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Structure Based Drug Desiginig of Neplanocin A

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ABSTRACT

Neplanocin A, a novel cyclopentenyl analog of adenosine, is a naturally occurring antibiotic which exhibits significant antitumor activity. In the present study we demonstrate that neplanocin A is also a potent inhibitor of S-adenosylhomocysteine (AdoHcy) hydrolase. Analysis of the apparent irreversible inactivation of AdoHcy hydrolase by neplanocin A indicates that the drug is a tight binding inhibitor, exhibiting a stoichiometry of one molecule of inhibitor to one molecule (tetramer) of enzyme. Also neplanocin A is a potent inhibitor of vaccinia virus (WR). The inhibition of virus multiplication by neplanocin A may be related to an inhibition of S-adenosylmethionine-dependent macromolecular methylation reactions which are essential to the production of new virus particles (e.g. viral messenger RNA). To decrease its adverse effects, we performed docking studies with different substitutes of the Neoplacin with GOLD software. Pharmacophore mapping and ludi interaction were calculated for strengthening the binding of ligand with S-adenosyl-L-homocysteine hydrolase.

Key words: *Neplanocin A, Modelling, S-adenosylhomocysteine, Docking studies*

INTRODUCTION

Neplanocin A, a novel cyclopentenyl analog of adenosine, is a naturally occurring antibiotic which exhibits significant anti-tumor activity. S-adenosyl-L-homocysteine hydrolase (AdoHcyase) is an enzyme of the activated methyl cycle, responsible for the reversible hydration of S-adenosyl-L-homocysteine into adenosine and homocysteine. AdoHcyase is an ubiquitous enzyme which binds and requires NAD^+ as a cofactor. AdoHcyase is a highly conserved protein of about 430 to 470 amino acids. The family contains a glycine-rich region in the central part of AdoHcyase; a region thought to be involved in NAD-binding.In recent years, S-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) has emerged as a specific target for the design of potential chemotherapeutic agents[1,2,3]. Such an approach has been prompted by the important role that this enzyme is known to play in regulating biological methylation reactions. AdoHcy hydrolase catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine. Although the equilibrium of the reaction favors synthesis, AdoHcy is efficiently hydrolyzed under physiological conditions because Ado and Hcy are simultaneously removed by several metabolic routes[4].

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Inhibition of AdoHcy hydrolase in intact cellular systems results in the accumulation of AdoHcy, a product inhibitor of AdoMet-dependent methylation reactions[5,7]. As a consequence of inhibiting AdoHcy metabolism cellular methylation reactions are perturbed, many of w which are required for maintenance of the normal metabolic integrity of the cell. An example of an essential methylation reaction is found in the maturation scheme of certain eukaryotic and viral messenger RNA molecules. It is known that, in many instances, these mRNA molecules must be both capped and methylated on their 5' terminus to promote active translation of the corresponding proteins[8]. Methylation of the 5'-cap structure has been demonstrated to enhance the efficiency of initiation of translation at the 5'-endo f the Mrna[8]. Moreover, it has been shown that the vaccinia virus-specific enzymes which catalyze these reactions for viral mRNAs *(i e.* guanine 7-methyltransferase; 2'-0-nucleoside methyltransferase) are susceptible to inhibition by AdoHcy[3,4]. It is not surprising, therefore, that potent inhibitors of AdoHcy hydrolase such as 3-deazaadenosine[5], 3-deazaaristeromycin[1,6], and adenosine &aldehyde2 elicit significant antiviral activity against viruses requiring a methylated 5'-cap structure on their mRNAs. Recently, the isolation and characterization of neplanocin A has been reported[7,8]. This compound, a novel carbocyclic analog of adenosine in which the ribose moiety is replaced by a cyclopentene ring (Fig. l), has been shown to possess anti-tumor properties with relatively low cytotoxicity. Considering its structural similarity to adenosine, it is conceivable that the pharmacological activity of neplanocin A may be mediated through interaction with an enzyme involved in adenosine metabolism, such as AdoHcy hydrolase. Neplanocin A is a potent inhibitor of AdoHcy hydrolase both *in vitro* and *in uiuo,* and that it elicits potent antiviral activity against vaccinia virus (WR) in mouse L929 cells.

MATERIALS AND METHODS

3D model building

The initial model of Structure of Human S-adenosylhomocysteine hydrolase was built by using homology-modeling methods and the MODELLER software; a program for comparative protein structur e modeling optimally satisfying spatial restraints derived from the alignment and expressed as probability density functions (pdfs) for the features restrained. The pdfs restrain Cα-Cα distances, main-chain N-O distances, main-chain and side-chain dihedral angles. The 3D model of a protein is obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf is derived as a combination of pdfs restraining individual spatial features of the whole molecule. The optimization procedure is a variable target function method that applies the conjugate gradients algorithm to positions of all non-hydrogen atoms. The query sequence from Homo sapiens was submitted to domain fishing server for Structure of Human S-adenosylhomocysteine hydrolase prediction. The predicted domain was searched to find out the related protein structure to be used as a template by the BLAST (Basic Local Alignment Search Tool) program against PDB(Protein Databank). Sequence that showed maximum identity with high score and less e-value were aligned (Figure 1) and was used as a reference structure to build a 3D model for Structure of Human S-adenosylhomocysteine hydrolase. The sequence of Structure of Human S-adenosylhomocysteine hydrolase was obtained from NCBI. The co-ordinates for the structurally conserved regions (SCRs) for Structure of Human S-adenosylhomocysteine hydrolase were assigned from the template using multiple sequence alignment, based on the Needleman-Wunsch algorithm. The structure having the least modeller objective function, obtained from the modeller was improved by molecular dynamics and equilibration methods using NAMD 2.5 software using CHARMM27 force field for lipids and proteins along with the TIP3P model for water (Figure 2B). The energy of

