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PHARMACOPHORE BASED DRUG DESIGNING OF PLANT METABOLITE AXILLARIN FROM *INULA BRITANNICA* AGAINST NATIVE HIV1 –PROTEASE

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ABSTRACT

This research focuses on the generation of analogy for axillarin from *Inula britannica*. Chemical based drugs have tendency to induce more side effects to the human body ,especially the drugs used for treating dreadful disease like HIV shows more side effects to the patients. Thus, the new discoveries of leads are necessary to prevent the patients from side effects. One such a way of achieving is computational drug discovery. The chemical features of axillarin are analyzed by catalyst - Hiphop algorithm and its derivatives are generated based on axillarin Pharmacophore using Minimaybridge. Hence, the lead S00165 shows good absorption, solubility, and high penetrating mode in blood brain barrier with no carcinogenicity and mutagenicity. The analogy S00165 docked with HIV-1 native protease using Ligand-Fit algorithm with Montecarlo simulations and the binding affinity possess -3.89kcal/mol with dock score of 69.785.

KEYWORDS: **Axillarin, HIV-1 native protease, Ligand-Fit, Natural sources, Pharmacophore.**

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INTRODUCTION

HIV infection in humans is considered pandemic by the World Health Organization. From its discovery in 1981 to 2006, AIDS killed more than 25 million people. HIV infects about 0.6% of the world's population. In 2009, AIDS claimed an estimated 1.8 million lives, down from a global peak of 2.1 million in 2004. Approximately 260,000 children died of AIDS in 2009. There are very few drugs available in market to treat dreadful disease HIV with more effects[1]. Thus, the need for a rapid search for small molecules that may bind the targets of biological interest is of crucial importance in the drug discovery process. One way of achieving this is the Insilico or virtual screening (VS) of large compound collections to identify a subset of compounds that contains relatively many hits against the target, compared to a random selection from the collection[2]. Secondary metabolites are chemicals produced by plants for which no role has yet been found in growth, photosynthesis, reproduction, or other "primary" functions. These chemicals are extremely diverse; many have been identified in several major classes. Each plant family, genus, and species produces a characteristic mix of these chemicals, and they can sometimes be used as taxonomic characters in classifying plants[3].

The first requirement to all of the approaches is to have biological assay for particular compounds. Any kind of Bioassay is meant for determining a biological system, relative to a controlled compound, whether a compound has a desired activity and if so, what the relative potency of the compound is. The pharmacological depends upon the activity and potency of drug. Activity is a particular biological or pharmacological effect[4]. There has been a renaissance in modern medicine with the introduction of structure-based drug design. Conventional drug designing was time consuming, expensive and did not always yield good results. In addition, there was also a lack of rationalism in the approach toward drug discovery[4-5].

In contrast, this new elegant technique promises high specificity and efficacy. Also of importance is the positive impact of these techniques on the economies of the pharmaceutical industry. The structure of proteins and nucleic acids are being increasingly known, opening new avenues for drug designing[6]. Structure based drug design has already yielded several drugs currently on the market. It is a now growing rapidly in research field in which many successes have reported in recent years. The progresses of genomics and proteomics and the rapid accumulation of structural information have provided hundreds of new targets and opportunities for further drug discovery [6-7].

Structure-based drug design (or direct drug design) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy[8]. Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques[9]. The time devoted to the structure based drug design process may represent only a fraction of the total time toward developing a marketable drug product, but structure-based drug design is essential and most powerful when it is a part of an entire drug lead discovery process[10]. The Docking is conducted with a procedure involving the shape-matching alignment of a molecule to a cavity followed by molecular-mechanics torsion optimization and energy minimization on both the molecule and the protein residues at the binding region[11-12]. A good score for a given molecule indicates that it is potentially a good binder. This process is repeated for all molecules in the collection, which are subsequently rank-ordered by their scores. This rank-ordered list is then used to select for purchase, synthesis, or biological investigation only those compounds that are predicted to be most active[13-15].Plant secondary metabolites are a generic term used for more than 30,000

different substances which are exclusively produced by plants. Secondary metabolites carry out a number of protective functions in the human body. Plant secondary metabolites can boost the immune system, protect the body from free radicals, and kill pathogenic germs and much more. Axillarin is an O-methylated flavonol. It can be found in *Pulicaria crispa*, *Filifolium sibiricum*, *Inula britannica*, *Wyethia bolanderi* in *Balsamorhiza macrophylla* and in *Tanacetum vulgare*. It can also be synthetized[16]. The structure of axillarin is represented in Figure 1

