

Pharmacophore Modelling of Brassicaceae Members as Potent HIF (Hypoxia Inducible Factor) Inhibitors Involved in Cancer Angiogenesis

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ABSTRACT

Angiogenesis is considered as an essential pathological feature of cancer due to its interplay between cancer and other diseases. Natural products found to act as antiangiogenic agents that mediate the angiogenic switch between pro and anti angiogenic factors. Among the different targets, HIF is an important and critical factor that stands as a key mediator between angiogenesis, inflammation and cancer. In our study different phytochemicals of Brassicaceae were analysed for their drug like properties and mapped for pharmacophore development. The developed pharmacophore was virtually screened and further subjected to Lipinski and ADMET filters. The molecular interaction studies of the 10 retrieved compounds were studied by binding with HIF. Among the compounds 1st drug like molecule HTS 0115 (C₁₅H₂₁BrN₂O₃) was found to have best docked score and its interaction was further validated using dynamics simulation. The compound found to share the pharmacophoric features with progoinin a biochemical form of glucosinolate with reported anticancer and anti thyroid activities. Thus the drug like compound HTS 0115 can be further optimised as a putative HIF inhibitor in tumor angiogenesis.

Key words: Pharmacophore, HIF, Angiogenesis, Brassicaceae, Molecular docking, Simulation.

INTRODUCTION

Angiogenesis is a physiological process involved in blood vessel formation related to several developmental process in embryo, placenta and wound healing. It is controlled by a tenuous balance between angiogenic and angiostatic factors.¹ In cancer, this imbalance is getting activated in the endothelial cell leading to metastatic proliferation. When a small dormant tumor initiates angiogenesis, referred to as the 'angiogenic switch', it secretes factors that induce sprouting and chemotaxis of endothelial cells (ECs) towards the tumor mass.² Within this hypoxic environment the transcription factor Hypoxia-Inducible-Factor-1 (HIF-1) is stabilized that activates the expression of multiple genes contributing to the angiogenic process. HIF-1 induced proteins include Vascular Endothelial Growth 2 Factor (VEGF) and Basic Fibroblast Growth Factor (bFGF), which promote vascular permeability and growth, respectively. Thus HIF expression is considered to be the key signalling factor in cancer angiogenesis. Other HIF-1 induced gene products include matrix metalloproteinases (MMPs) that breakdown the extracellular matrix to facilitate extracellular migration release growth factors such as integrins and tumor-associated macrophages (TAMs).³ Cruciferous vegetables were

extensively studied for their anticancer properties due to the presence of glucosinolates and sulforaphane.⁴ Glucosinolates are hydrolyzed to isothiocyanates (ITCs). (ITCs), a bioactive component in cruciferous vegetables that are being investigated for their chemopreventive and chemotherapeutic effects. These chemicals are responsible for the pungent aroma and bitter flavour of cruciferous vegetables. Indole-3-carbinol (an indole) and sulforaphane (an isothiocyanate) are the most frequently examined phytochemicals for their anticancer effects.⁶ The purpose of the study is to design a pharmacophore model based on the interaction of different phytoconstituents of Brassicaceae with HIF and optimising it as a lead against angiogenesis. The model was developed using common pharmacophore and ligand mapping followed by its validation using Molecular Dynamics (MD) simulation.

MATERIALS AND METHODS

The study is designed for pharmacophore candidate from set of potent ligands with reported activities. Pharmacophore features were devised from set of ligands using PHARMAGIST an online web server.

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It involves an indirect approach which requires a 3D training set that serves as a pivot point for alignment. The input set include the phytochemicals that are known to bind to HIF. The output consists of candidate pharmacophores that are generated by MFA (molecular field analysis). It employs the flexibility of input ligands in a deterministic manner within the alignment process.

Identification and screening of phytoconstituents and their studies

Anticancer and antiangiogenic compounds from different members of Brassicaceae were collected and reviewed. From the collection the compounds with reported anti angiogenic properties were separated and thoroughly studied using wide literature collection. From the list, constituents were screened for drug like properties using Lipinski rule of five. The resultant ligands were devised for their pharmacophore features using PHARMAGIST.

Pharmacophore model development and virtual screening

The compounds with potent antiangiogenic properties were used as base template for the generation of pharmacophore using COMMON PHARMACOPHORE GENERATION of Accelrys Discovery Studio 2.0. (ADS). Different hypotheses were developed with different parameters such as H-bond acceptor, donor and hydrophobic characteristics in common. The ligand pharmacophore and mapping protocol of ADS was used for mapping the best compounds with distinct pharmacophore features. Virtual screening of compounds was done with the developed pharmacophore in MINIMAY bridge database of ADS. The best pharmacophore models were chosen based on their structure likeness with the developed pharmacophore. All retrieved compounds were validated using drug likeness, ADMET descriptors and TOPKAT module of ADS.^{7,8}

Binding site analysis and Molecular docking

The binding site was identified from receptor cavities using flood-filling algorithms as implemented in ADS.⁹ A grid resolution of 0.5 which is an indication of grid spacing and minimum number of grid points used for this analysis.^{10,11} The compounds with good drug likeness and ADMET properties were chosen and docked to the binding site of HIF using AUTODOCK of ADS 2.0. The resulting docked complexes were selected based on dock score and PMF (potential of mean force).