the structure was minimized with 1,00,00 steps. A cutoff of 12 Å (switching function starting at 10 Å) for van der Waals interactions was assumed. No periodic boundary conditions were included in this study. An integration time step of 2 fs was used, permitting a multiple time-stepping algorithm to be employed in which interactions involving covalent bonds were computed every time step, short-range nonbonded interactions were computed every two time steps, and long-range electrostatic forces were computed every four time steps. The pair list of the nonbonded interaction was recalculated every ten time steps with a pair list distance of 13.5 Å. The short-range nonbonded interactions were defined as van der Waals and electrostatics interactions between particles within 12 Å. A smoothing function was employed for the van der Waals interactions at a distance of 10 Å. CHARMM27 [force-field parameters were used in all simulations in this study. The equilibrated system was simulated for 1 ps with a 500 kcal/mol/Å2 restraint on the protein backbone under 1 atm constant pressure and 310 K constant temperature (NPT) and the Langevin damping coefficient was set to 5 ps unless otherwise stated . Finally, the structure having the least energy with low RMSD (Root Mean Square Deviation) was used for further studies. In this step, the quality of the initial model was improved. The final structure obtained was analyzed by Ramachandran's map using PROCHECK (Programs to check the Stereo chemical Quality of Protein Structures) and environment profile using ERRAT graph (Structure Evaluation server). This model was used for the identification of active site and for docking of the substrate with the enzyme.

Active site Identification

Active site of Structure of Human S-adenosylhomocysteine hydrolase was identified using CASTp server. A new program, CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings.

Docking method

The ligands, including all hydrogen atoms, were built and optimized with chemsketch software suite. Extremely Fast Rigid Exhaustive Docking (FRED) version 2.1 was used for docking studies (OpenEye Scientific Software, Santa Fe, NM). It is an implementation of multiconformer docking, meaning that a conformational search of the ligand is first carried out, and all relevant low-energy conformations are then rigidly placed in the binding site. This two-step process allows only the remaining six rotational and translational degrees of freedom for the rigid conformer to be considered. The FRED process uses a series of shape-based filters, and the default scoring function is based on Gaussian shape fitting.

RESULTS AND DISCUSSION

Homology Modeling of Structure of Human S-adenosylhomocysteine hydrolase

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only two-reference proteins, including 2RF2 A (Chain A, Crystal Structure and assembly of eukaryotic small heat shock protein) has a high level of sequence identity and the identity of the reference protein with the domain are 85%. Structurally conserved regions (SCRs) for the model and the template were determined by superimposition of the two structures and multiple sequence alignment.

Fig. 1: CLUSTAL W multiple sequence alignment

```
pro     PQITLWQRPLVTIKVGGQLKEALLDTGADDTVLEDINLPGKWKPKMIGGI 50
temp    --------------------------------------------------

pro     GGFIKVKQYDNICIDICGHKATGTVLVGPTPVNIIGRNLLTQLGCTLNFP 100
temp    ----------------------------------------------MNSP 4
                                                         :* *
pro     ISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALTEICTEMEKEGKISKIG 150
temp    ISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKEGKISKIG 54
                 ********************************** **********

pro     PENPYNTPVFAIKKKDSTKWRKLVDFRELNKRTQDFWEVQLGIPHPAGLK 200
temp    PENPYNTPVFAIKKKDSTKWRKLVDFRELNKRTQDFWEVQLGIPHPAGLK 104
                 **************************************************

pro     KKKSVTVLDVGDAYFSVPLDKDFRKYTAFTIPSVNNETPGIRYQYNVLPQ 250
temp    KKKSVTVLDVGDAYFSVPLDEDFRKYTAFTIPSINNETPGIRYQYNVLPQ 154
                 **********************:**********:**********

pro     GWKGSPAIFQCSMTKILEPFRKQNPDIVIYQYMDDLYVGSDLEIGQHRTK 300
temp    GWKGSPAIFQSSMTKILEPFRKQNPDIVIYQYMDDLYVGSDLEIGQHRTK 204
                 **********.**************************************

pro     IEELRQHLLRWGFTTPDKKHQKEPPFLWMGYELHPDKWTVQPIVLPEKDS 350
temp    IEELRQHLLRWGLTTPDKKHQKEPPFLWMGYELHPDKWTVQPIVLPEKDS 254
                 **********:*****************************************

pro     WTVNDIQKLVGKLNWASQIYSGIKVRQLCRLLRGAKALTEVIQLTKEAEL 400
temp    WTVNDIQKLVGKLNWASQIYPGIKVRQLCKLLRGTKALTEVIPLTEEAEL 304
                 ********************.********:*****:******** **:*****

pro     ELAENREILKEPVHGVYYDPSKDLIAEIQKQGQGQWTYQIYQEPFKNLKT 450
temp    ELAENREILKEPVHGVYYDPSKDLIAEIQKQGQGQWTYQIYQEPFKNLKT 354
                 **************************************************

pro     GKYARMRGAHTNDVKQLTEAVQKIATESIVIWGKTPKFRLPXLKETWDT- 499
temp    GKYARMRGAHTNDVKQLTEAVQKITTESIVIWGKTPKFKLPIQKETWETW 404
                 **************************:**************:**  *****:*

pro     --------------------------------------------------
temp    WTEYWQATWIPEWEFVNTPPLVKLWYQLEKEPIVGAETFYVDGAANRETK 454
```

