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Virtual screening of antitumour phytochemical against peroxisome proliferators activated receptor proteins PPARs

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Abstract: Cancers are caused by the defects in apoptosis process which leads to uncontrolled proliferation, therefore, most attractive drug target discovery strategy is to find ligands which have the ability to activate or regulate the apoptotic machinery. Peroxisome-proliferator-activated receptors (PPARs) are nuclear hormone receptors their over expression is observed in many tumours and contributes to chemotherapy resistance. The goal of this study to scrutinized antitumor phytochemicals from *Alysicarpus bupleurifolius*, *Piper nigrum* and *Plumeria obtuse* and potential energy values render from interactions between active site residues and ligands. The potential phytochemicals with significant binding affinity are ursolic acid, cis-4-decenoic acid and p-coumaric acid respectively most effective compounds in high throughput virtual screening belongs to *Plumeria obtuse* against PPARs associated with tumour development and progression. This modern drug designing modeling *in silico* approach, therefore, identifies the potential leads against over expressed tumours.

Keywords: *Alysicarpus bupleurifolius*, *Piper nigrum*, *Plumeria obtuse*, PPARs, antitumor, molecular modeling.

INTRODUCTION

Cancer is the most devastating disease of this century with the maximum rate of morbidity and mortality. According to an estimate by 2030, the number of cancer cases is expected to be 21 million (Bray *et al*., 2018). The uncontrolled proliferation of cells leads to cellular accumulation structural transformation and genetic instabilities ultimately result in the formation of malignant cells. Apoptosis resistance is a hallmark of human cancer and targeting key anti-apoptosis regulators with the goal of restoring apoptosis in cancer cells is a promising new therapeutic approach to cancer (Fathi *et al*., 2018). The Molecular docking algorithms are concerned with the evaluation, generation and interpretation of possible intermolecular structural complexes. The Crystal structure of Bcl-XL, PPARs gamma (peroxisome proliferators-activated receptor proteins) PDB ID; 2YXJ is responsible for the apoptosis defects which leads to uncontrolled proliferation and tumour generation (Javaid *et al*., 2018). Therefore, one the most important drug development strategy is to find ligands which can implicate and regulate the apoptotic machinery. Nevertheless, phytochemicals are remarkable in targeting tumour cells and remain ineffective to normal cells (Razaghi *et al*., 2018). The Medicinal Plant selected and there derived secondary metabolites, flavonoids, flavonols, saponins, terpenoids, alkaloids etc has a significant role either in inhibiting or suppressing onco-proteins available (Yu *et al*., 2018). Recently, *Plumeria obtuse* has been studied for significant potential drug compounds multiple pharmacological activities including activation of cell apoptosis through ROCK/PTEN mediated mitochondrial translocation in cancerous cells (Gai *et al*., 2016). The *Alysicarpus bupleurifolius*, plant extracts reported to possess anti-inflammatory, antibacterial, antiseptic and anticancer potential (Malar *et al*., 2019). Moreover, *Piper nigrum* has been testified globally for its medicinal effect and known to possess promising phytochemicals against various ailments including anticancer potential on human cancers cell lines (Manayi et al., 2019). The docking algorithms in this study were utilized to screen phytochemicals, evaluate druggability and design potential antitumour leads.

MATERIALS AND METHODS

Ligand retrieval and preparation

To design *in silico c*hemotherapeutic drug, medicinal plants were selected from different climatic zones of Pakistan based on undocumented indigenous knowledge. These databases provide concise, validated information and chemical structure in 2D, 3D and different formats like XML, SDF, MOL etc. All 3D structures were download in the SDF or MOL format and converted to the PDB format a by using the Online Molecular Format Converter Open Babel (http://openbabel.org/wiki) to search and convert data for molecular modeling. Phytochemicals were retrieved from Pub chem. data base a library of 200 phytochemicals were generated reference to the plant source. Optimization of ligand involves energy minimization.

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Protein retrieval and preparation

Similarly the 3D structures of BCL-XL protein (PDB ID; 2XYJ) with resolution of 2.3 A° was retrieved from Protein Data Bank (PDB) (http://www.rcsb.org). To visualize protein structure PyMOL user software which allows graphical representation of protein in cartoon ribbons format were generated alteration in protein residues structures and colours. The pre-bond ligand and water molecules were removed. Protein structure was refined with addition of H-atoms to the residues and minimization of energy. The protonated states of residues, hydrogens on hydroxyls and thiols optimize H-bond network.

