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Virtual Screening of Tricyclic Compounds as DNA Intercalators

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ABSTRACT

In present days, cancer is one of the key contributors to the overall mortality rate. Several approaches are made in controlling the fatality. DNA intercalation has already been considered one of the highly applied approaches. A more rational design and application of novel DNA intercalators, with both higher efficiency and selectivity, constitutes an urgent task in medicinal chemistry. In order to develop an understanding of binding pattern between both DNA and intercalators, molecular docking study was conducted. Considering the structural feature of the cocrystal, i.e., BFA50 (9-bromo-phenazine-1-carboxylic acid (2-dimethylamino-ethyl)-amide) three different series of thirty compounds were designed, in which the side chain and the central tricyclic system, both were altered. Binding enrgy and docking were the two parameters set to assess the quality of the virtually developed compounds. Highest alignment was observed to those compounds which possess either hydroxyl or methyl group in their side chain. Therefore, it can be concluded that owing to their binding pattern, compounds as2, ps2, ps3, ps7, ps8, ss2, ss8 were the highly active among all.

Keywords: Molecular docking, Intercalators, Phenazine

INTRODUCTION

Cancer is a disorder in which a group of abnormal cells grow uncontrollably by disregarding the normal way of cell division. Normal cells are regularly subject to signals that decide whether the cell should grow, divided into two individuals or die. Cancer cells impose an autonomy to these signals, which ultimately results into uninterrupted growth and expansion. 90 % of world cancer affected individuals are dead due to tumour spreading, which is clinically known as metastasis. It is well known to the molecular biologists that all mammalian cells share nearly similar molecular networks which modulate cell proliferation, differentiation and cellular death. There it can be clearly said that variation in this networks at any respective level, this distortion can occur. Modern science defined cancer in a simplest way, a disease which involves mutation in the cell genome. This alteration, DNA mutations generates proteins that eventually cause disruption of this intricate cellular between cell division and dormancy, resulting in production of dividing cells cause cancer [1]. Considering the increasing contribution of cancer to the overall mortality rate, a more rational design and application of novel DNA intercalators, with both higher efficiency and selectivity, constitutes an urgent task in medicinal chemistry. It is more than likely that DNA-binding properties of an intercalator new drug may play a key role in different chemotherapies. DNA is considered the ultimate cellular target of many anticancer drugs. Since Watson and Crick's three-dimensional model of DNA and related studies, many efforts and progress were made to provide a deeper understanding on its 3D arrangement and conformation. The discovery and development of novel therapeutic intercalators for the treatment of malignancy are some of the most important goals in modern medicinal chemistry. It is important to highlight that upon intercalation, the intercalator causes a distortion on DNA structure. In general, the angle of the phosphate groups changes (opening) allowing for the intercalation. The unwinding of the double strand leads to a lengthening of the helix by approximately 3.4 Å, which causes a conformational change of some sugar moieties involved. Intercalation occurs when ligands of an appropriate size and chemical nature fit themselves in between base pair of DNA. These ligands are mostly polycyclic, aromatic, planar and therefore often make good nucleic acid strains. As a consequence of the intercalation, the so-called "neighbor exclusion principle" takes place. This principle determines that after intercalation of a structure, the access of another intercalator to the binding site next to the neighboring intercalation pocket is now hindered, and it does not occur. This fact is relatively obvious since an intercalation results in significant local DNA structural changes, which means that deep alterations in the nucleotide secondary structure occur. A complete characterization of DNA binding agents requires that their mode of binding to DNA be established. Actually, it may be a hard task to be performed. Experiments such as spectrophotometric and/or spectrofluorimetric titrations or fluorescence polarization measurements are very useful in order to help the scientists to elucidate the general binding interaction between the guest molecule and DNA. Another approach in developing the understanding of interaction is molecular docking study.

The hurdles in obtaining experimentally structural data of target complexes have prompted the development of computational predictive methods. Molecular docking (also known as in silico molecular docking) is a computational science aiming at predicting the optimal binding mode and conformation of participating molecules in space, and to assess the stability of their complex. Molecular docking predicts whether or not the two molecules interact, the target as receptor/ enzyme/DNA and small/ligand molecule. Computational docking is an essential tool in modern drug discovery. Over the last few decades, it has been routinely and successfully applied in most pharmaceutical and biotech companies for a huge number of applications. Computational docking expresses the concept of molecular similarity. The structures interact like a hand within a glove, where both the shape and the physico-chemical properties of the structures contribute to the binding. Shape and size complementarity is the primary criterion for evaluating the fit in the computational docking of two candidate molecules. In addition to shape compatibility, chemical and physicochemical complementarity is also important criteria in the docking between candidate structures. Docking comprises two distinct tasks, The first one being the prediction of favorable binding geometries for a small molecule in the binding site of a target protein and the second one being the estimation of the binding free energy of the complex so formed, which is usually referred to as scoring. The purpose of the scoring procedure is the identification of the correct binding pose by its lowest energy value, and the ranking of protein-ligand complexes according to their binding affinities [2-4].