In the following study, we have chosen 2RF2 A as a reference structure for modeling S-adenosylhomocysteine domain. Coordinates from the reference protein (2RF2 A) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. The energy unit will be in kilo joule. All side chains of the model protein were set by rotamers.

The final stable structure of the Structure of Human S-adenosylhomocysteine hydrolase protein obtained is shown in Figure 2. By the help of SPDBV it is evident that Structure of Human S-adenosylhomocysteine hydrolase domain has 14 helices and 17 sheets and it is shown in the Figure 2.

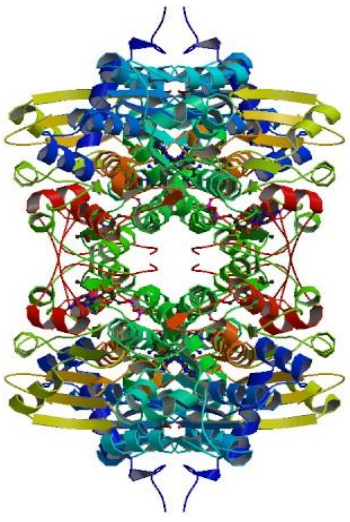


Fig. 2: Final refined structure of Structure of Human S-adenosylhomocysteine hydrolase

The final structure was further checked by verify3D graph and the results have been shown in Figure 3a. The overall scores indicates acceptable protein environment.

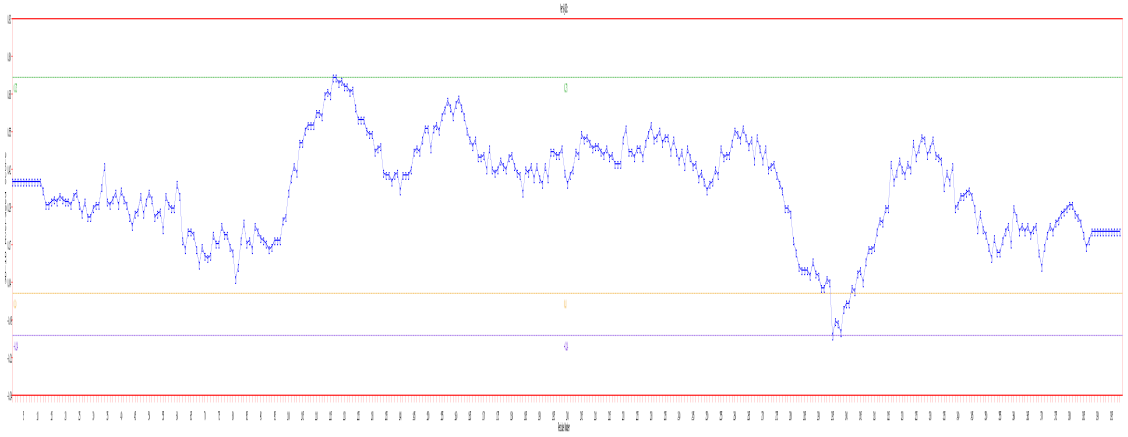


Fig. 3a : The 3D profiles verified results of Structure of Human S-adenosylhomocysteine hydrolase model; overall quality score indicates residues are reasonably folded

Validation of Domain

After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program. The π and ψ distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table 1. The RMSD (Root Mean Square deviation) deviation for covalent bonds and covalent angles relative to the standard dictionary of Structure of Human S-adenosylhomocysteine hydrolase was -3.56 and -0.17 Å. Altogether 96.3% of the residues of Structure of Human S-adenosylhomocysteine hydrolase was in favored and allowed regions. The overall PROCHECK G-factor of Structure of Human S-adenosylhomocysteine hydrolase was – 1.32 and verify3D environment profile was good.

