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Ligand based Virtual screening on natural compounds for discovering active ligands

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ABSTRACT

Ligand Based Virtual screening (LBVS) methods have emerged as an adaptive response to massive throughput synthesis and screening technologies. Based on the structure-permeability paradigm, the Lipinski rule of five has become a standard property filtering protocol for virtual screening. Seven compounds, which are already reported for their antidiabetic activity from different plants had considered for the present study, whose structure and Lipinski Rule of Five was calculated through Chemsketch and TSAR (Accelrys) respectively. The ADME/T was studied using Accord Excel, an Accelry's product. Among the seven, only saponin, terpenoid, lupeol and eremanthin show violation in Lipinski Rule of Five and ADME/T. It infers that the other three compounds are eligible as drug likeness. Further studies are required to modify the violated compounds to become a good drug.

Keywords: Ligand based Virtual screening, Lipinski Rule of Five, Chemsketch, TSAR, ADME/T .

INTRODUCTION

The quest for the new chemical entities and novel structural scaffolds with applications in the therapeutic areas is always at the heart of pharmaceutical chemistry. While arriving at these structures traditionally involve arduous, careful and systematic synthesis of several putative structures or screening of natural products. Most of these efforts may be categorized as the chance discovery rather than a rational approach. The enthusiasm to embrace rational approaches is triggered in recent years following tremendous advances in the computations and protein crystallography [1,2,3]. Thus *in-silico* approaches have gained immense popularity and have

become an integral part of the industrial and academic research, directing drug design and discovery [4,5,6,7,8]. Genomics, proteomics, bioinformatics and, chemoinformatics are among the few recent terms that refer to relatively new and rapid growing of drug discovery process [9]. The ideal goal of the drug discovery process is to identify potent, novel compounds with favorable drug-like characteristics specifically defined by pharmaceutic, pharmacokinetic, and drug safety profiles. The compound should possess some properties to be accepted as a drug [10]. For lead identification and optimization, wet lab and dry lab (*in-silico*) methods are effectively applied to speed-up the process of drug discovery [11]. The lead molecules modulate the function of the target proteins and later optimized to therapeutic drug against a specific disease[12]. Now a days, to check the binding affinity of the target receptor with the library compounds computational screening method like virtual High throughput Screening (HTS) is widely applied and used by many researchers to save wet lab economy and time [13]. In Ligand Based Virtual Screening process, the most effective biologically active lead molecule is detected using structural or topological similarity or pharmacophoric similarity search. In the screening techniques, a single molecule comparison takes a considerable amount of time. Hence the descriptor representation of the molecules is introduced and being used for searching; which has been proved to be more efficient aid in searching the large chemical databases. Descriptors can be generated by means of statistical correlation techniques like quantitative structure activity relationship (QSAR). In combination with Lipinski rule [14] the molecular descriptors provide a very useful approach for drug designing. Here, we attempt to virtually screen the bioactive (novel and known) compounds isolated from medicinal plants and established for their anti-diabetic potential in our laboratory, based on the ligand based virtual screening method.

The use of medicinal plants has been a central component of health care in many cultures for centuries, dating as far back as 5,000 years. The World Health Organization estimates that up to 80 percent of the world now relies on medicinal plants as their main source of health care. Currently, more than 120 pharmaceutical drugs on the market contain extracts from medicinal plants .They have been used for centuries as remedies for human diseases because they contain components of the therapeutic value. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer [15]. Natural therapies, such as the use of plant-derived products, may reduce adverse side effects. Herbal drugs are considered less toxic and free from side effects than synthetic drugs [16].The world Health organization has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe modern drugs. Studies have shown that phytochemical isolated from plant sources have been used for the prevention and treatment of cancer, heart disease, diabetes mellitus, and high blood pressure [17].

In this context, we have isolated Dihydroxy gymnemic triacetate [18],Gymnemic diacetate [19] and Gymnemic triacetate [20] from *Gymnemic sylvestre*, Saponin [21] form *Eugenea jambolana* and terpenoid [22] from *Elephantopus scaber* which are found to be novel compounds (Filed for patenting) and established for their anti- diabetic activities[23,24]. Further more we have isolated Eremanthin form *Costus specious* [25] and Lupeol from *Elephantopus scaber* which were also established for their anti-diabetic activities. We have adopted the bioassay guided fractionation for the isolation of these compounds and nuclear magnetic resonance (NMR), mass spectrometry (MS), ultra violet (UV) and infra red (IR) spectrometry studies for the structural elucidation.

In the present study these bioactive conformers are subjected for ligand based virtual screening including Lipinski's Rule of Five and ADME/T without any prior knowledge about the nature of interaction and targets binding sites for the ranking of the drug like efficacy of the compounds.