OBJECTIVES

DNA intercalators, by virtue of their lethal instinct over cytotoxicity have already been considered the first line treatment in cancer therapy. Still researchers are looking after even better intercalators, as uses of most of the DNA intercalators involves health hazards. The above discussion dealt with both the intercalators and possible way to develop better intercalator by means of molecular docking. From an overall perusal of the above mentioned information, it is well understood that how docking methodology is exploited in developing the initial idea about ligand-target interaction. Out of several crystallographic structures of diversified targets, DNA has been chosen to be the less touched one as it (crystallographic structure) is not readily available. Therefore our present study is directed towards designing a novel class of DNA intercalators applying docking methodology. The cocrystal, BFA50 [9-Bromo-phenazine-1-carboxylic acid (2-dimethylamino-ethyl)-amide] present in the crystal structure of D(CG(5-BRU)ACG) complex, obtained from protein data bank was considered as template. Considering the topological description of DNA and the 2D pose view of cocrystal within the active site (Figure 1), we have designed three different series of compounds (as, ps and ss) (Table 1) by altering the ring atoms as well as side chain pattern.

As the research is aimed at initial designing, therefore, before subjecting them to synthesis, we have carried out *in silico* study using AUTODOCK VINA to develop an understanding about their binding mode.

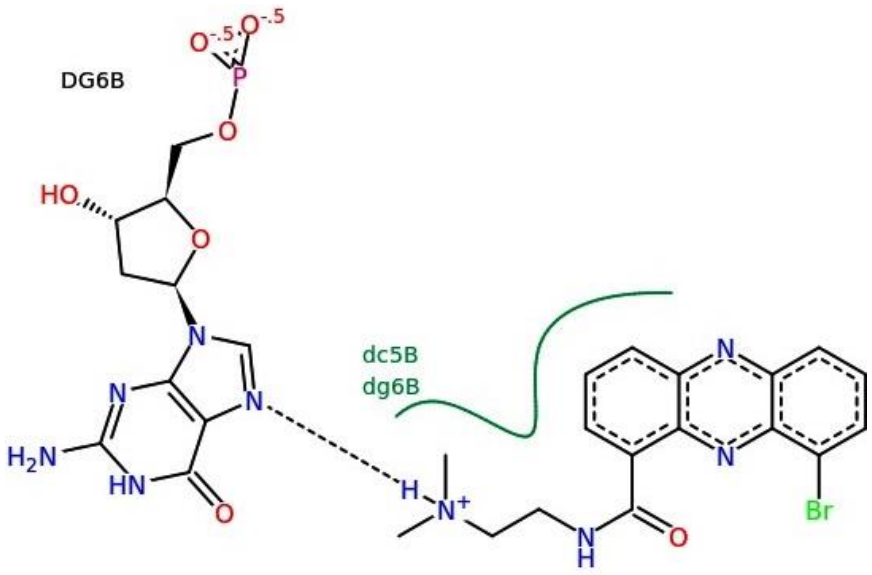
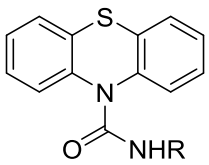
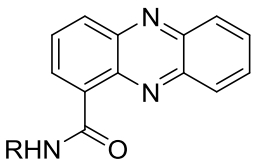
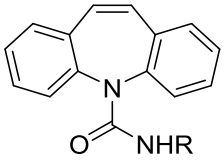


Figure 1: 2D pose view of co-crystal with the binding site.

Table 1: Three different series of compounds considered for the docking study.

Series Template		
 (as)	 (ps)	 (ss)
Compound Code	**R**	
as1, ps1, ss1	$-CH_2CH(CH_3)N(CH_3)_2$	
as2, ps2, ss2	$-CH(CH_3)CH_2N(CH_3)_2$	
as3, ps3, ss3	$-CH(OH)CH_2N(CH_3)_2$	
as4, ps4, ss4	$-(CH_2)_3N(CH_3)_2$	
as5, ps5, ss5	$-(CH_2)_2N(C_2H_5)_2$	
as6, ps6, ss6	$-CH_2CH(CH_3)N(C_2H_5)_2$	
as7, ps7, ss7	$-CH(CH_3)CH_2N(C_2H_5)_2$	
as8, ps8, ss8	$-CH(OH)CH_2N(C_2H_5)_2$	
as9, ps9, ss9	$-(CH_2)_3N(C_2H_5)_2$	
as10, ps10, ss10	$-(CH_2)_2NHCH(CH_3)_2$	

EXPERIMENTAL PROCEDURE

DNA modeling

The DNA model was built by using AutoDock Tools- 1.5.4 and MGL Tools-1.5.4 packages (The Scripps Research Institute, Molecular Graphics Laboratory, 10550 North Torrey Pines Road, CA, 92037) running on Windows based Dell system (3.4 GHz processor, 2GB RAM, 320 GB Hard disk). It consists of several steps. The structure of the D(CG(5-BRU)ACG) complex was obtained from Brookhaven Protein Data Bank [5] (entry code 1eg6) [6] (PDB; http: //www.rcsb. org/pdb) and loaded to python molecular viewer. The nonbonded oxygen atoms of waters, present in the crystal structure were removed. After assigning the bond orders, missing hydrogen atoms were added, then the partial atomic charges was calculated using Gasteiger-Marsili method [7]. Kollman [8] united atom charges were assigned, non-polar hydrogens were merged, and rotatable bonds were assigned, considering all the amide bonds as non-rotatable. The receptor file was converted to pdbqt format, which is pdb plus ‘‘q’’ charges and ‘‘t’’ AutoDock type. (To confirm the AutoDock types, polar hydrogens should be present, whereas non-polar hydrogens and lone pair should be merged, each atom should be assigned Gasteiger partial charges).