Figure 1
The structure of Axillarin

MATERIALS AND METHODS

(i) Common Pharmacophore generation

Common feature pharmacophores are generated using the HipHop algorithm. Hip Hop identifies configurations or three-dimensional spatial arrangements of chemical features that are common to molecules in a training set. The configurations are identified by a pruned exhaustive search, starting with small sets of features and extending them until no larger common configuration is found.

(ii) Generation of ligands using Search 3D

The hiphop are ranked based on the Fit value, best rank with maximum fit is taken for generation of similar ligands like axillarin, a secondary metabolite from plant *Pulicaria crispa*, using Minimaybridge database available in accelrys.

(iii) Prediction of pharmacokinetics properties

ADME is the one of the protocol which is available through acclerys for the pharmacokinetics studies, which explains what drug does to the body, the parameters as Absorption, Distribution, Metabolism, Excretion, is determined by protocol in two ways such as probability and levels. The ligands/lead molecule, which falls within the limit of levels, was further taken in consideration for future analysis.

(iv) Prediction of pharmacodynamics properties

TOPKAT is used to analyze what body does to the drug; It uses the animal models and sub models to predict important parameters like mutagen, carcinogenicity, irritancy etc.., based on QSTR, Bayesian methods and fingerprints of chemical molecule.

(v) Structure based drug designing

In this docking both receptor and ligand is known to perform the respective steps using the receptor ligand fit protocol which is available through Acclerys DS 2.5 . In this current study the PDB ID -1ODW (Native HIV1 -Protease) is taken for docking studies with analogy of axillarin generated from Minimaybridge database. The process of ligand fit algorithm was explained in terms of following flowchart (Figure 2)

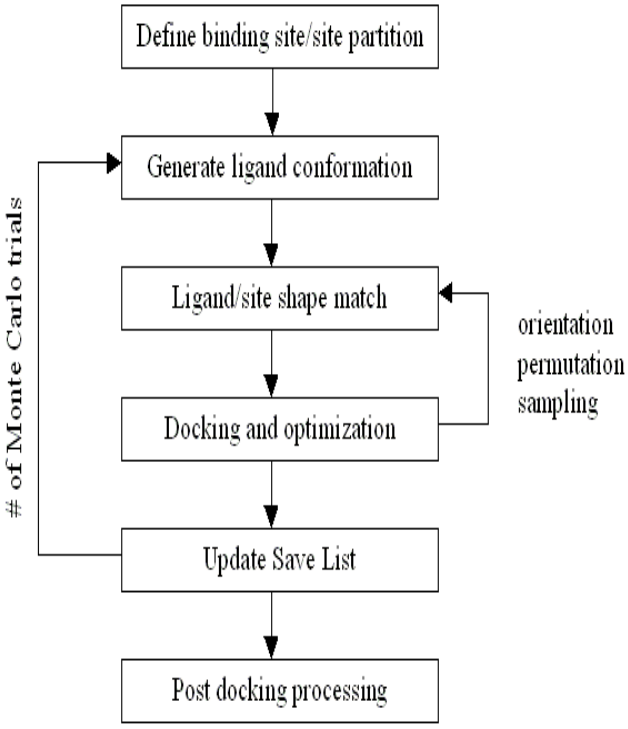

Figure 2
Flow chart of ligandfit

RESULTS AND DISCUSSION

(i) Pharmacophore generation for Axillarin

The chemical features of the Axillarin molecules is generated using common pharamcophore analysis was shown in the (Figure 3**)** individual maximum best fit value were generated for axillarin is 6 with direct pharma hit as 1 using Hiphop-catalyst algorithm. The catalyst feature of axillarin contains two HBD (Hydrogen bond donor) and four hydrophobic regions in elemental structure was displayed in the (Figure 4**)** The qualitatively 10 hip hop hypothesis were generated using catalyst hiphop algorithm is shown in the below along with rank and fit value is shown below in **(** Figure 5**)**