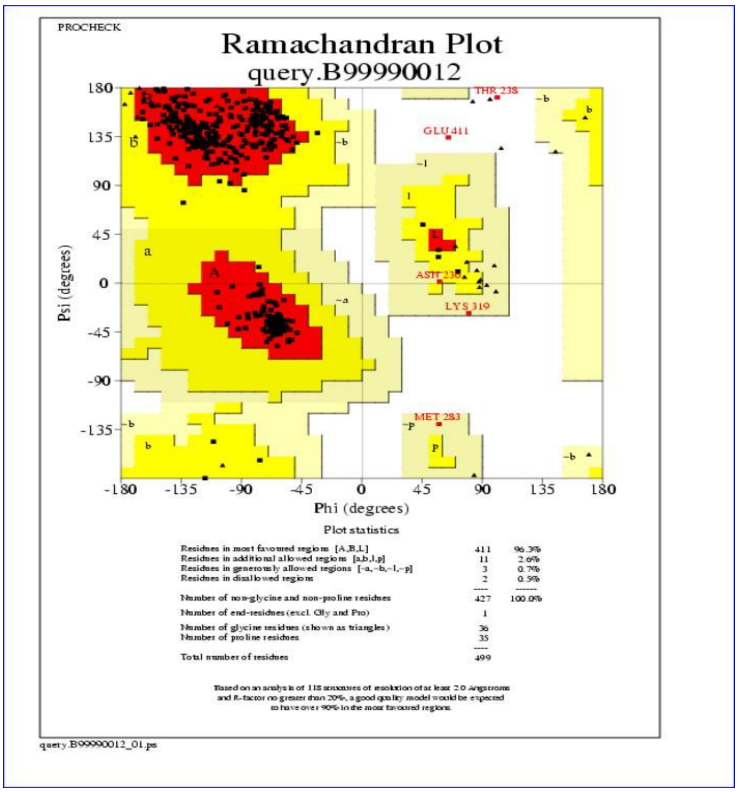


Fig. 3b: Ramachandran Plot

Superimposition of 2RF2 A with Structure of Human S-adenosylhomocysteine hydrolase The structural superimposition of C trace of template and S-adenosylhomocysteine is shown in Figure 4. The weighted root mean square deviation of C α trace between the template and final refined models 0.65A^{o}. This final refined model was used for the identification of active site and for docking of the substrate with the domain Structure of Human S-adenosylhomocysteine hydrolase.

Fig. 4: super imposition

Active site Identification of Structure of Human S-adenosylhomocysteine hydrolase After the final model was built, the possible binding sites of Structure of Human S-adenosylhomocysteine hydrolase was searched based on the structural comparison of template and the model build and also with CASTp server and was shown in Figure 5. Since, S-adenosylhomocysteine and the 2RF2 A are well conserved in both sequence and structure; their biological function should be identical. Infact from the structure-structure comparison of template, final refined model of Structure of Human S-adenosylhomocysteine hydrolase rse Transcriptase domain using SPDBV program and was shown in Figure3. It was found that secondary structures are highly conserved and the residues,. PHE 99, TRP 123, ILE 136, GLU 139, MET 140, GLU 143, LYS 145, PHE 160, ILE 162, LYS 163, LYS 164, LYS 165, ASP 166, LYS 169, TRP 170, ARG 171, LYS 172, LEU 173, VAL 174, ASP 175, PHE 176, ARG 177, ASN 180, VAL 189, GLN 190, ILE 193, VAL 207, ASP 209, VAL 210, GLY 211, ASP 12, ALA 213, TYR 214, PHE 215, PRO 249, GLN 250, GLY 251, TRP 252, SER 255, PRO 256, PHE 259, GLN 260, MET 263, GLN 281, TYR 282, MET 283, ASP 284, ASP 285, TYR 287, LYS 322, GLU 323, PRO 325, PHE 326, LEU 327, TRP 328, MET 329, GLY 330, GLN 341, PRO 342, ILE 343, VAL 344, ASN 354, LYS 358, GLY 361, LYS 362, TRP 365, LEU 385.