Validation of the docking protocol in autodock

The most suitable method of evaluating the accuracy of a docking procedure is to determine how closely the least energy conformation predicted by the scoring function resembles an experimental binding mode as determined by X-ray crystallography. In the present study, the docking of phenazine which was extracted previously from 1EG6, DNA duplex was performed to test the reliability and reproducibility of the docking protocol for our study. We found a very good agreement between the localization of the intercalator, phenazine upon docking and from

The crystal structure

The root mean square deviations (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation of cocrystal phenazine equaled 1.65 Å by Autodock. This indicated the reliability of the docking method in reproducing the experimentally observed binding mode for DNA intercalator.

Ligand receptor modeling

CS ChemDraw 4.5 (Cambridge Soft.Com, 100 Cambridge park drive, Cambridge, MA 02140, USA) was used to draw 2D structures of different ligands. Ligands were further refined and cleaned in 3D by addition of explicit hydrogens by OpenBabel-

2.2.1 [9]. All the structures were written in pdb file format. Autodock requires that ligands got partial atomic charges and Autodock atom types for each atom; it also requires a description of the rotatable bond in the ligand. In the final execution the ligand has to be rewritten as ligand.pdbqt .

Molecular docking studies

All the docking experiments were performed using Autodock vina [10] installed in a Dell system (3.4 GHz processor, 2GB RAM, 320 GB Hard disk). Autodock vina was designed and implemented by Dr. Oleg Trott at the Scripps Research Institute. The residues within 7 Å from the ligand's atom were used to define the binding site of guest molecule. For the docking procedure, a grid spacing of 1 Å and 24 X 24 X 24 number of points was used. The grid was centered on the mass center of the experimentally bound BFA50 coordinates. Autodock generated 9 possible binding conformations for each ligand. The optimized 3D structure of ligands was used for the docking studies. The binding mode and interactions were analyzed for the significant compounds with their virtually bioactive conformer (s).

RESULTS AND DISCUSSION

Molecular docking study of three different series of tricyclic system was conducted to develop an understanding of complementarities between target as DNA and their corresponding ligands. The structural features were developed considering the structural geometry of the cocrystal present within the active site of duplex strand. Three different template systems were developed implementing the application of bioisosterism and side chain modification was made considering the usual pattern followed in the development of different category of drugs.

The tricyclic systems chosen for the study were phenothiazine (as) (N-[substituted]-4a,10a-dihydro-10H-phenothiazine-10-carboxamide), phenazine (ps) (N-[substituted]phenazine-1-carboxamide) and dibenzazepine (ss) (N-[substituted]-5H-dibenzo[b,f]azepine-5-carboxamide). Ten different types of side chain were inserted. Docking energy was not considered the only criteria to assess the drug like feature of the compounds under evaluation; rather the conformer(s) which are almost superimposed onto the original cocrystal has been retained for further analysis and future study. The docking energy of significant conformers of the docked compounds is given in Table 2.

Table 2: Binding energy details of the compounds as1-as10, ps1-ps10 and ss1-ss10

Compound	Conformer No	Observed binding energy (Kcal/mole)
as1	-	-
as2	6	-4.9
as3	-	-
as4	-	-
as5	-	-
as6	-	-
as7	-	-
as8	-	-
as9	4 & 8	-5.0 & -4.8
as10	-	-
ps1	2	-6.1
ps2	1	-5.6
ps3	1	-6.3
ps4	2	-5.9
ps5	2	-6.0
ps6	-	-
ps7	1 & 2	-5.9 & -5.6
ps8	9	-5.3
ps9	-	-
ps10	1	-6.5
ss1	-	-
ss2	8	-6.4
ss3	-	-
ss4	5 & 9	-6.1 & -5.9
ss5	3	-6.2
ss6	-	-
ss7	6	-5.7
ss8	4 & 5	-5.9 & -5.9
ss9	4	-6.1
ss10	8	-5.7

After performing the individual docking study it has been observed that in series ‘as’, compounds as2, (N-[1-(dimethylamino) propan-2-yl]-4a,10a-dihydro-10H-phenothiazine-10-carboxamide) and as9 (N-[3-(diethylamino)propyl]-4a,10a-dihydro-10H-phenothiazine-10-carboxamide) with their respective conformer (s) were partially superimposed onto the cocrystal, BFA50 (Figures 2 and 3), whereas rest of the compounds in the same series were fallen apart from the active site. Figure 4 show the cluster of conformers of compound as1 was far away from the cocrystal, phenazine, thus found to be inactive as an intercalator. The affinity of significant conformers may be attributed to the spacer as both straight chain hydrocarbon as well as branched chain. It is assumed that the distance between the ring nitrogen and the teriminal tertiary amino does have some significance in the interaction mode within the minor groove of DNA.

