Val326, Thr323, Ser578, Trp579 and Phe580 around ligands may contribute better stability to the complex. They interact with the catalytic residue Glu444[10]. The top three ligands docked pose are depicted in Figure 1, Figure 2 and Figure 3. And the chosen ligands are docked together in the targeted site are depicted in Figure 4.

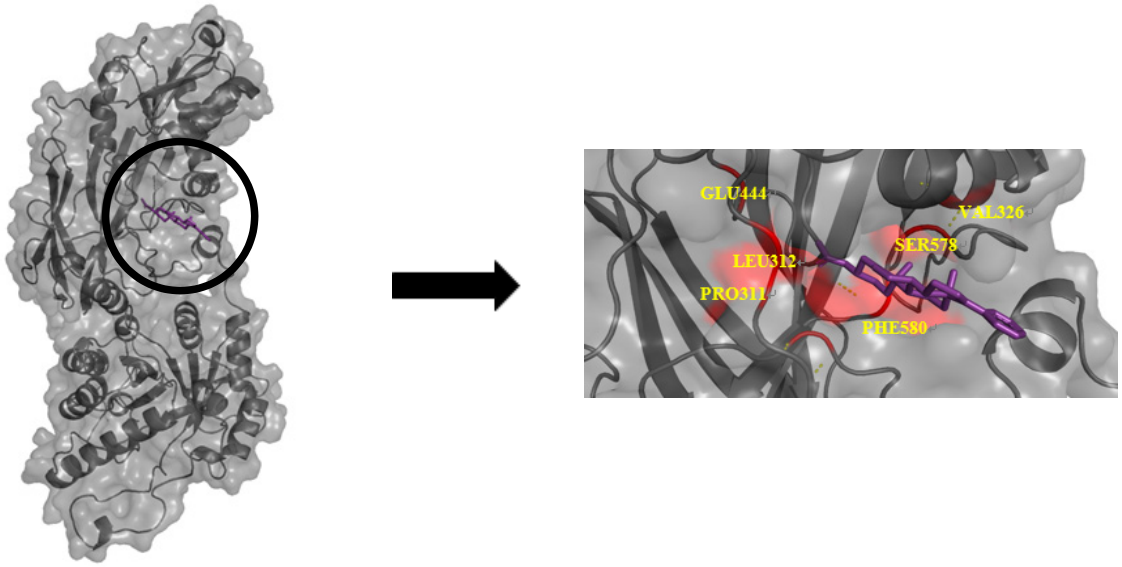
Table 1. Binding affinities of ChEMBL compounds with the PRMT5 protein along with the H-bond interactions and hydrophobic interactions made with the amino acid residues

FDA drug Compounds	Binding affinity(kcal/mol)	H-bond interactions	Hydrophobic interactions
Lifitegrast	-10.2	GLN309, LEU412, GLY438, SER439	PHE580, GLN309,VAL326, TRP579, SER578, PHE577, GLN322, SER439, PHE327
Venetoclax	-10.2	GLN511, HIS510,ARG505	
Avapritinib	-10.0	LYS259, HIS507, GLY575, ASN295	
Alectinib	-9.8	GLN289,SER288	
Atovaquone	-9.7	GLN309,SER578	
Abiraterone acetate	-9.6	LEU312, PRO311	
Norethynodrel	-9.6	GLY438, SER439	
Capmatimib	-9.5	SER439, GLY438, THR323	
Stanzolol	-9.5	SER439	
Solifenacin	-9.5	GLN309, SER310	