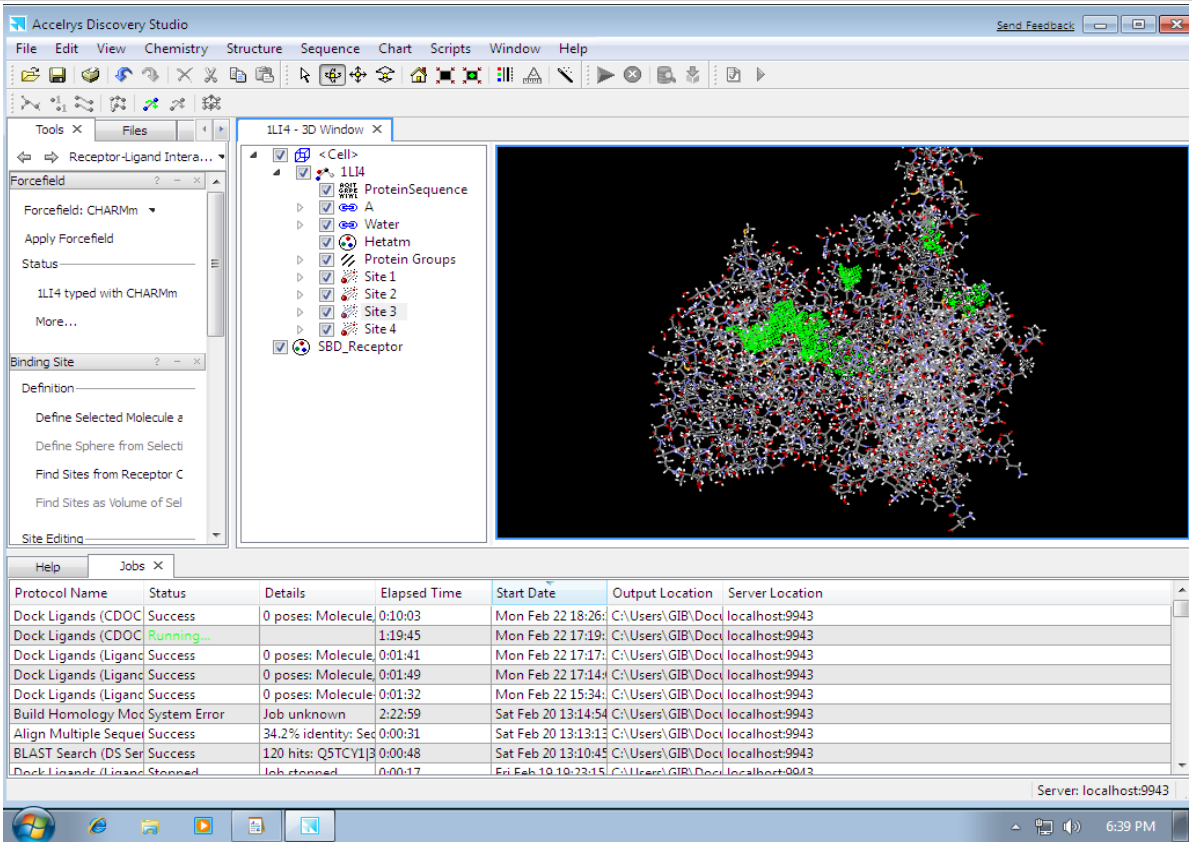


Fig. 5: active site identification

The Ligand (inhibitor) molecules used for Docking studies

Structure of Neplanocin A:

The structure of Neplanocin A was designed using chemsketch software.

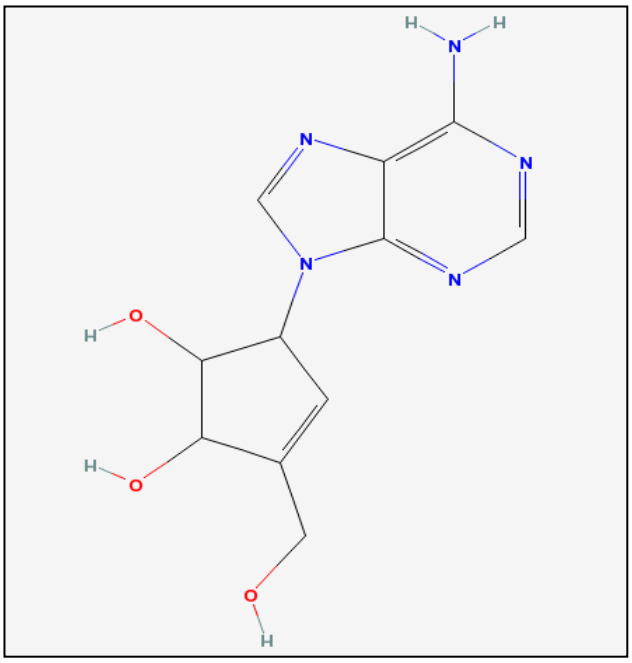
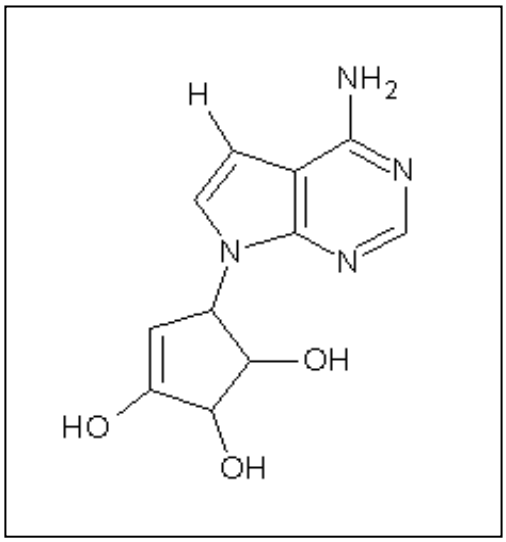


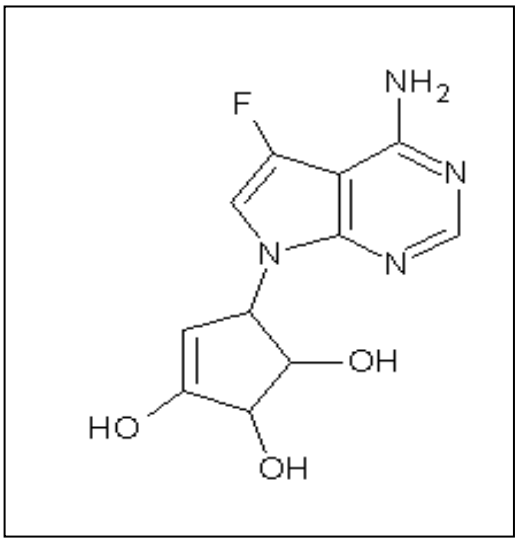
Fig. 6: shows structure of Neplanocin A has taken as Scafold

Drug Derivatives and Structures of Neplanocin A:

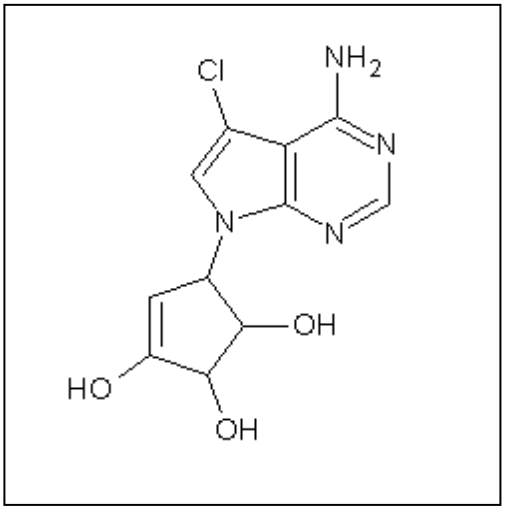
NEPLANOCIN-A: H DERIVATIVE



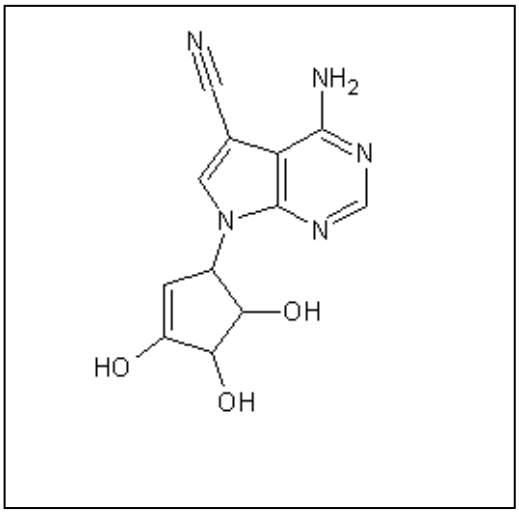
NEPLANOCIN-A: F DERIVATIVE



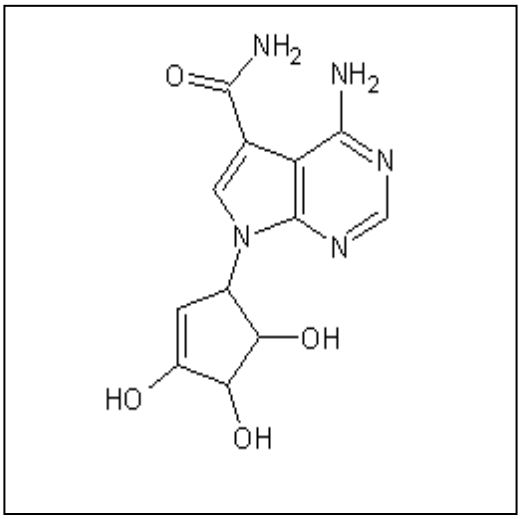
NEPLANOCIN-A: Cl DERIVATIVE



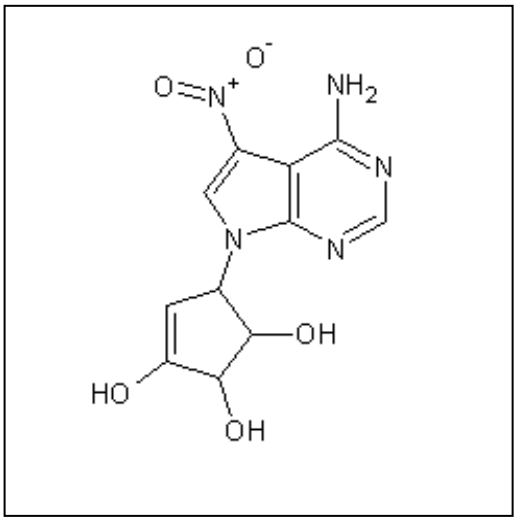
NEPLANOCIN-A: CN DERIVATIVE



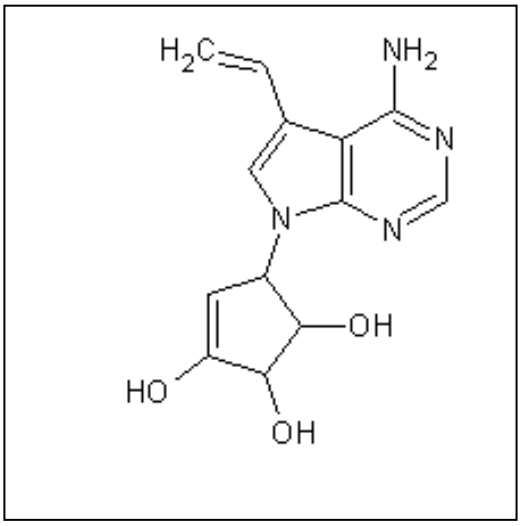
NEPLANOCIN- A: $CONH_2$ DERIVATIVE



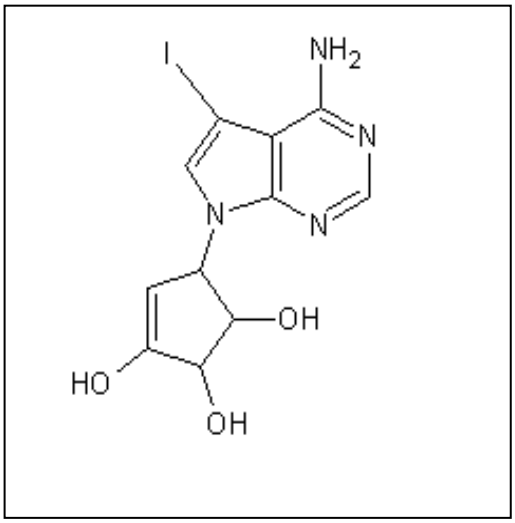
NEPLANOCIN- A: NO_2 DERIVATIVE



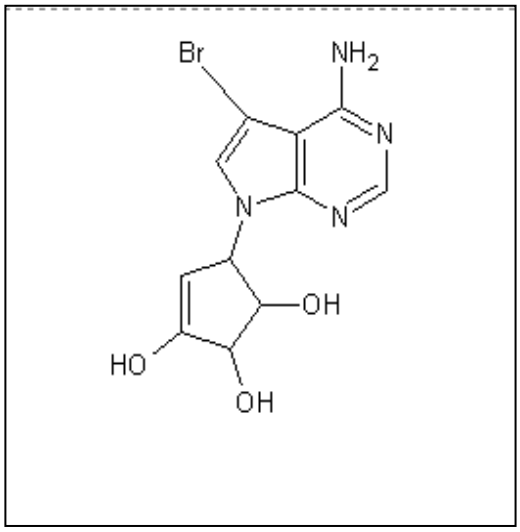
NEPLANOCIN- A: VINYL DERIVATIVE



NEPLANOCIN-A: I DERIVATIVE



NEPLANOCIN-A :Br DERIVATIVE



NEPLANOCIN-A:ETHYNYL DERIVATIVE

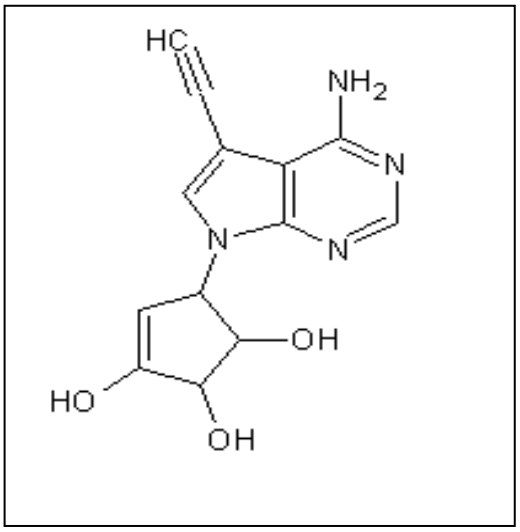


Table 1: Drug Deratives

COMPD	R	EC50(um)	IC50(um)	SI 50
2	H	2.5	>100	>40
12*	F	9.5%	112.7%	
13	CL	14.7	50.1	3.4
14	Br	16.7	41.7	2.5
15	I	2.1	15.0	7.1
18	NO2	31.5	101.5%	
22	Vinyl	49.2%	99.7%	
24	Ethynyl	20.1	48.9	2.4
26	CN	0.5%	103.3%	
27	CONH2	1.8	11.8	6.6
2'-c-Me-A		0.15	>10.0	>66.7

Docking of inhibitors with the active site of Human S-adenosylhomocysteine hydrolase

Docking of the inhibitors given in Figure 6 with Human S-adenosylhomocysteine hydrolase was performed using FRED v 2.1, which is based on Rigid Body Shape-Fitting (Open Eye Scientific