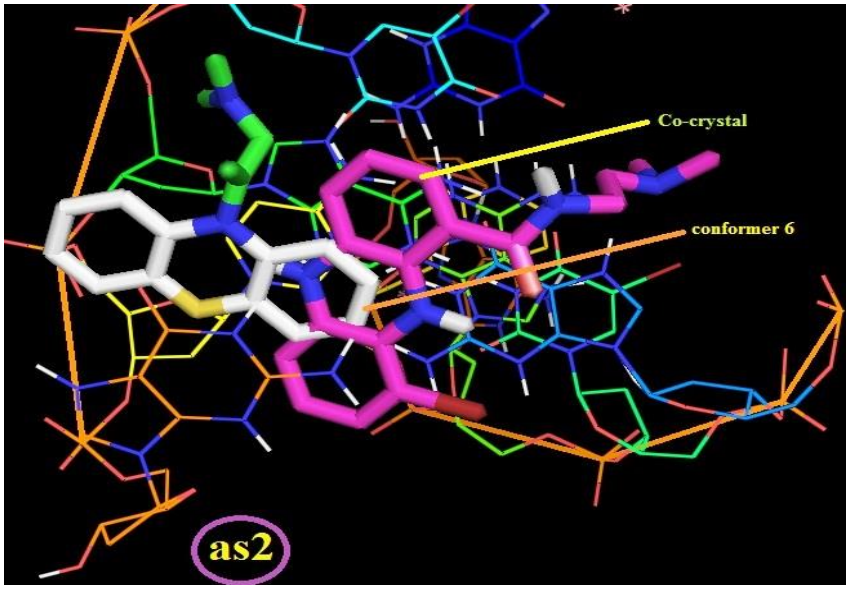


Figure 2: Docking pose of as2 (conformer 6) within the active site of the target 1eg6.

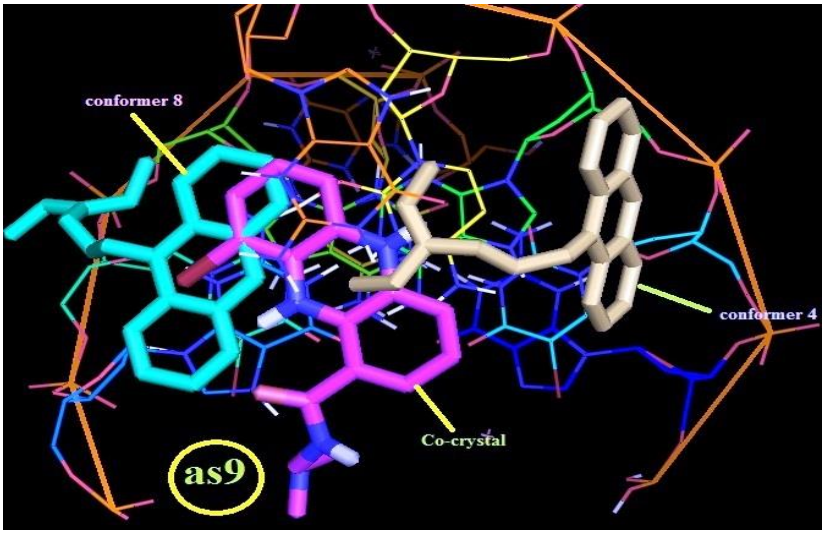


Figure 3: Docking pose of as9 (conformers 4 & 8) within the active site of the target 1eg6.

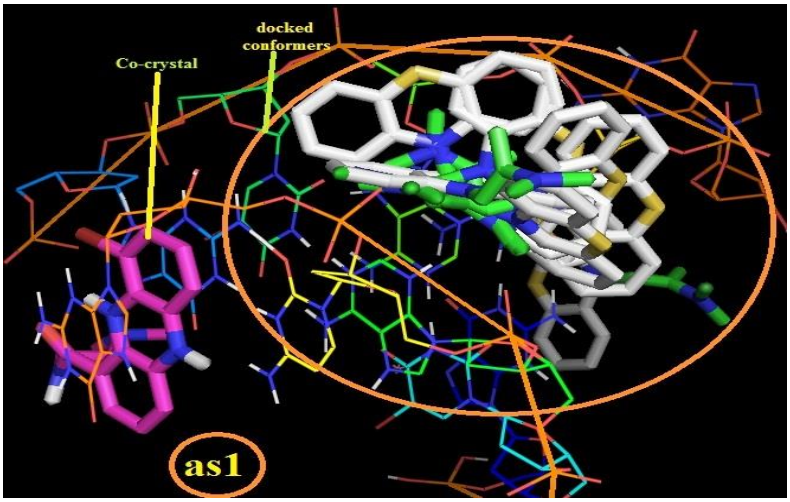


Figure 4: Docking pose of as1 (no conformer) within the active site of the target 1eg6.

