EMBELIN DERIVATIVE AS ANTICANCER AGENT FOR BREAST CANCER – IN SILICO SCREENING AND DOCKING STUDIES

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ABSTRACT:

Breast cancer is the second leading cause of cancer death in women, next to lung cancer. Two commonly used drugs for breast cancer treatment are Doxorubicin and Tamoxifen. But they are associated with several side effects. Embelin is a naturally occurring hydroxybenzoquinone whose anti-tumor and anti-cancer properties are well known. In this study, Embelin and its 4-aminoantipyrine derivative (E-AAP) are screened for anticancer activity using HBL-100 (ICLC NO: HTL 00004) breast myoepithelial tumor cell lines using Doxorubicin and Tamoxifen as standards. In silico ADMET studies carried out using Med Chem Designer software and docking studies using iGEMDOCK software revealedthat E-AAP may be a potent anticancer drug against breast cancer. Gln: 100, Lys: 164 and Ser: 166could be the most important residues for potential drug targets.

INTRODUCTION

Cancer is a big threat to our society in spite of good advancements for diagnosis and treatment. It accounts for about 23% deaths in USA and 7% deaths in India. It is believed that in near future the number of cancer patients will increase in the developing and under developed countries. Most frequently observed cancers in Indian population are of lungs, breast, colon, rectum, stomach and liver [1]. Breast cancer is a global disease. Practically, one fourth of all female cancer cases are breast cancers. Two commonly used drugs for the treatment of breast cancer are Doxorubicin and Tamoxifen but these drugs are found to be associated with several side effects. Serious side effects of Tamoxifen include a small increased risk of uterine cancer, stroke, vision problems, and pulmonary embolism. Common adverse effects of doxorubicin include hair loss, myelosuppression (a compromised ability of the body's bone marrow to produce new blood cells), nausea , vomiting, diarrhoea, skin reactions and localised swelling and redness along the vein in which the drug is delivered[2][3].

Embelia ribes Burm f. (Myrsinaceae) is an important traditional medicinal plant of Indian origin and its berries are used for numerous traditional medicinal remedies. Embelin (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone), isolated from the berries of Embelia ribes is a bioactive constituent having a wide spectrum of biological activitiesand has been reported to have tremendous antitumor and anticancer properties.

Today the process of drug discovery has been revolutionized with the help of efficient technologies like virtual screening, in silico ADMET screening and structure-based drug design. In silico methods helps to identify drug targets using bioinformatics tools and also analyze target structures for possible binding sites. ADMET studies can be carried out using several commercial as well as free softwares like MedChem Designer, CLOGP, DrugMatrix etc and docking studies can be performed using GOLD, GLIDE, AutoDock, iGEMDOCK etc.

In the present study, a comparative evaluation of Embelin and its 4-Aminoantipyrine derivative is carried out with the two standard drugs- Tamoxifen and Doxorubicin using molecular docking with the target

protein p53 having PDB:ID 2X0W. The p53 protein is very important in multicellular organisms for regulating the cell cycle and thus, functions as a tumor suppressor, preventing cancer[4][5] . Docking studies are done using the free software iGEMDOCK andIn silico ADMET screening are performed using MedChem designer to study the pharmacokinetic parameters. An attempt to identify possible lead molecules as anticancer drug using computational techniques is made here.

MATERIALS AND METHODS

Target Protein identification and preparation:
Three dimensional structure of the protein p53 was obtained from the RCBS Protein data bank (http://www.pdb.org/). The protein was pre- processed by deleting the ligand as well as the crystallographically observed water molecules [6].

Ligand preparation:
4-aminoantipyrine derivative of embelin (E-AAP) was synthesized in the laboratory and its structure was characterized by various spectroscopic techniques.

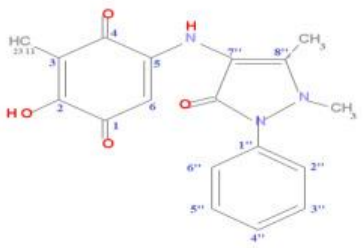


Fig 1

Docking module:
Docking was performed using the graphical environment iGEMDOCK.It carries out virtual screening and helps in recognizing pharmacological interactions that are useful for identifying lead molecules. A set of active compounds are acquired experimentally and ligand binding mechanism of a therapeutic target is inferred mostly from these set of active compounds.iGEMDOCK generates protein-compound interaction profiles and based on these, it infers the pharmacological interactions. Experiment results show that the success rate of iGEMDOCK is 78% on 305 protein-compound complexes(http://gemdock.life.nctu.edu.tw/).

Docking mechanism:
The target protein after preparation was subjected to energy minimization. All the potential active sites on p53 were located using Q-SiteFinder Server and docking was performed on all these sites with the inhibitors. The compound which gives the lowest binding energy is chosen as the best inhibitor [7].

Docking visualizer:
The docking images are visualized using Discovery Studio Visualizer 3.5which is free and very easy to use. It also provides good quality images.