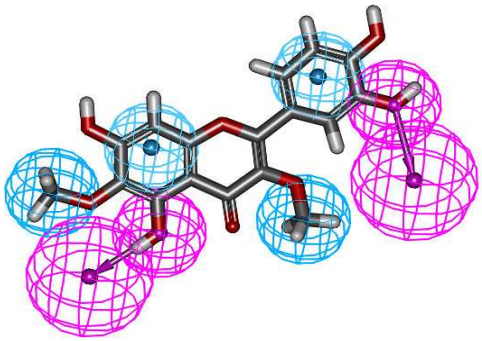

Figure 3
Axillarin with chemical features

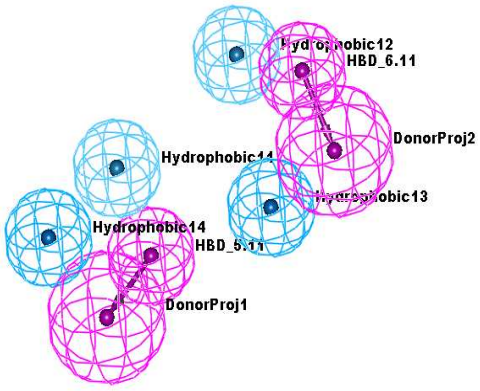

Figure 4
Catalyst-Hiphop of Axillarin

	Features	Rank	Direct Hit	Partial Hit	Max Fit
01	YYZZDD	16.544	1	0	6
02	YYZZDD	16.544	1	0	6
03	YYZZDA	16.344	1	0	6
04	YYZZDA	16.344	1	0	6
05	YYZZDA	16.344	1	0	6
06	YYZZDA	16.344	1	0	6
07	YZZDDA	16.159	1	0	6
08	YZZDDA	16.159	1	0	6
09	YZZDDA	16.155	1	0	6
10	YZZDDA	16.155	1	0	6

Figure 5
Summary of pharmacophore generation

(ii) Pharmacophore based analog generation

The analogs were generated based on the hiphop1 catalyst file as a input to search within the pre available integrated commercial database. The generated analog were retrieved from minimaybrige database contains, it 2000 molecules from that only four molecules shows the maximum fit value compared with original Pharmacophore of axillarin. The analog namely RDR 02049, JFD02181, PD00533, S00165. These numerical identities are meant for future reference. The structure of the each molecule is shown above with respective database identity notation is shown in the Figure 6