In the second series, the alteration was made only with the side chain pattern, retaining the centroid similar as that of the cocrystal. The docking output and the pose obtained out of it was shown in Figures 5-12. Only the compounds ps6 (N-[2-(diethylamino) propyl]phenazine-1-carboxamide) and ps9 (N-[3-(diethylamino)propyl]phenazine-1-carboxamide) were found to be ineffective as none of the conformers found close to the active site. Rest of the compounds with their multiple conformers occupied the minor groove of duplex strand, thus may be considered for future study. Most of the compounds with their respective conformers were found within the proximal vicinity of the active site. This might because of the relative position of the side chain and the tricyclic system.

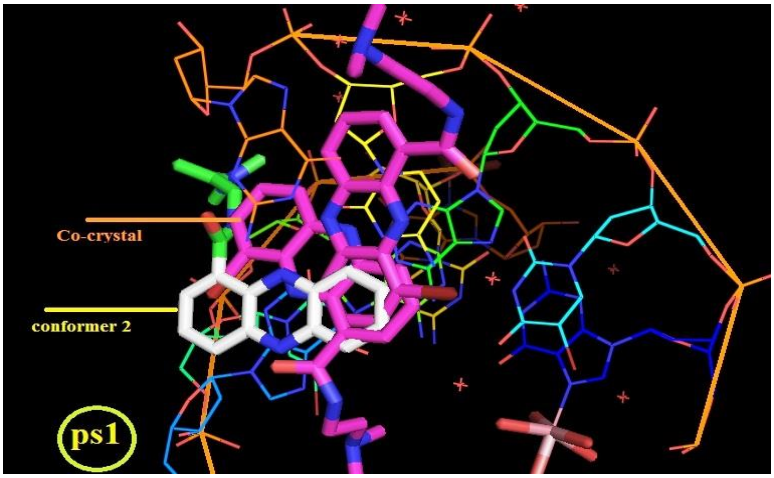


Figure 5: Docking pose of ps1 (conformer 2) within the active site of the target 1eg6.

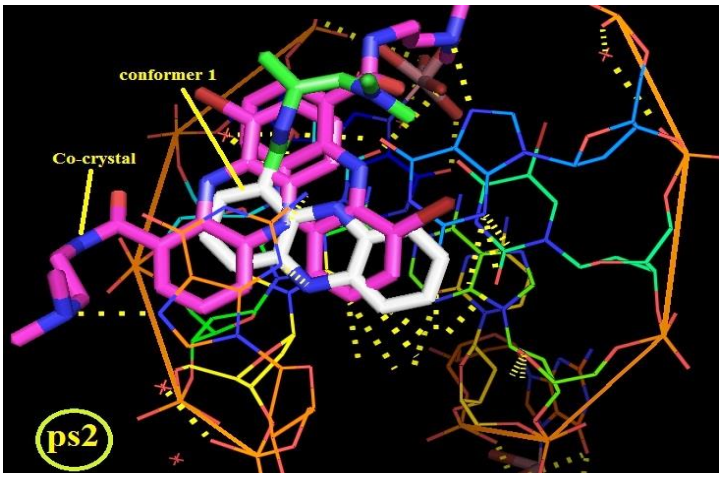


Figure 6: Docking pose of ps2 (conformer 1) within the active site of the target 1eg6.

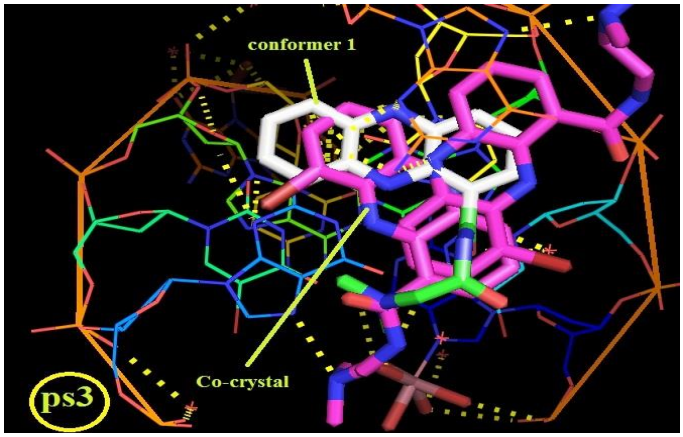


Figure 7: Docking pose of ps3 (conformer 1) within the active site of the target 1eg6.

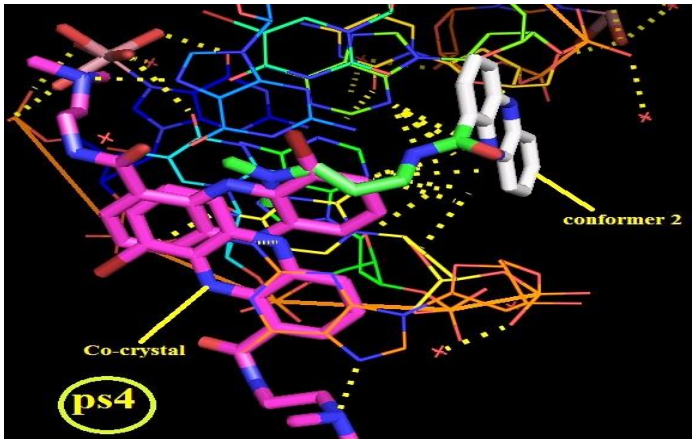


Figure 8: Docking pose of ps4 (conformer 2) within the active site of the target 1eg6.