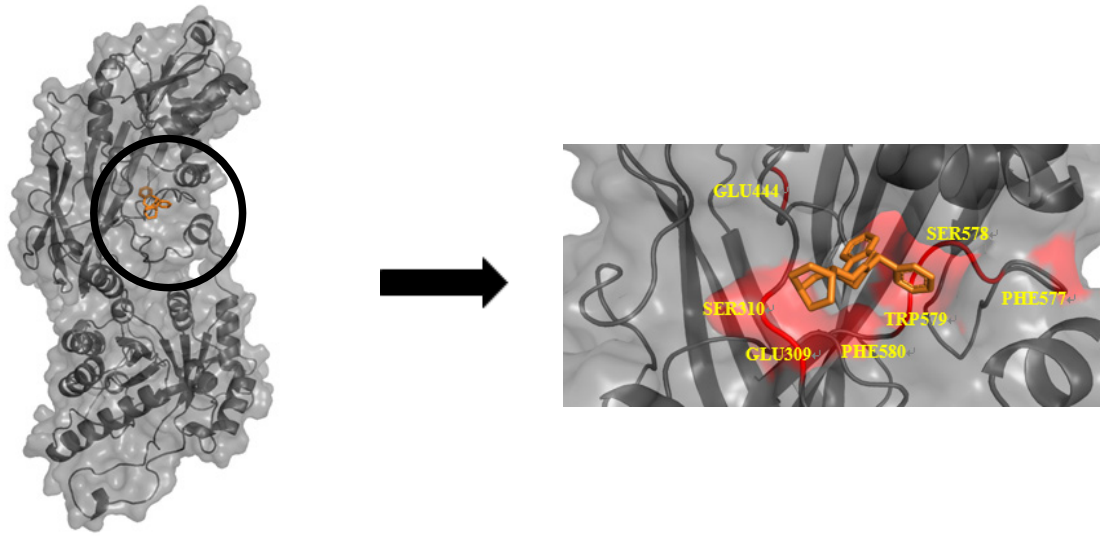
(A) (B)

Fig. 1. (A) Binding pose of Lifitegrast (purple blue) in PRMT5
(B) The hydrogen bonding with active site residues GLN309, LEU312, GLU444, GLN322, VAL326, THR323, SER578, TRP579, PHE580 has been depicted in red.



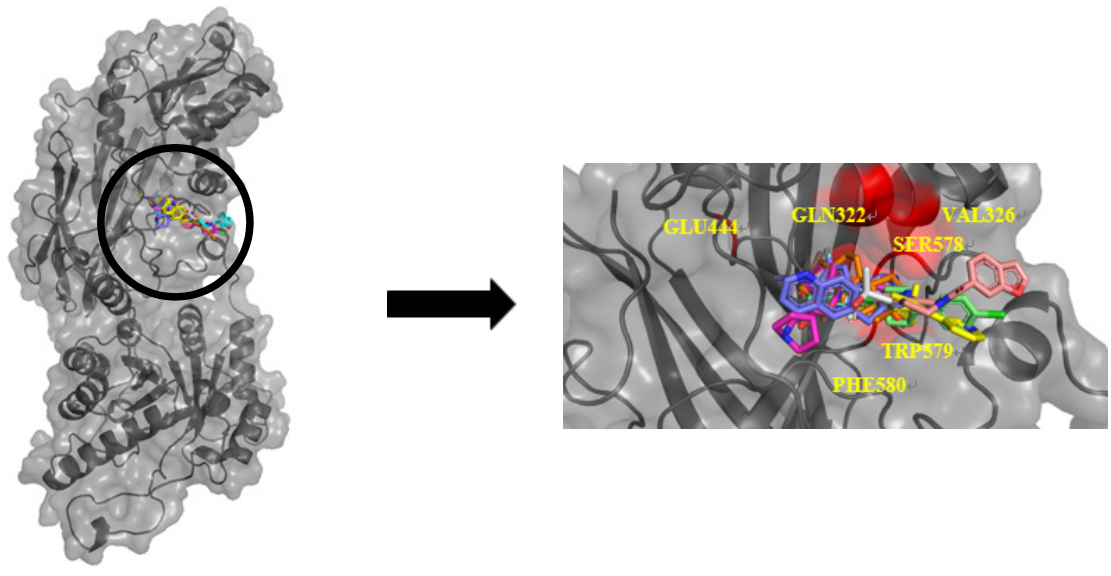
(A) (B)

Fig. 2. (A) Binding pose of Abiraterone acetate (deep violet) in PRMT5
(B) The hydrogen bonding with active site residues PRO311, LEU312, GLU444, VAL326, SER578, PHE580 has been depicted in red.



(A) (B)

Fig. 3. (A)Binding pose of Solifenacin (bright orange) in PRMT5
(B) The hydrogen bonding with active site residues GLU309, SER310, GLU444, PHE577, SER578, SER579, PHE580 has been depicted in red.