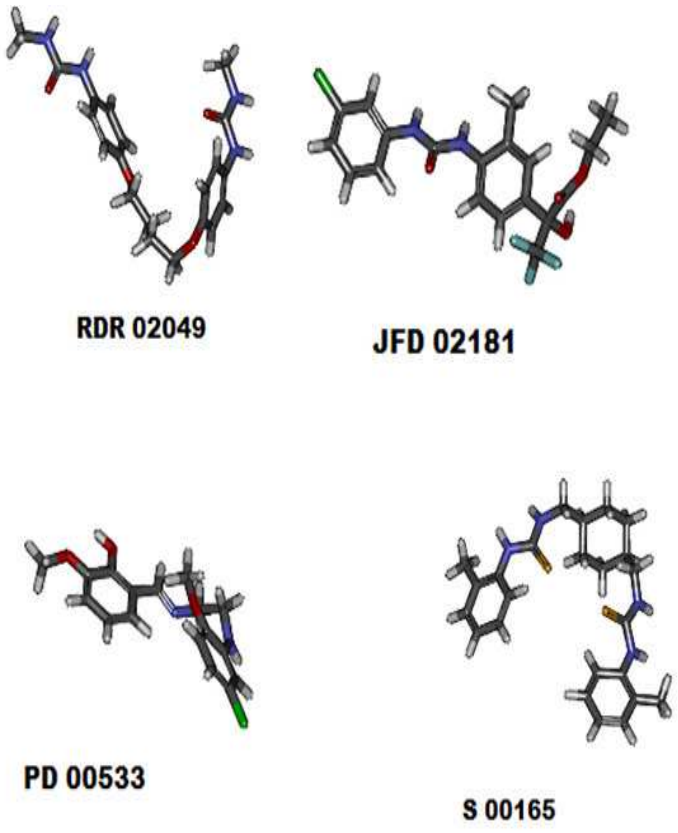

Figure 6
Pharmacophore based analog of Axillarin

(iii)Optimization of analogy using Pharmacokinetics and dynamics

Insilico ADME studies were expected to reduce the risk of late-stage attrition of drug development and to optimize screening and testing. Hence, among four generated compounds as like as axillarin chemical features, were initially screened based on pharmacokinetic property. Each analog follows their own pattern of levels in ADMET properties. The pharmacokinetics results for the obtained leads are tabulated with respective indices in Table 1.

Table 1
ADMET studies of lead molecules

Analog	BBB	Absorption	Solubility	Hepatotoxicity
RDR 02049	Low Penetrate	Low	Optimum	Toxic
JFD 02181	Undefined	Low	Good	Toxic
PD 00533	Low Penetrate	Low	Good	Toxic
S 00165	High Penetrate	Good	Good	Non-Toxic

The numerical generated values using ADMET protocol were compared with the predefined description and respective indices[17]. Finally the analog S00165 is hepatotoxic free compound and good absorption and good solubility with High BBB(Blood Brain Barrier) penetration was taken for further leads optimization technique. Thus the other three leads are omitted due to hepatotoxic, which affect the liver cells severely as per Insilco analysis. Pharmacodynamics is the next step used for optimizing the leads using various properties like based on carcinogenicity and mutagenicity using Topkat protocol which is available through Acclerys, which predicts computationally. The pharmacodynamic properties of the leads molecule based on QSAR studies, the following results clearly states the compound S00165 was free from carcinogenicity and mutagenic property are the most side effect which affects the human body system is shown in Figure 7

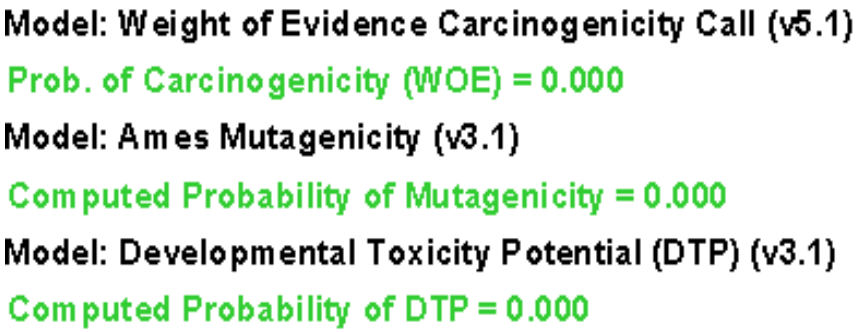

Figure 7
Pharmacodynamics property for S00165

Table 2
Limit value for pharmacodynamics

Property	Numerical indication	Result
Carcinogen and mutagen	0 to 0.29	Non carcinogen and Non Mutagen
	0.3 to 0.69	Intermediate carcinogen and mutagen
	0.7 to 1	Highly mutagen and carcinogen

The respective limit values as per generated using QSTR[18], with various number of test and training set as in Table 2. Hence, analog S 00165 found to be non-carcinogen and mutagen as per indication from the Table 2

(iv) Drug target protein

HIV-1 protease is an obligatory enzyme in the replication process of the HIV virus. The abundance of structural information on HIV-1PR has made the enzyme an attractive target for computer-aided drug design strategies. So far there is no report for the natural secondary metabolite analog based lead have been reported for the treating the HIV. The structure of the drug target protein Native HIV 1-Protease is shown below in the Figure 8.