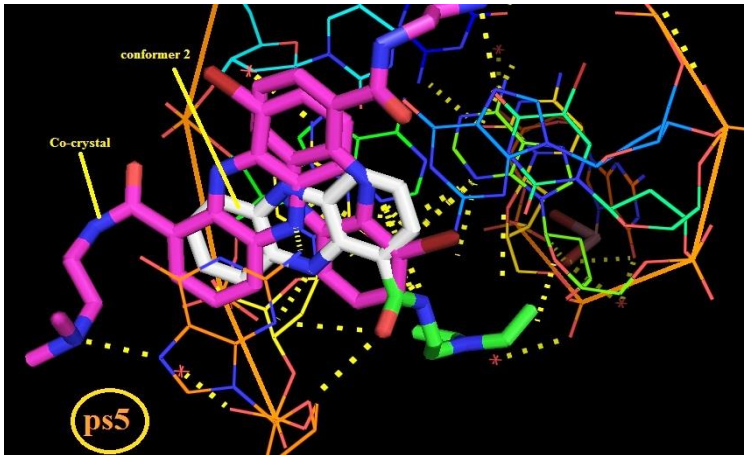


Figure 9: Docking pose of ps5 (conformer 2) within the active site of the target 1eg6.

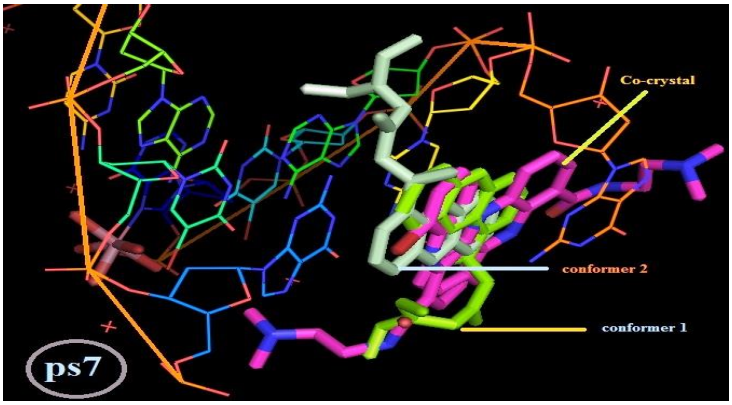


Figure 10: Docking pose of ps7 (conformers 1 & 2) within the active site of the target 1eg6.

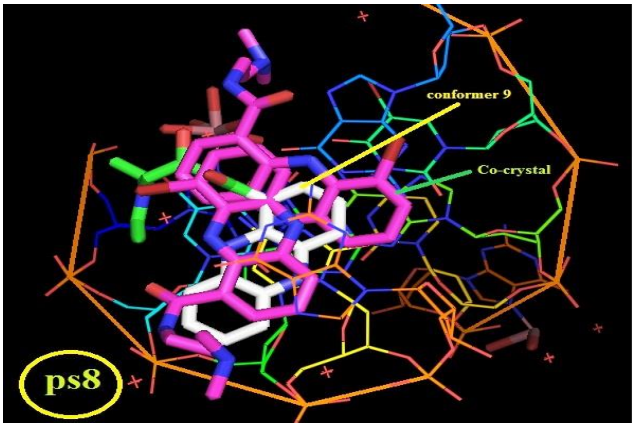


Figure 11: Docking pose of ps8 (conformer 9) within the active site of the target 1eg6.

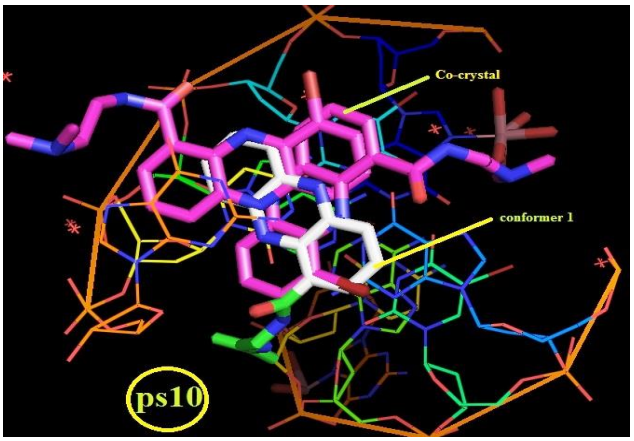


Figure 12: Docking pose of ps10 (conformer 1) within the active site of the target 1eg6.

In the final series, the central ring was expanded to a seven member heterocyclic, azepine with possible alteration as made in the earlier series in the side chain was made. The result obtained out of it was noteworthy as all the compounds, except ss1 (N-[2-(dimethylamino)propyl]-5H-dibenzo[b,f]azepine-5-carboxamide), ss3 (N-[2-(dimethylamino)-1-hydroxyethyl]-5H-dibenzo[b,f]azepine-5-carboxamide), and ss6 (N-[2-(diethylamino)propyl]-5H-dibenzo[b,f]azepine-5-carboxamide) with their conformer(s) occupied the minor groove, aligning with the cocrystal (Figures 13-19). Complete superimposition is noticed in compound ss8 (N-[2-(diethylamino)-1-hydroxyethyl]-5H-dibenzo [b,f]azepine-5-carboxamide (Figure 17), which may be attributed to the side chain hydroxyl group and the hydrocarbon chain.

