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Molecular docking studies using Sinigrin and Tamoxifen

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Abstract
Drug design is a process which involves the identification of a compound that displays a biological profile and ends when the biological profile and chemical synthesis of the new chemical entity are optimized. The present work deals with a comparative *in silico* docking analysis using sinigrin, an aliphatic glucosinolate and tamoxifen, the commonly used oral anticancer drug. Protein-ligand docking studies were performed to explore the anti-cancer property of sinigrin. The results revealed that Libdock scores were high for sinigrin when compared to tamoxifen. The protein, iNOS docked with sinigrin possessed a high Libdock score. Sinigrin and tamoxifen passed the Lipinski's rule of five which evaluates the drug-likeness of plant derived compounds. The suitability of sinigrin as a lead candidate for the drug industry was revealed by ADMET and TOPKAT studies.

Keywords: sinigrin, tamoxifen, libdock, ADMET, TOPKAT, lipinski

Introduction
Drug discovery and designing is an expensive process due to the high costs involved in Research & Development (R&D) and the final release of a useful drug. While drug development involves the identification of targets and suitable soluble candidates that block or activate the target; drug design involves the actual designing of small molecules, exhibiting pharmacological interactions with biological receptors [1]. Computer assisted drug design (CADD) involves all the computer-assisted techniques used to design, discover and optimize biologically active compounds [2].
Traditionally it is difficult to select the best chemical moiety of compound that plays an effective role in treating diseases, so computational strategies including molecular docking, ADMET and virtual toxicity studies are essential for identifying potential protein targets of various phytochemicals.
Obviously, *in silico* technique is inexpensive and shortens the time required for testing drug efficacy. The present study is concerned with sinigrin and its potential, relative to the synthetic drug, tamoxifen as an anticancer agent. Sinigrin is an aliphatic glucosinolate and is the precursor of the anticancer compound, allyl isothiocyanate [3]. Tamoxifen is a drug, taken orally as a tablet, which interferes with the activity of estrogen [4]. For the molecular docking studies, eight cancer protein receptors were selected and tested for their interactions with sinigrin as well as tamoxifen along with evaluation of Lipinski's rule of five, ADMET and TOPKAT properties.

Materials and Methods
Ligand preparation
The compound sinigrin (compound ID: 23682211) and the synthetic drug tamoxifen (compound ID: 2733526) were used in the present study. The structure of the compounds was retrieved from the PubChem database (http//pubchem.ncbi.nlm.nih.gov/) as .sdf files. The .sdf files were then converted to .pdb files using smiles online translator.

Protein preparation
The proteins with their PDB ID were retrieved from RCSB protein data bank (www.rcsb.org), crystallographic water molecules were removed from the proteins and the chemistry of the proteins was corrected for missing hydrogen. The eight proteins used in the study along with their PDB ID were 1. α-β tubulin (1JFF), 2. iNOS (1M9K), 3. PTP1B (1Q1M), 4. hppARγ (3VI8), 5. VEGF (1FLT), 6. VEGF2 (2X1X), 7. VEGFR2 (1Y6A) and 8. PIGF-1 (1FZV).
All the analyses were conducted using the facilities available in Accelrys Discovery Studio 4.0 (Ligandfit - docking, Lipinski's drug filter - Lipinski' rule of five, ADMET descriptors-ADMET properties, TOPKAT parameters – virtual toxicity)

Docking analysis

The interaction study was carried out in Ligandfit. The binding sites of the protein were predicted using 'find cavities' from the receptor site parameter of the tool. Then Libdock procedure was applied to position the conformation of the ligand correctly in the active site. The procedure was performed using libdock module. The binding results could be displayed by scoring ligand poses and several scoring functions used for measuring the goodness of a docking study to find a top ranked pose for ligands. In this study, absolute energy and Libdock scores were calculated. The number of hydrogen bonds involved in the interaction along with amino acids involved in the hydrogen bonding was also estimated.

Lipinski's rule of five (Drug likeliness evaluation)

The drug likeliness of sinigrin and tamoxifen was evaluated with the help of Lipinski's drug filter [5]. The rules are molecular weight < 500 daltons, number of hydrogen bond donors <5, number of hydrogen bond acceptors <10 and calculated partition coefficient between n-octanol and water (Log P) <5.

ADMET property studies

ADMET values were predicted for sinigrin and tamoxifen using ADMET descriptors. In this module, six mathematical models [Aqueous solubility, blood-brain barrier penetration (BBB), cytochrome P450 2D6 inhibition (CYP450 2D6), hepatotoxicity, human intestinal absorption and plasma protein binding (PPB)] were used to quantitatively predict properties of a set of rules that specify ADMET characteristics of the chemical structure of the molecules.