ADMET properties prediction:
The adverse properties such as Absorption, Distribution, Metabolism, Excretion and Toxicity of the ligands are predicted usingin silico methods to know whether the ligands has the
potential of adverse effect in human. The free version of MedChem Designer used here predicts the followingproperties:
a) S+logP
b) S+logDbased on S+logP.
c) MlogP ie Moriguchi estimation of logP.
These are descriptors related to partition coefficient. Other informations like molecular weight(MWt), hydrogen bond donor(HBDH), hydrogen bond acceptors(M_NO), topological surface area (TPSA) and Lipinski rule are also obtained.

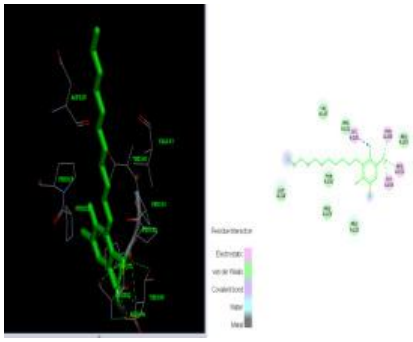
RESULTS AND DISCUSSION

Embelin, its derivative E-AAP, Tamoxifen and Doxorubicin are docked with p53 and the resulting docking scores are represented in **table 1.**

The docking poses of embelin and E-AAP are shown in **fig 1 and 2** respectively.

Table 1: Docking studies

Compound	Total energy (kcal/mol)	V	H	E
Embelin	-85.88	-67.26	-18.62	0
E-AAP	-90.05	-75.9	-14.15	0
Doxorubicin	-102.36	-91.68	-10.68	0
Tamoxifen	-79.66	-75.74	-3.91	0



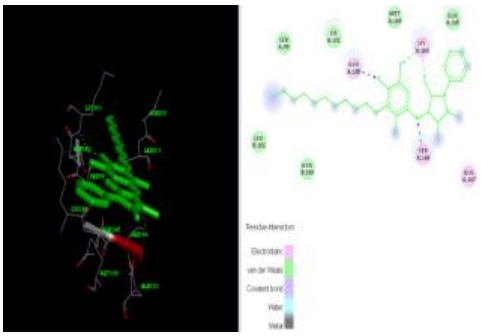


Fig 2

Table 2:ADMET studies

Structure name	Embelin	E-AAP	Doxorubicin	Tamoxifen	Limiting Value
S+log P	4.386	5.842	0.651	6.674	< 5.6
S+log D	2.517	4.194	0.524	5.635	< 4.1
MlogP	1.906	3.917	-0.816	5.201	< 4.15
MWt	294.394	479.623	543.531	371.526	< 500
HBDH	2.000	2.000	7.000	0.000	< 5
M_NO	4.000	7.000	12.000	2.000	< 10
T_PSA	74.600	93.330	206.070	12.470	< 140
Rule of5	0.000	0.000	3.000	1.000	0.000

How well a drug binds to the target macromolecule is determined by the binding energy of the protein-ligand interactions. Out of the selected ligands, E-AAP has a much lower binding energy(-90.05 Kcal/mol) than embelin (-85.88Kcal/mol) as well as the currently used anticancer drug Tamoxifen(-79.66Kcal/mol). Gln:100, Lys:164, Ser:166 are found to be the most important residues for drug target.ADMET profile is evaluated using the MedChem Designer database and results obtained are represented in **table 2**.

The logarithm of partition coefficient represented by log P indicates lipophilicity and upto a certain limit, compounds with higher lipophilicity has a higher permeation across biological membranes. Permissible lipophilicity may range from -0.4 to +5.6 though desired value may not exceed 5. Log P describes the partition of unionized form of the compound and log D describes the total partition of both ionized and unionized forms. Usually logD at pH 7.4 is considered as an index of the behavior of the compound in plasma (www.admescope.com). MlogP is Moriguchi estimation of logP. The number of hydrogen bond donor protons is given by HBDH and the total number of hydrogen bond acceptors is indicated byM_NO. More than 5 hydrogen bond donors and more than 10 hydrogen bond acceptors are not permitted for an orally active drug. T_PSA indicates the topological polar surface area in square angstroms.It should not be greater than 140 $Å^2$.Rule of five indicates Lipinski's Rule of Five [8]which is a score indicating the number of potential problems a structure might have with passive oral absorption. The presence of a Rule of Five Code means that the corresponding Lipinski rule is violated.

ADMET studies indicate that embelin and E-AAP shows no violation of Lipinski's rule. Both these compounds show better values for all parameters especially when compared to the standard drug Tamoxifen.

CONCLUSIONS

The naturally occurring hydroxybenzoquinone Embelin and its 4- aminoantipyrine derivative (E-AAP) were subjected to computational analysis to understand their mechanism of

interaction and binding affinity with the protein p53. Doxorubicin and Tamoxifen were taken as standards. The embelin derivative (E-AAP) showed much lower binding energy compared to the parent compound as well as the standard drug tamoxifen. The ADMET studies also points E-AAP as a potential lead molecule for the inhibition of breast myoepithelial tumor cell lines . Gln:100, Lys:164, Ser:166 could be the most important residues for drug target.This study may be subjected to experimental validation and clinical trials to establish E-AAP as a more potent drug for the treatment of breast cancer.

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