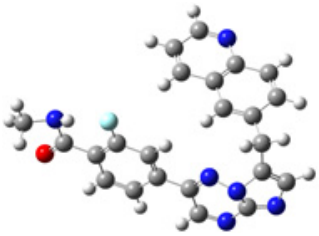
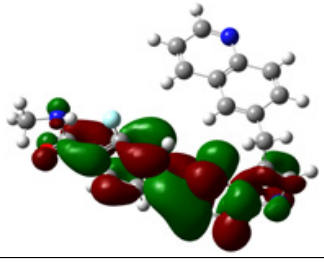
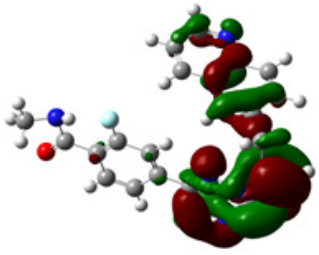
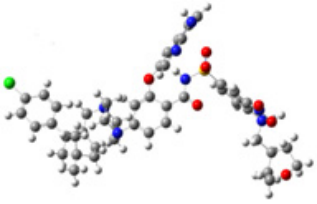
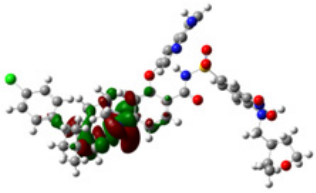
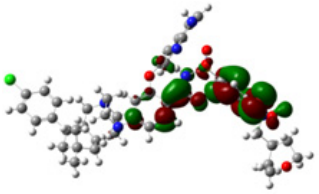
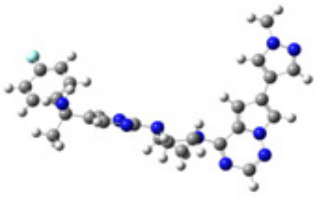
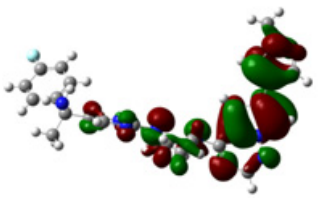
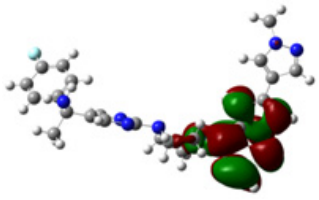
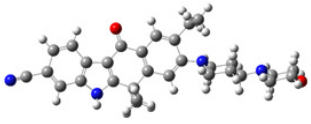
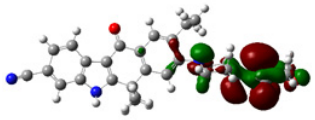
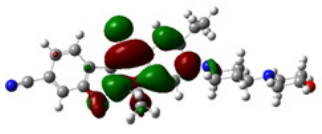


(A) (B)

Fig. 4. (A)Binding poses of the selected ligands (represented in distinct colours) in the binding pocket of PRMT5 protein has been depicted
(B) The binding pocket area has been zoomed in, to visualize the residues in the vicinity.

3.3 Conceptual DFT

The selected ligands were optimized using B3LYP function with a 6-31G (d) basis in Gaussian 16. Molecular orbital energies like HOMO energy (EHOMO) and LUMO energy (ELUMO) were calculated. Table 2 represents the EHOMO and ELUMO values of the selected ligands. Figure 5 represent the electron density maps of HOMO and LUMO of selected ChEMBL database compounds. With the following formula, the energy gap (E) between the molecules' molecular orbitals was calculated: E = ELUMO - EHOMO. It is significant to note that the energy gap is inversely connected with the reactivity of the compounds. Lifitegrast and Atovaquone showed the lowest energy gap between HOMO and LUMO with a ÄE value of 3.67 and 3.15 eV and also shows interaction with catalytic residues Glu444. Stanzolol showed maximum energy difference with a value of 6.27 eV and other compounds like Venetoclax, Avapritinib, Alectinib, Abiraterone acetate, Norethynodrel, Capmatimib and Solifenacin scored ÄE lesser than 6.0 eV.