Software, Santa Fe, NM). This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site. This box was defined by extending the size of a cocrystalized ligand by 4 Å (addbox parameter of FRED). This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with OMEGA (same protocol as above)(OpenEye Scientific Software, Santa Fe, NM). To this set, the substrate (generation of multiconformer with Omega) corresponding to the modeled protein were added.

Table 2: The total energies of Chemguass score, Chemscore, PLP score and shapeguass score of the best-docked conformations of Human S-adenosylhomocysteine hydrolase

Molecule name	Chemgausses	Chemscore	PLP	Screenscore	Shapegausses	TOTAL
1	-45.68	-12.52	-36.79	-84.38	-337.22	-516.59
2	-41.37	-6.93	-34.63	-76.08	-338.53	-497.54
3	-44.65	-12.42	-37.86	-83.04	-325.72	-503.69
4	-47.29	-7.86	-21.54	-62.22	-349.95	-488.86
5	-44.57	-5.76	-34.78	-87.17	-349.63	-521.91
6	-46.84	-4.88	-30.58	-70.54	-346.81	-499.65
7	-39.28	-6.22	-33.97	-68.03	-350.42	-497.92
8	-46.2	-8.68	-35.1	-73.83	-368.12	-531.93
9	-49.58	-6.8	-29.98	-69.51	-375.15	-531.02
10	-42.28	-7.26	-28.26	-67.49	-316.71	-462
11	-43.97	-11.87	-32.32	-63.54	-338.65	-490.35
12	-47.8	-7.79	-38.41	-76.22	-377.19	-547.41