MATERIALS AND METHODS

In the present study, the structures were drawn using Chemsketch. ACD/ChemSketch software is an integrated software package from Advanced Chemistry Development Inc. for drawing chemical structures, 3D optimization algorithm allows the planar (2D) structure from ChemSketch to be rapidly translated into a realistic 3-dimensional structure. It is based on the modified molecular mechanics which take into account, bond stretching, angle bending, internal rotation and Van der Waals non-bonded interactions. The 3D optimization algorithm is a proprietary version of molecular mechanics with the force field initially based on CHARMM parameterization [26,27]. CHARMM is a program used for macromolecular energy, minimization, and dynamics calculations. By the help of chemsketch software the molecular properties of the compounds were generated. TSAR, an Accelrys software package mainly describes Quantitative Structure –activity relationship which was used for the calculation of Lipinski Rule of Five. Accord Excel another Accelry's Package was used for the prediction of ADME properties of the compounds.

RESULTS

The 3D structures of Gymnemic diacetate, Gymnemic triacetate and Dihydroxy Gymnemic Triacetate are presented in Fig-1. Fig-2 represents the 3D structures of Terpenoid and Lupeol, fig-3 describes the 3D structure of Eremanthin and fig-4 shows the 3D structure of Saponin. All the structures were drawn using Chemsketch, in which red color indicates Oxygen atom, blue color represents Carbon atom and white color denotes Hydrogen atom of the compounds.

Fig.1 Compounds Isolated from Gymnemic sylvestre

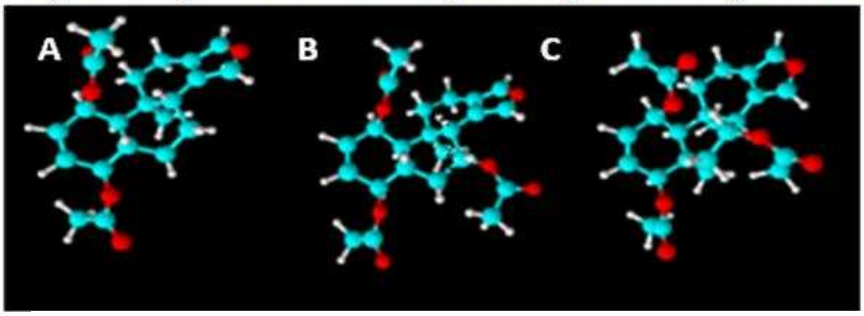
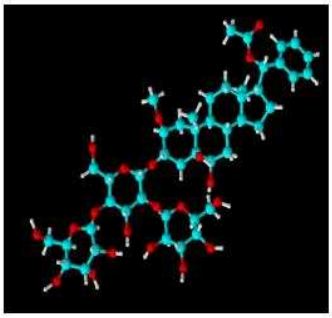


Fig.4. Compound Isolated from Eugenea Jambolana



Saponin

Table.1 "Lipinski Rule of Five" of the compounds and identified

S.I.	Compounds	HAcce	Hdon	LogP	Mol. Weight
1	**DihyrGymnemicTriacetate**	9	2	0.7009	462.54
2	**Gymnemic diacetate**	5	0	3.102	358.47
3	**Gymnemic triacetate**	7	0	2.7509	430.54
4	**Lupeol**	1	1	8.0281	426.801
5	**Eremanthin**	3	0	1.5586	218.27
6	**Saponin**	21	12	-0.187103	973.191
7	**Terpenoid**	2	0	6.3443	394.65

These molecules are imported in TSAR and calculated "Lipinski Rule of Five" and is presented in table-1. The ADME properties of the compounds predicted using Accord excel are given in Table-2.

Table.2 "ADME/T" of the compounds isolated and identified

S.I	Compounds	2DFPSA	Aq.Sol. Lev	BBB penetration	CYP2D	HepTox	HIA	Carrier Prot.BinLev
1	**DihyrGymnemic triacetate**	Good absorption	Good	**Undefined**	Non-inhibitor	Non-toxic	moderate	<90%
2	**Gymnemic diacetate**	Good absorption	Low	Medium	Non-inhibitor	Non-toxic	Good	<90%
3	**Gymnemic triacetate**	Good absorption	Low	low	Non-inhibitor	Non-toxic	0good	<90%
4	**Lupeol**	Good absorption	Extremely low	undefined	Non-inhibitor	Non-toxic	Very low	>=95%
5	**Eremanthin**	Good absorption	Good	**Medium**	Non-inhibitor	Toxic	Good	<90%
6	**Saponin**	Very low absorption	Low	undefined	Non-inhibitor	Toxic	Very low	<90%
7	**Terpenoid**	Good absorption	Very low	Very high	Non-inhibitor	Toxic	Moderate	>=95%

DISCUSSION

Virtual screening has become an integral part of contemporary drug research. A variety of computational tools are being developed and refined to effectively employ fast screening methods to yield potent hits. The last few years have witnessed an explosive growth in the successful applications employing a wide ranging methods, spanning similarity analysis, pharmacophore based search, graph theoretical approaches, machine learning tools, etc. Efforts are also being made to employ the drug likeliness of a given compound. There seem to be a lot of issues related to pharmaco-kinetic, pharmaco-dynamic and toxicity aspects which may have to be considered in the virtual screening approaches. The interplay between computational modelling and experimental research seem to have reached a decisive stage where the inputs from each of these disciplines are essential for their mutual growth. In view of this Doddareddy et al. 2006[28] carried out virtual screening by combination of ligand based 3D pharmacophore and biological assays which resulted in the identification of potent and selective T-type calcium channel blockers .