Virtual toxicity (TOPKAT) studies

To predict a variety of toxicities that are often used in drug development, various models are used and calculated through TOPKAT parameters. The toxicity profile of sinigrin and tamoxifen were predicted using TOPKAT which uses a range of Quantitative Structure Toxicity Relationship (QSTR) models for assessing special toxicological endpoints. Toxicity profiles include NTP (National Toxicology Programme) carcinogenicity for male and female rat, mutagenicity, developmental toxicity, Rat Oral LD_{50}, Rat inhalational LC_{50}, Rat chronic LOAEL (Lowest Observed Adverse Effect Level) and skin, occular irritation tests.

Results

Docking analysis

Molecular docking study was carried out to identify the putative binding sites of sinigrin and tamoxifen (ligands) onto the various cancer causing proteins. The absolute energy, libdock score between the proteins and ligands, and the number of hydrogen bonds was also calculated. The results have been tabulated (Table 1).

Table 1: Ligand-protein docking Libdock scores of sinigrin and tamoxifen

Proteins	Ligands	Absolute energy	Libdock score	No of hydrogen bonds
α-β tubulin	sinigrin	25.197	121.002	10
	tamoxifen	88.745	109.978	4
iNOS	sinigrin	33.054	128.974	1
	tamoxifen	100.408	122.337	4
PTP1B	sinigrin	32.407	120.002	6
	tamoxifen	90.918	95.871	4
hppARγ	sinigrin	29.876	115.093	4
	tamoxifen	99.512	97.855	6
VEGF	sinigrin	25.157	121.929	5
	tamoxifen	100.099	107.474	6
VEGF2	Sinigrin	32.074	109.339	6
	tamoxifen	83.152	99.681	4
VEGFR2	sinigrin	29.979	99.583	6
	tamoxifen	85.533	97.508	3
PIGF 1	sinigrin	35.105	81.151	5
	tamoxifen	-	No docking	-

The protein iNOS docked with sinigrin and possessed the highest Libdock score. However, PIGF1 docked with sinigrin showed the lowest dock score. Tamoxifen could not dock onto PIGF1. The Libdock scores of sinigrin were higher than tamoxifen in all the proteins studied.

The possible binding sites of the proteins were THR104, GLU47, ASN101, SER140, THR179, ASN206, TYR224, LYS254, ASN228, GLY186, TRP356, GLY355, PHE182, SER216, GLN266, ASP181, ALA217, TYR46, THR279, SER280, ASN219, LEU331, TYR334, CYS275, ALA333, ALA147, ASP276, THR150, GLY141, ASN274, PHE152, GLU64, ASP34, ARG224, ASP63, THR226, SER1035, THR862, PHE916 and CYS917.

Lipinski's rule of five

In the present study, sinigrin and tamoxifen passed the Lipinski's rule of five. The molecular weight of both compounds was less than 500 daltons, the LogP value was less than 5 and the number of hydrogen bond acceptors was less than 10, but the number of hydrogen bond donors was greater than 5 (Table 2).

Table 2: Lipinski's rule of five for sinigrin and tamoxifen

Ligands	Molecular weight	Log P	H-bond donors	H-bond acceptors
Sinigrin	<500	-1.873	>5	<10
Tamoxifen	<500	4.319	>5	<10

ADMET property studies

The ADMET studies provided insight into the pharmacokinetic property of the compounds with the help of six precalculated ADMET models. All the parameters calculated are tabulated (Table 3).

Table 3: ADMET property studies in sinigrin and tamoxifen

Ligand	Solubi-lity	BBB	Hepatotoxi-city	Absorp-tion	CYP450 2D6 binding	PPB	Log P
Sinigrin	4	4	False (0.00)	1	False (0)	1 (True)	-1.873
Tamoxifen	1	0	True (0.970)	1	False (0)	1 (True)	4.319

Sinigrin possessed better solubility compared to tamoxifen. The blood brain barrier (BBB) values showed that sinigrin may not be able to penetrate, whereas tamoxifen could penetrate. Tamoxifen showed hepatotoxicity, whereas sinigrin could be non-toxic. Both tamoxifen and sinigrin are shown to possess moderate intestinal absorption. The compounds are not likely to inhibit CYP450 enzyme during metabolism and have the capacity to cross the membrane and bind to plasma protein as noted by the PPB scores.

Virtual toxicity (TOPKAT) studies

TOPKAT in Accelrys predicts endpoints based on chemical structure. Models which satisfy all the validation criteria for the compounds were computed and results are recorded (Table 4). From the toxicity analysis, sinigrin and tamoxifen are proven to be non-toxic (non-carcinogenic) in the case of NTP and FDA carcinogenicity assays, non-mutagenic and are not likely to exhibit skin and ocular irritancy. In case of developmental toxicity, tamoxifen showed indeterminate toxicity, whereas sinigrin was non-toxic.