Compound	DFT optimized structure	HOMO	LUMO
Lifitegrast			
Venetoclax			
Avapritinib			
Alectinib			

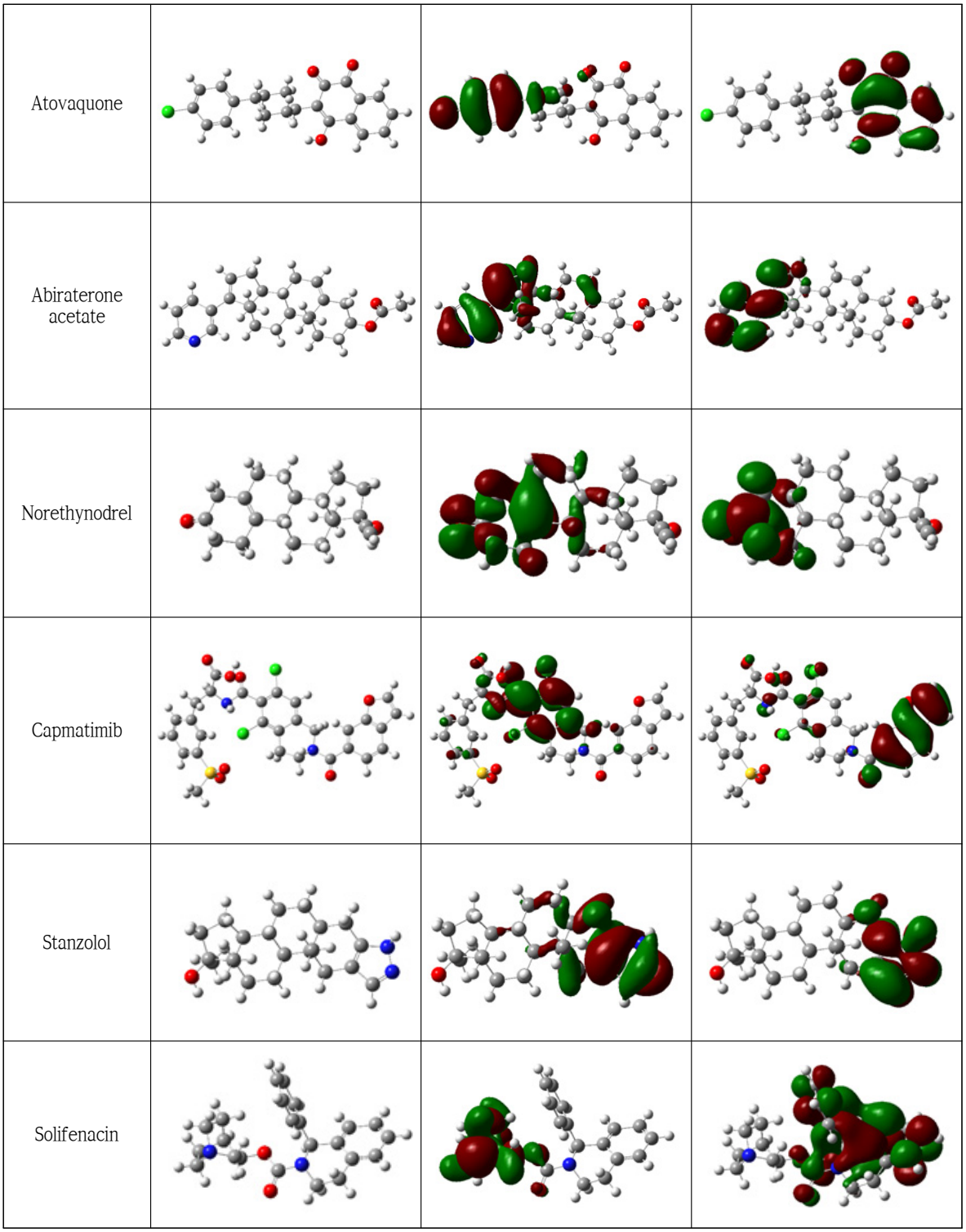


Fig.5. Electron density maps of HOMO and LUMO of selected ChEMBL compounds

4. Conclusion

The PRMT family, as one of the most promising biological anticancer targets. PRMT5 has attracted more and more attention. In this study, through virtual screening and DFT, compound Lifitegrast, Abiraterone acetate, Solifenacin may serve as a potential inhibitors for PRMT5 protein. They predominately occupied the SAM-binding pocket and interact with the residue Glu444 which is one of methyltransferase catalytic residues. This study has provided a reliable virtual screening and DFT method for finding novel PRMT5 inhibitors.

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