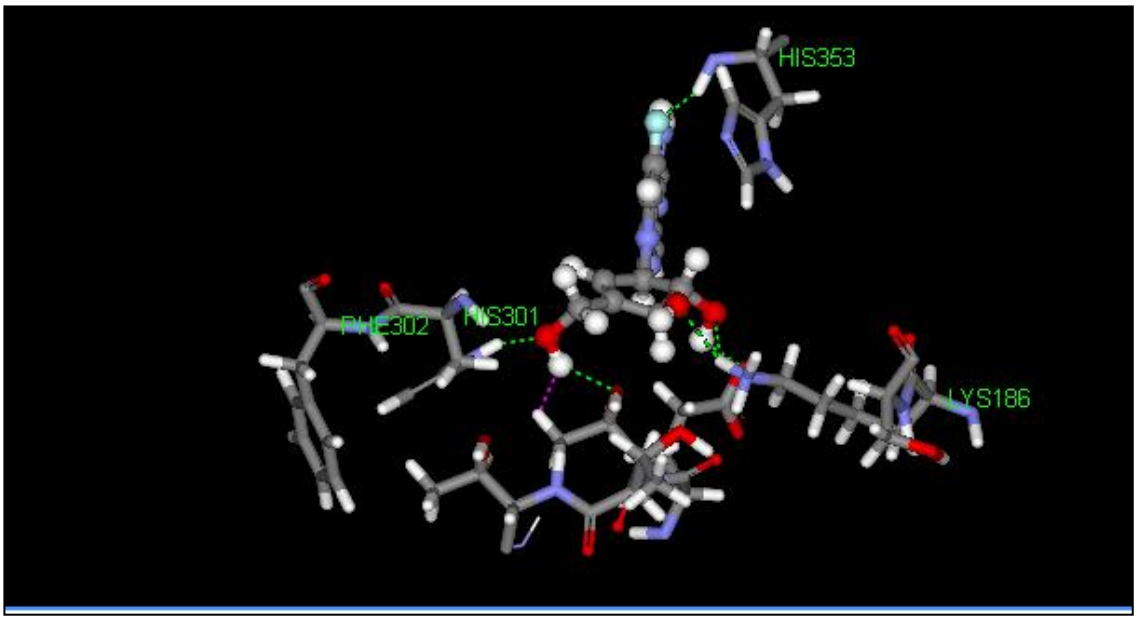


Fig. 7: Neplanocin derivative docked with Human S-adenosylhomocysteine hydrolase

CONCLUSION

The present study was carried out to find effective drug derivatives which show better activity than Neplanocin A. A virtual screening of suitable drugs was performed, which identified better neplanocin A derivative proven to result in improved inhibition of S-adenosyl-L-homocysteine hydrolase and active amino acid residues, which will be useful in designing other potent drugs and drug analogs. This study provides new insights into the identification of drugs in the in vitro laboratory. To understand the possible binding mode Neoplacin A with S-adenosyl-L-homocysteine hydrolase (EC: 3.3.1.1) (AdoHcyase), different substituted Neoplacin were used for docking with GOLD software. In the case of 5'-substitued fluoro -Neplanocin A highest binding energy was observed (38.23 kcal/mol). Pharmacophore mapping and ludi interaction were calculated for strengthening the binding of ligand with S-adenosyl-L-homocysteine hydrolase. The compound 5'-fluoro-Neplanocin A has got better fit score, present case study need to be investigated for better conclusion.

REFERENCES

1. Bohm, H.J., The computer program LUDI: A new method for the de novo design of enzyme inhibitors. *J. Comp-Aided Molec. Design*. **6:** 61- 78 (1992).
2. Bohm, H.J., LUDI: rule based automatic design of new substituents for enzyme inhibitor leads. *J. Comp-Aided Molec. Design*. **6:** 593-606 (1992).
3. Bohm, H.J., The development of a simple empirical scoring function to estimate the binding constant for a protein-ligand complex of known three-dimensional structure. *J. Comp-Aided Molec. Design*. **8:** 243-56 (1994).
4. Bohm, H.J., On the use of LUDI to search the Fine Chemicals Directory for ligands of proteins of known three-dimensional structure. *J. Comp-Aided Molec. Design*. **8:** 623-32 (1994).
5. Bohm, H.J., Prediction of binding constants of protein ligands: a fast method for the prioritization of hits obtained from de novo design or 3D database search programs. *J. Comp-Aided Molec. Design*. **12:** 1-15 (1998).
6. Dorsey, J.F., Jove, R., Kraker, A.J. and Wu, J., The pyrido[2,3-d]pyrimidine derivative PD180970 inhibits p210Bcr-Abl tyrosine kinase and induces apoptosis of K562 leukemic cells. *Cancer Res.*, **60:** 3127 (2000).
7. Nagar, B., Bornmann, W.G., Pellicena, P., Schindler, T. and Veach, D.R. *et al*., Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res*. **62:** 4236 (2002).
8. Pendergast, A.M., Gishizky, M.L., Havlik, M.H. and Witte, O.N., SH1 domain autophosphorylation of P210 BCR/ABL is required for transformation but not growth factor independence. *Mol Cell Biol*., **13:** 1728 (1993).