Table 4: TOPKAT values for sinigrin and tamoxifen

Toxicity models	Sinigrin	Tamoxifen
NTP Carcinogenicity Call (Female rat) (g/kg body weight)	0.00	0.00
NTP Carcinogenicity Call (male rat) (g/kg body weight)	0.00	0.00
FDA Carcinogenicity Male Rat Non vs Carc (g/kg body weight)	0.00	0.00
FDA Carcinogenicity Female Rat Non vs Carc (g/kg body weight)	0.00	0.00
FDA Carcinogenicity Male Rat Single vs Mult (g/kg body weight)	0.00	0.00
FDA Carcinogenicity Female Rat Single vs Mult (g/kg body weight)	0.00	0.00
Developmental toxicity potential	0.00	0.35
Ames Mutagenicity	0.00	0.00
Rat Oral LD50 (g/kg body weight)	2.933	2.856
Rat inhalational LC50($mg/m^3/h$)	5.966	3.028
Rat maximum tolerated dose-feed/water (g/kg body weight)	4.105	3.594
Rat chronic LOAEL (g/kg body weight)	0.012	0.009
Skin irritation	0.00	0.00
Ocular irritancy	0.00	0.00

Discussion

Most approaches for drug discovery start with the identification of a target, which plays an important role in the protein interaction network of a particular disease. Thus an ideal target is essential for designing inhibitory drugs and in the present study, eight cancer causing proteins were chosen as target molecules and their interaction with both sinigrin and tamoxifen was tested.

Molecular docking studies were performed to generate the bioactive binding poses of inhibitor molecules in the active sites of the chosen protein target molecules using Libdock program from Accelrys Discovery Studio 4.0. The protein-ligand complexes were analyzed to understand the interactions between protein residues and bound ligands. The analysis of the protein-ligand complexes revealed binding site residue, including amino acid residues, water molecules and metal atoms. The present study, revealed that sinigrin could bind at the active site of all the eight cancer related proteins. As a result of docking studies, different conformations were generated for sinigrin and tamoxifen. To correlate the biological activity of the proteins and the site directed docking of sinigrin and tamoxifen, Libdock scores were calculated. Higher Libdock scores indicate stronger receptor-ligand binding affinity. In the present study, sinigrin docked with all the eight proteins, but tamoxifen could not dock with PIGF1. Though, sinigrin docked with PIGF1, the score was very low (35.105). Highest Libdock score (128.974) was obtained for sinigrin docked with iNOS (Table 1).

Sinigrin has been used as a nutrition supplement and as a preventive against some types of cancers and other diseases [3]. Tamoxifen is taken as an oral tablet, but is known to interfere with the activity of estrogen. Some of the most common side effects of tamoxifen include blood clots, strokes, uterine cancer and cataracts [4]. Ironically, for more than 25 years, tamoxifen has been the gold standard for the endocrine treatment of all stages of estrogen-receptor-positive breast cancer, and the World Health Organization lists tamoxifen as an essential drug for the treatment of breast cancer.

According to [6], compounds should possess certain properties to be accepted as drug as formulated by [7]. Lipinski's 'rule of 5' as it is called, describes molecular properties important for a drug's pharmacokinetics in the human body and provides the information regarding the utilization of the ligand as a drug [5]. All the chemical structures are evaluated for good oral bioavailability in order to be an effective drug-like compound. Also, a drug-like molecule should have not more than one of the following violations such as no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, molecular weight no more than 500 daltons and Log P value not more than 5. Among the four parameters, if two rules are out of range, poor absorption or permeability may be possible.

In the present study, sinigrin and tamoxifen could qualify the Lipinski's rule of five, eventhough the number of hydrogen bond donors were greater than 5 (Table 2). Higher molecular weight (MWT) compounds are in general less likely to be orally active than lower MWT compounds. The molecular weight of sinigrin and tamoxifen was less than 500 daltons. An excessive number of hydrogen bond donor groups may impair permeability across a membrane bi-layer [8]. Hydrogen donor ability can be measured indirectly by the partition coefficient between strongly hydrogen bonding solvents like water or ethylene glycol and a non-hydrogen bond accepting solvent like a hydrocarbon or as the log of the ratio of octanol to hydrocarbon partitioning. Too many hydrogen bond acceptor groups also hinder permeability across a membrane bi-layer. The sum of Ns and Os is a rough measure of H-bond accepting ability. In the present study the number of H-bond donors was more than five and H-bond acceptors were less than ten in for both the compounds.

LogP value is a measure of lipophilicity and is the ratio of the solubility of the compound in octanol compared to its solubility in water. In the present study, the values of LogP were less than 5 for both compounds indicating a better oral bioavailability. These properties are then typically used to construct predictive ADMET models [9].