According to Johnson and Wolfgang, 2000[10], compounds should possess certain properties to be accepted as drug. Those properties were formulated by Christopher A Lipinski in 1997[29]. It is a rule of thumb to evaluate drug likeness, or to determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely active drug. The properties are: The molecule should not have not more than 5 hydrogen bond donors (OH and NH groups), Not more than 10 hydrogen bond acceptors (notably N and O) and molecule should have a molecular weight under 500 g/mol , a partition coefficient log *P* less than 5. Using a simplified, yet efficient version of the QSAR paradigm for structure-permeability [30] suggested that poor absorption or permeation is more likely to occur when the molecular weight (MW) is over 500,the calculated [31] octanol/water partition coefficient (CLOGP) is over 5, there are more than 5 H-bond (hydrogen bond) donors (HDO – expressed as the sum of O-H and N-H groups) and there are more than 10 H-bond acceptors (HAC – expressed as the sum of N and O atoms). Lipinski et al., 2001[28] suggested that any pair wise combination of the following conditions: MW > 500, LOGP > 5, HDO > 5, and HAC > 10, may result in compounds with poor permeability (exceptions are actively transported compounds and peptides).

In connection to this, it is very much clear from the table -1 that, of the seven compounds screened, Novel Saponin exhibits a highest degree of violating the Lipinski's Rule of Five with a very high molecular weight of 973.1 with 21 HAC and 12 HCO which are not even in the boundary level of the rule. It confirms that it has a very low absorption and permeability. At the same time Novel Terpenoid and lupeol shows the sign of second level of violating the rule with a high LogP value of 6.34 and 8.0 respectively. The novel compounds Gymnemic diacetate , Gymnemic triacetate and Dihydroxy gymnemic triacetate and Eremanthin comply perfectly with the Lipinski,s Rule of Five and are considered to have a good was absorption and permeability.

According to Egan and Lauri, 2002[32], ADMET predicts the Human Intestinal Absorption (HIA) after oral administration. Intestinal absorption is defined as a percentage absorbed rather than as a ratio of concentrations (cf. blood-brain penetration). A well-absorbed compound is one that is absorbed at least 90% into the bloodstream in humans. Chen and Merz, 2003[33], suggested that, ADMET describes the aqueous solubility (Aq.Sol.Lev)using linear regression to predict the solubility of each compounds in water at 25 °C. ADMET - Blood brain barrier is a model that predicts blood-brain penetration (blood-brain barrier, BBB) of the compounds after oral administration. ADMET- The cytochrome P450 2D6 model predicts CYP2D6 enzyme inhibition using 2D chemical structure as input [34]. Dixon and Villar, 1999 [35] developed the ADMET hepatotoxicity model which predicts potential organ toxicity for a wide range of structurally diverse compounds. Dixon and Merz, 2001[36] developed the ADMET-plasma protein binding model that predicts whether a compound is likely to be highly bound to carrier proteins in the blood.

In these contexts, it is more obvious from our results that, saponin is exhibiting a poor ADMET –HIA, absorption, Aq.Sol.Lev and blood-brain barrier penetration with a hepato-toxic effect. This is followed by eremanthin and terpenoid which also have a hepato-toxic effect. Though Lupeol is found to be non-toxic, it possesses a low Aq.Sol.Lev and HIA. At the same time the other 3 compounds namely Gymnemic diacetate, Gymnemic triacetate and Dihydroxy

gymnemic triacetate shows a good absorption, HIA, Aq.Sol.Lev blood-brain barrier penetration without hepato-toxicity.

Since saponin is strongly violating both the aspects of screening tools, it is not found to possesses drug like property. This is followed by terpenoid and Lupeol in which the same trend of saponin is followed with a medium violation. Since eremanthin is strongly violating the ADMET-hepato-toxic nature it also lost its drug likeliness. But the novel compounds Gymnemic diacetate, Gymnemic triacetate and Dihydroxy gymnemic triacetate shows a better drug likeliness without any violation.

CONCLUSION

The present study confirms that Gymnemic diacetate, Gymnemic triacetate and Dihydroxy gymnemic triacetate, the 3 novel compounds isolated from *Gymnema sylvestre* are eligible to be developed into potent oral drugs for diabetes and cancer without further modifications. Docking studies are required for the target identification for these ligands. In addition the study confirms that, all the other compounds require further modifications to improve their absorption, permeability and ADMET-properties to develop into potent oral drugs.

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