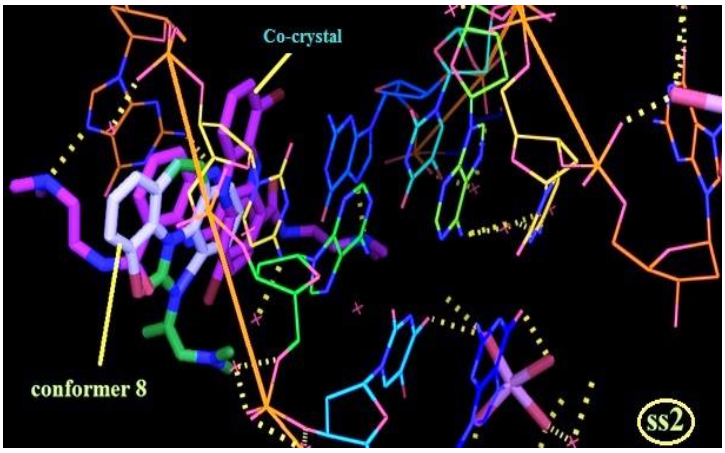


Figure 13: Docking pose of ss2 (conformer 8) within the active site of the target 1eg6.

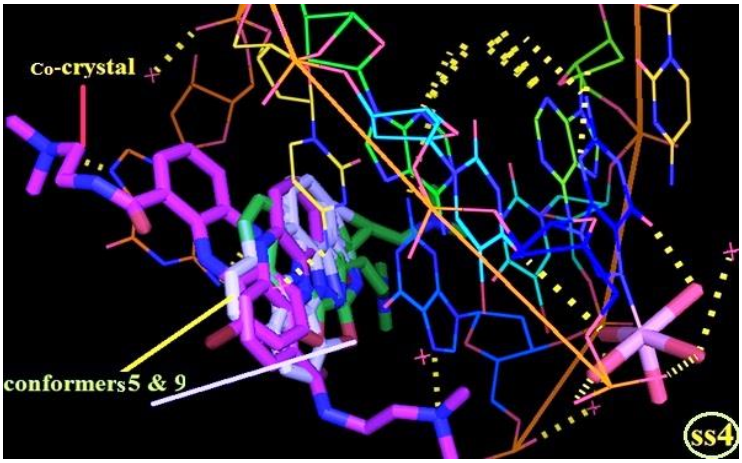


Figure 14: Docking pose of ss4 (conformers 5 & 9) within the active site of the target 1eg6.

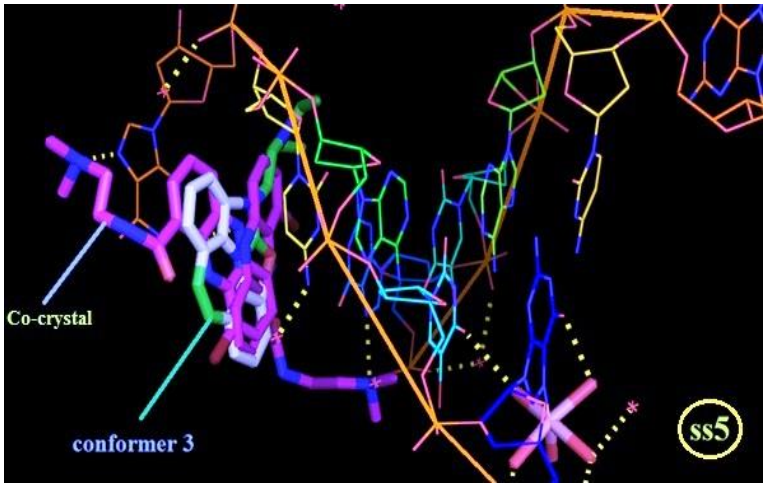


Figure 15: Docking pose of ss5 (conformer 3) within the active site of the target 1eg6.

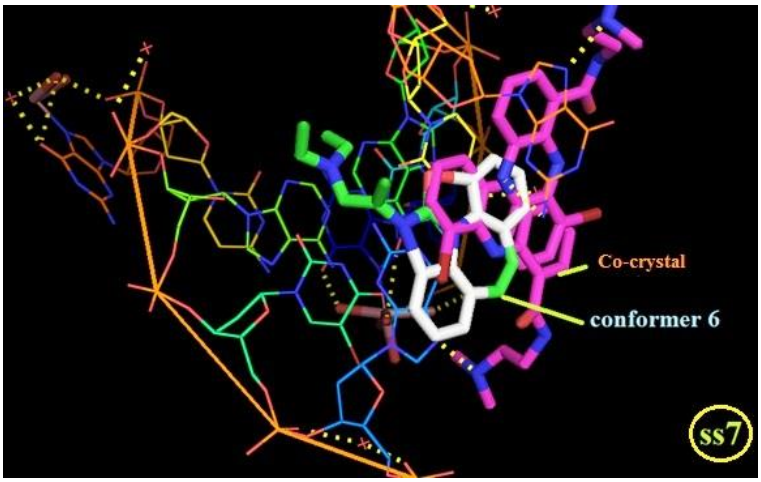


Figure 16: Docking pose of ss7 (conformer 6) within the active site of the target 1eg6.