The prediction of ADMET properties plays an important role in the drug design process. Drugs for which the ADMET were not determined resulted in almost 60% failures of all drugs in the clinical phases. ADMET is applied at an early phase of drug development process in order to remove the molecules with poor ADMET properties and also leads to the significant savings in research and developmental costs, thereby avoiding

expensive reformulation before synthesis [10]. For this, aqueous solubility, BBB, cytochrome P450 2D6 binding, hepatotoxicity, intestinal absorption and PPB were calculated (Table 3).

In the present study, sinigrin possessed better solubility in comparison to tamoxifen. The aqueous solubility predictions showed that sinigrin was soluble in water. A poor aqueous solubility is likely to result in absorption problems, since the flux of drug across the intestinal membrane is proportional to its concentration gradient between the intestinal lumen and the blood. Aqueous solubility, in turn, is dependent on several factors such as size and shape of the molecule, hydrophobicity, hydrogen bonding, crystalline/amorphous state and others [11]. Blood brain barrier (BBB) permeability is a crucial factor which needs careful consideration in the ADMET profiling. Central Nervous System drugs must cross BBB to exhibit therapeutic effect whereas non-CNS drugs are expected not to cross the BBB to avoid unwanted side effects [12]. Blood Brain Barrier level of a compound varies from 0 to 4. In the present study, the BBB values showed that sinigrin may not be able to penetrate the blood brain barrier and as a result, the chances of CNS side-effects are lower or absent.

Hepatotoxicity plays a crucial role in drug discovery. If the hepatotoxic value is 0, then the compound is non-toxic, but if the value is 1, the compound is toxic [13]. Tamoxifen showed hepatotoxicity, whereas sinigrin was non-toxic. The drugs which are orally administered must be absorbed by the intestine. Intestinal absorption is defined in terms of percentage absorbed rather than as a ratio of concentrations. According to [14], ADMET predicts the Human Intestinal Absorption (HIA) after oral administration. A well-absorbed compound is one that is absorbed at least 90% into the bloodstream in humans. In the present study, both the compounds possessed only moderate intestinal absorption.

Majority of the drugs are either substrates or inhibitors of the CYP enzymes [15]. The most important implication of either inhibition/induction of CYP family proteins is clinically significant and at times, potentially fatal due to drug-drug interactions. The values predicted for non-inhibitors and inhibitors of CYP450 2D6 was '0' and '1' respectively [13]. The compounds in the present study were non-inhibitors of cytochrome P450 2D6, which indicates that both the compounds are likely to be metabolized efficiently in the Phase I metabolism with almost no side-effects.

Plasma protein binding level is a very important factor for finding the distribution rate of the compound and is significant with respect to the toxicity, pharmacology and pharmacokinetics of the drugs. The values assigned for PPB are 0 (≤90%), 1(≥90%) and 2 (≥95%) according to [16]. In the present study, PPB scores showed that the compounds have good binding capacity to cross the membrane and bind to plasma protein hence there is a high probability that these compounds can reach the desired targets. The excretion process that eliminates the compounds from the human body depends on LogP.

TOPKAT predictions help in optimizing therapeutic ratios of lead compounds for further development and assessing their potential safety concerns. They help in evaluating intermediates, metabolites and pollutants along with setting dose range for animal assays [17]. Toxicity studies include mutagenicity, NTP and FDA carcinogenicity and developmental toxicity assays. Mutagenicity predicts the ability of the drug to cause mutation to human cells. Carcinogenicity assay predicts the ability of the compound to cause cancer to normal human cells. Carcinogenicity test are carried for male and female rat models. Skin irritation tests provide information on the use of the compound for topical application. Computational probability is used to determine toxicity. If the value is between 0 and 0.29 the compound is non-toxic, if it is between 0.3 and 0.69 the result is indeterminate and if the score is between 0.7 and 1, the compound is toxic [9]. From the toxicity analysis, sinigrin and tamoxifen have proved to be non-carcinogenic in case of NTP and FDA carcinogenicity assays, non-mutagenic and have no skin and ocular irritancy. In case of developmental toxicity, tamoxifen showed indeterminate toxicity, whereas sinigrin was non-toxic (Table 4).

Conclusion

The study highlights the potential of sinigrin as an anticancer drug in comparison to tamoxifen. The results of *in silico* docking study clearly revealed that sinigrin has a greater anticancer potential than tamoxifen with good Libdock scores for the eight cancer proteins studied. The present work also establishes the suitability of sinigrin as a lead compound for the drug industry due to its desirable Lipinski properties, ADMET and toxicity screening, through which efficiency of drugs and side effects can be determined at early stages in drug discovery. Thus, the computer aided method plays a rapid and significant screening approach of drug discovery by selecting the lead molecules with good pharmacological property in order to bind effectively with target protein. This study also explores the molecular mechanism by which sinigrin can be further utilized with better activity by rational modifications. However both *in vitro* and *in vivo* studies should be taken up to better characterize sinigrin before taking up the clinical studies.

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