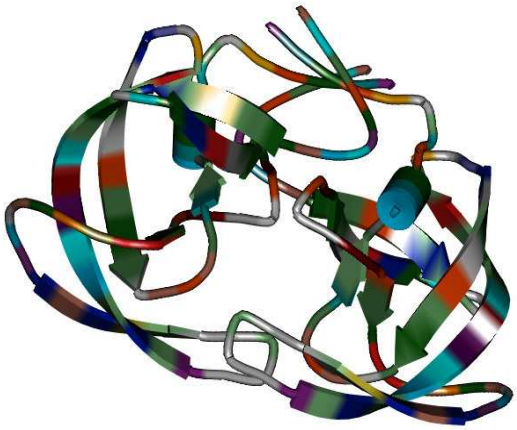

Figure 8
Schematic representation of drug target protein

(v) Docking and Interaction

Molecular docking using an appropriate docking program to check the binding affinity between the screened analog and HIV-1 protease is performed using Monte Carlo simulation. Conformations of each analogy were created with Monte Carlo simulation (15 000 trials) and flexible fit was selected. The RMSD threshold and score threshold were set to 1.5 and 20 kcal ⁄ mol, respectively, for avoiding identical conformation. Each of the saved conformations was evaluated and ranked using Internal energy with Dock score, analog S 00165 were docked in the active site of the drug target protein. Binding of ligand with protein is shown in the Figure 9**.** S00165 shows least binding energy and maximum dock score is shown in the Table 3 .Atomic interaction of active site amino acid of the HIV-1 protease with drug candidate analog is shown in the Table 4

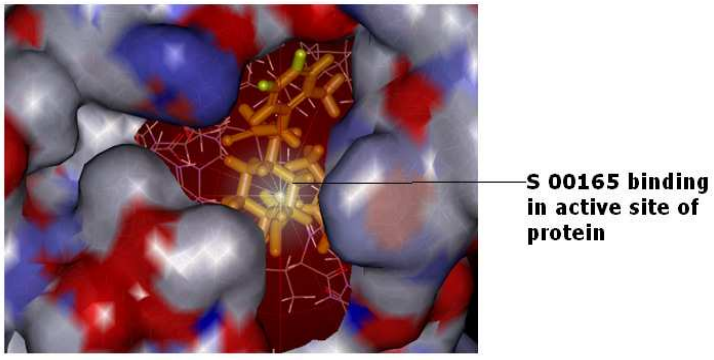

Figure 9
Binding of ligand with protein

Table 3
Dock score with binding energy

Analog	Internal Energy(Kcal/Mol)	Dock Score
S 00165	-3.89	**69.785**

Table 4
Amino acid interaction with active site of protein with its distance

Protein-PDB ID	Amino acid	Distance of drug and protein(Å)	Dock score
1ODW	ASP 29:A chain	2.171	**69.785**
	GLY 27:A chain	2.461	
	GLY 27:B chain	2.382	

The Pharmacophore analog S00165 is actively binds to active site amino acid GLY 27 in both A and B chains of the protein, so the any change or mutation in the GLY27 may affect the binding affinity of analog S 00165.The binding affinity and its interaction of analog with drug candidate is shown below in the Figure 10

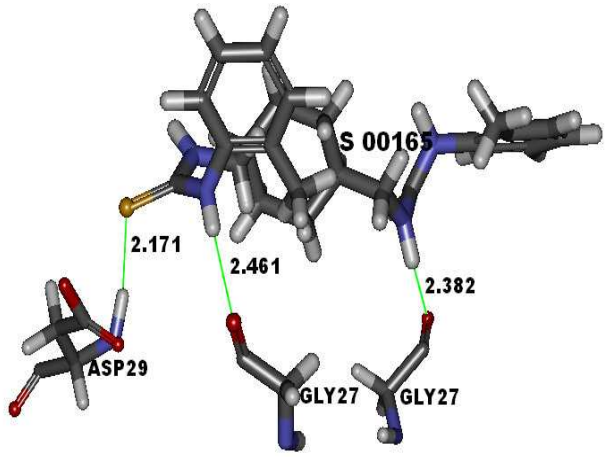

Figure 10
Atomic interaction of ligand with amino acid present in the active site of protein

CONCLUSION

Thus the present studies revealed that the analogy generated from axillarin also posses the biological property to inhibit the protein HIV-1 protease. The analogy S 00165 such a lead molecule obtained through Pharmacophore based drug designing with maximum fit value compared with axillarin. Since it is naturally based analogy screening with lead optimization it may have low side effects with drug like property which can be a better drug candidate for the treatment of HIV virus. Hence, this lead molecule can be further taken for invitro studies to proven this inhibition potency against HIV virus.

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