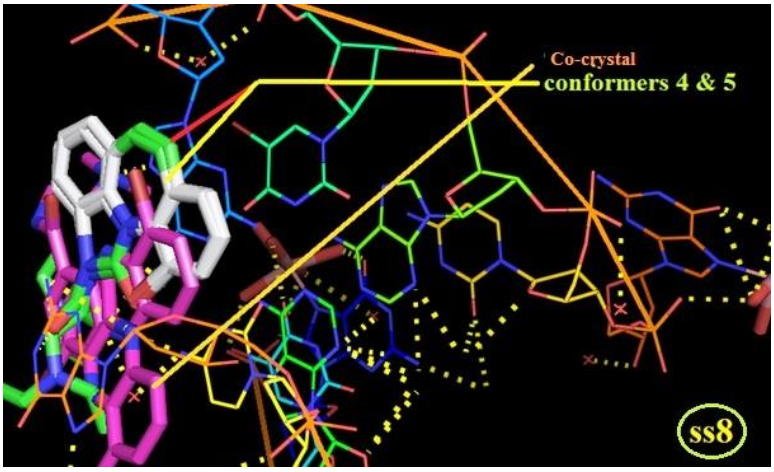


Figure 17: Docking pose of ss8 (conformers 4 & 5) within the active site of the target 1eg6.

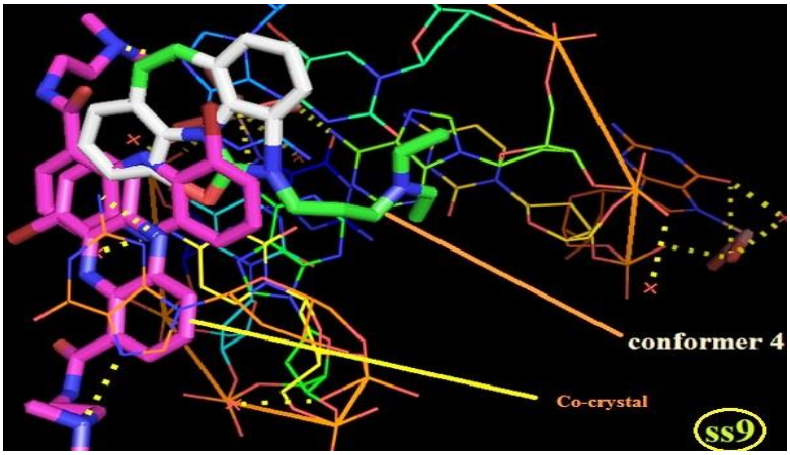


Figure 18: Docking pose of ss9 (conformer 4) within the active site of the target 1eg6.

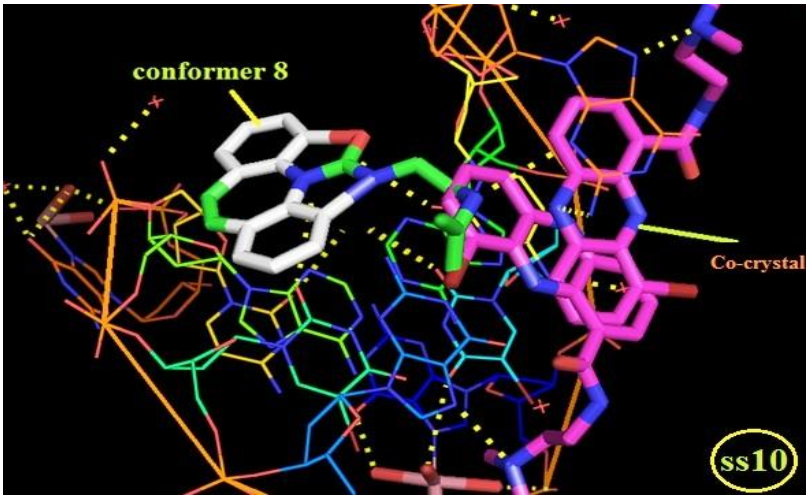


Figure 19: Docking pose of ss10 (conformer 8) within the active site of the target 1eg6.

CONCLUSION

The reliability and reproducibility of the docking parameters for our study was done through redocking the co-crystal present in the target. The closeness in data has established the authenticity of docking process. In addition to the scoring function, alignment with the cocrystal was another major criteria set for the selection of quality compound. The alteration of the tricyclic system in respective series did make some difference in the active site occupancy but, maximum alignment was attributed to the side chain pattern, though it cannot be justified through electron density, but it can be substantiated through bioisosteric modification. Highest alignment was observed to those compounds which possess either hydroxyl or methyl group in their side chain. Therefore, it can be concluded that owing to their binding pattern, compounds as2, ps2, ps3, ps7, ps8, ss2, ss8 were the highly active among all. However, these results are speculative and further study may be required to understand the exact molecular mechanism of action of these compounds.

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