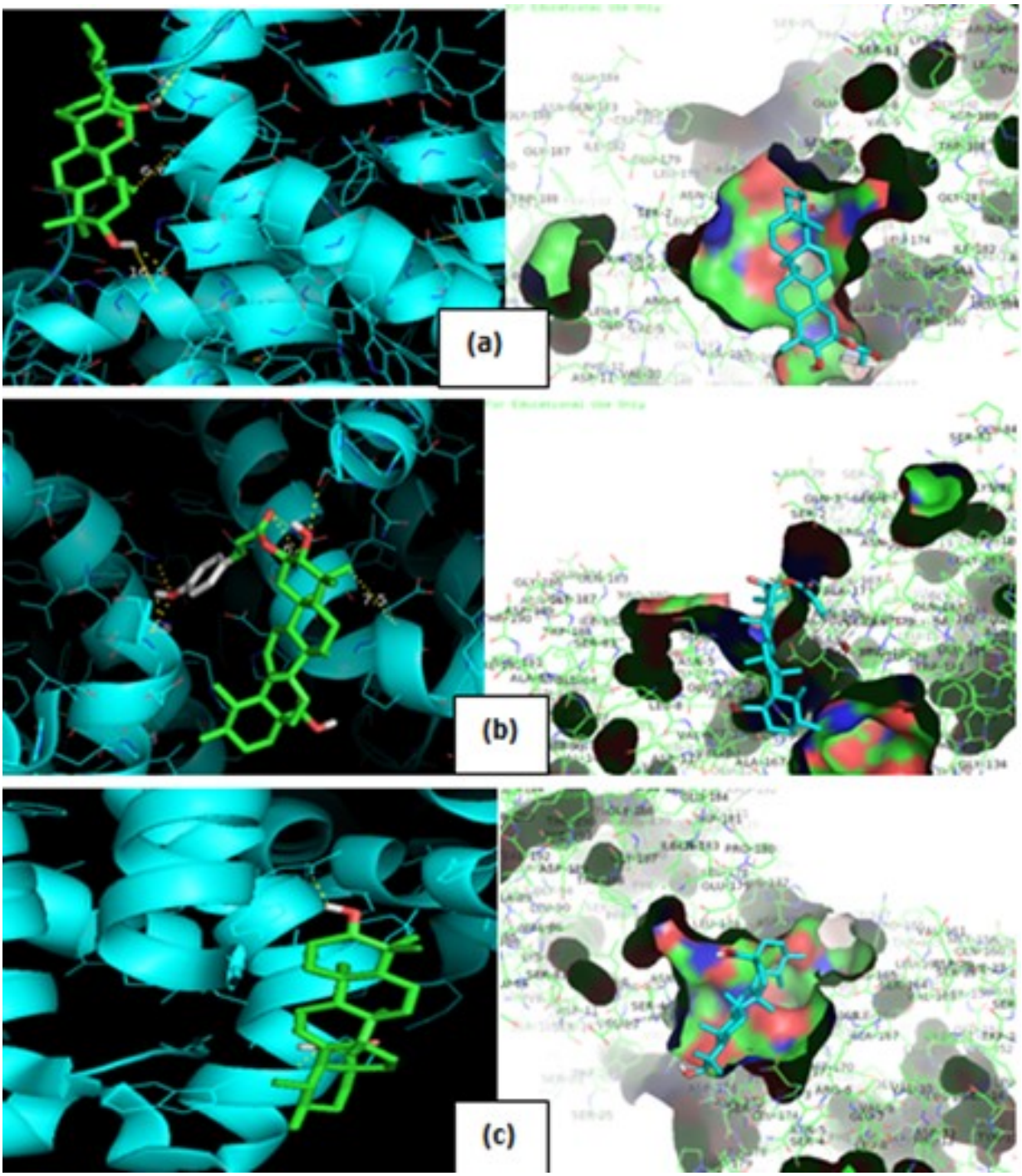
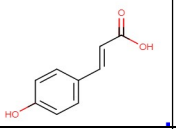
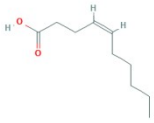
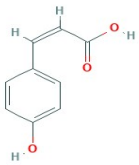


Fig. 1: 3D binding interactions and cavity pockets of (a) cis-4-decenoic acid (b) p-coumaric acid (c) ursolic acid docked with peroxisome proliferators activated receptor proteins (PPARs) images were generated with PyMOL 1.7.4.

Table 1: Molecular docking binding affinities and residual interactions of promising phytochemicals from *Alysicarpus bupleurifolius*, *Piper nigrum* and *Plumeria obtuse* against peroxisome proliferators activated receptor proteins (PPARs)

Pub chem ID	IUPAC names	Binding Affinity Kcal/mol	Residues Interact via H-bonding	Residues in contact to ligand
Alysicarpus bupleurifoliu				
4444098	2-(3-Methylanilino)-N'-[1-(4-Nitrothiophen-2)Ethenyl]Acetohydrazide	-7.0	PHE143, PHE1444, MET170	VAL86, LYS87, AGR91, GNN89
Piper nigrum				
4444129	2-[2-[(3-ethyl-6-fluoro-1,3-benzothiazol-2-ylidene)amino]-2-oxoethyl]sulfonyl-~(Ashfaq et al.)-(5-methyl-1,2-oxazol-3-yl)acetamide	-7.1	GLN182, ALA185	VAL161, SER164, ARG165, ALA 171
22536985	1-[6-(5-chloro-2,4-dimethoxyanilino)-5-nitropyrimidin-4-yl]-2,2-diphenylaceto-hydrazide	-7.0	HIS177, ILE6166, MET170	THR115, PRO116, THR119, GLU123
Plumeria obtuse				
8363	benzyl 2-hydroxybenzoate	-7.2	ILE113, GLY118	THR115, PRO116, THR119, GLU123, PHE122
637542	p-Coumaric acid	-9.2	PR0160, ASN168	PHE143, PEF143, GLY147, VAL152, GLN161
5312351	cis-4-Decenoic acid	-9.7	GLN121,ALA119	LEU108, GNK111, TYR1177, VAL126
64945	Ursolic acid	-10	ASP176, TYR173	ALA118, GLN121, SER122, VAL127, ASN128

Table 2: Predicted Lipinski's rule of five parameters for top hit scoring phytochemicals.

Pub Chem ID	2D Structural Formula	IUPAC Names	Mol. Weight	H-bond		CLogP	Molar Ref.
				Acceptor	Donor		
637542		p-coumaric acid.	298	2	3	6.420	91.56
5312351		cis-4-Decenoic acid	302	2	5	0.974	73.91
64945		Ursolic acid	456	2	3	7.0895	132.61

Molecular Docking

The Molecular docking was performed by Autodock Vina 4.2 a virtual molecular docking program, Phytochemical ligands were maintained in library the active site and binding pocket of protein was successfully retrieved from PDB Sum For implementation of docking algorithm, 3D conformations of ligand-receptor produce automatically nine best docking possess generated in solution file. The binding affinities were evaluated by conformations for each hit, scores; interface area of the complex; minimum binding energies and 3D transformation were also assessed.

Drug-likeness Prediction

Top scoring hits produced by screened phytochemical ligands were further subjected to analyze the five classical

drug-likeness properties which includes molecular mass, high lipophilicity CLogP, H-bond donors, H-bond acceptors and molecular using virtual tools Lipinski filter (http://www.scfbio-iitd.res.in/software/utility/Lipinski Filters.jsp) provides information about the effectiveness of the possible drug as shown in table 2.

RESULTS

Docking study was conducted using selecting a pocket all the conformations of docked ligands were analyzed in output files using PyMOL. The ligands with strong binding potential to the active site of PPARs gamma all possible conformations were developed as output files and analyzed by PyMOL 1.7.4. as illustrated in fig. 1.

Molecular docking efficiency of phytochemicals with PPARs gamma protein was further validated by polar bonds, binding affinities and residual interactions. The scoring function provides information about *in silico* efficiency of potential drug thus binding affinity score -7 kcal/mol was considered to be significant, the score value come out in the order of Ursolic acid > cis-4-Decenoic acid > p-Coumaric acid as shown in table 1.

DISCUSSION

Chemotherapeutic drugs are developed for inhibition of rapid growth of abnormal cells by apoptosis, also known as programmed cell death. In this study, structure-based drug designing was performed by peroxisome proliferators-activated receptor proteins PPARs gamma selected for molecular docking analysis was found to play an important role in uncontrolled proliferation of abnormal tumour cells (Gou *et al*., 2017). Over the years molecular docking has developed as a major field in drug designing and development (Ashfaq *et al*., 2016). The molecular docking interactions between ligand and active site of protein is conducting minimization of energy on a set of van der waals electrostatic grids to reduce the high energy produced as a result of interatomic interactions (García-Nieto *et al*., 2019). The docking simulations between peroxisome proliferators activated receptor proteins PPARs gamma and phytochemicals interpreted by measuring diameter to nearest protein residue and generation of binding pocket activities between ligands and protein. Top hits scoring phytochemicals with highest binding affinities, polar bonds and interactions with active residues of receptor protein were subjected to *in silico* evaluation for five classical drug-likeness and absorption, distribution, metabolism and elimination or excretion (ADME) properties (Zhao and Zhou, 2015, Grygorenko *et al*., 2019). These results of molecular docking inhibition are supported by *in vitro* evaluations on tumour cell lines and mouse model. Previously, p-coumaric and ursolic acid was found to be effective on HeLa, HCT116, and HT29 carcinoma cell lines in apoptosis induction assay, more interestingly the non-cancerous Vero cells remained in the same study (Gai *et al*., 2016, El-Naggar *et al*., 2018). Moreover, 10-hydroxy-2-decenoic acid (10-HAD) one of the major component of royal jelly, reported possessing anticancer potential in various malignancies (Miyata and Sakai, 2018). Our findings in this report show robust interaction of phytochemical leads with PPAR gamma proteins with indispensable drug-likeness properties. Therefore, this study will play a pivotal role, in the development of potential PPAR gamma inhibitory therapeutic drug against tumour in human after *in vitro* and *in vivo* study.

CONCLUSION

Current molecular docking study analysis reveals binding affinity, druggability and the order of highest scoring inhibitor compounds is ursolic acid, cis-4-Decenoic acid and p-coumaric acid. Interestingly, all the three most effective compounds in high throughput virtual screening belong to the same plant *Plumeria obtuse*. These phytochemical inhibitors with specific binding potential to successfully block PPRAs gamma proteins and can provide a basis to develop a promising antitumour drug with modest structural modifications.

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