Molecular dynamics simulation

Molecular dynamics simulation is a computational method that calculates the time-dependent behaviour of a molecular system. Molecular dynamics simulations provide detailed information on the fluctuations and conformational changes of proteins and nucleic acids. These methods are used

to investigate the structure, and thermodynamics of biological molecules and their complexes. The complex of drug like compound with target protein HIF was analyzed by producing simulation at 200 K for 1000 ns of the complex. The dynamics was then analyzed and the interacting residues were found.^{12,13}

RESULTS AND DISCUSSION

Target protein preparation

The 3 D structure of HIF was retrieved from PDB and stabilised for docking. The structure was minimised with conjugate gradient to resolve steric clashes and unwanted interactions.

Identification and screening of phytoconstituents and their studies

From the literature search different ligands from Brassicaceae were selected. 29 ligands were screened using Lipinski filter and the structures retrieved from PUBCHEM were used for the analysis. The list of ligands used for pharmacophore mapping were shown in Table 1.

Pharmacophore model generation

The compounds with good drug likeness were used as base structures for the generation of pharmacophore using PHARMAGIST. The pharmacophoric features were analysed for the 19 ligands using PHARMAGIST. Pharmacophore model generation was done using COMMON FEATURE PHARMACOPHORE TOOL IN ADS.¹⁴ Four ligands such as Kaempferol, Glucoraphanin, Flavanol, Progoitrin were found to have best pairwise alignment with flavanol as the pivotal molecule. Ligands such as Progoitrin and Kaempferol were found to be best mapped with generated pharmacophore. Drug likeness of the above two compounds were further screened for their analogues using database. Compounds with best fit values were subjected to toxicity prediction and pharmacokinetics using ADS as shown in Figure 1(a) and (b).

HIF was found to have 10 binding sites and the docking was done using ligand fit module by Monte Carlo methods.¹⁵ The final docked poses were evaluated based on dock scores and hydrogen bonding interaction with the binding site residues as shown in Table 2 and Figure 2.¹⁵

Molecular Dynamics simulation

Molecular dynamics (MD) is a method of studying the motions in the conformational space of molecular systems. MD simulation was performed over period of 1000 ns with the temperature at 200 K for all selected top ten drug molecules with HIF. The model produced after simulation shows that the HIS115, SER 11 and TYR 98 of HIF interact with HIS 56, ALA 3 and TYR 5 of the 1st drug like compound. The 10 mol of compound showed the interaction of ALA 3 and LEU 2. The binding complex of 1st mole with HIF complex showed stable conformation throughout the simulation process. The drug like compound HTS 0115 (C₁₅H₂₁BrN₂O₃) found to have the highest dock score with the receptor as shown in Figure 3. It has the same pharmacophoric features as PROGOITRIN.

Progoitrin is a biochemical form of the glucosinolate (2 hydroxy 3 butenyl) derivative predominantly found in cabbage, broccoli, brussel sprouts and other members of Brassicaceae.¹⁶ It is reported to possess anticancer and antiangiogenic properties. It is hydrolysed by myrosinase to form cyclic thiocarbamate goitrin which is found to reduce the production of thyroid hormone. There exists an inverse relationship between the thyroid vasculature and the local availability of iodine mediated by trace element dependant regulation of VEGF (vascular endothelial growth factor). HIF is responsible for VEGF activation by perceiving decreased iodide concentration as pseudo hypoxic stimulus.^{17,18} Therefore, we conclude



Figure 1: (a) Common pharmacophore 1 (b) Ligand mapping.

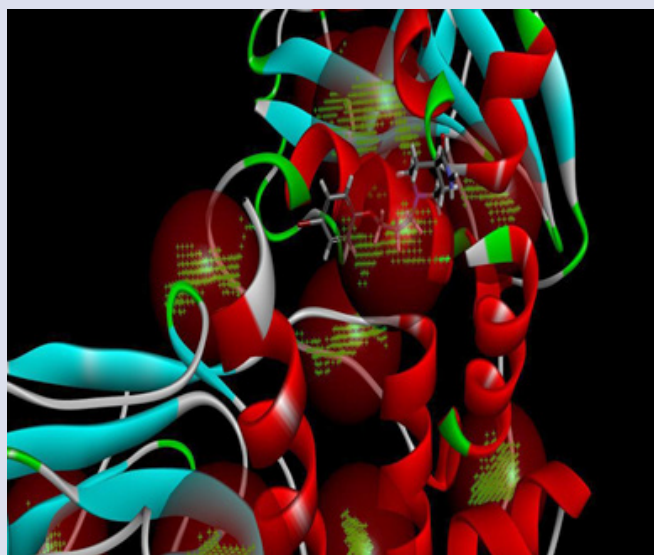


Figure 2: Molecular docking of HIF with developed pharmacophore (HTS 0115 molecule).

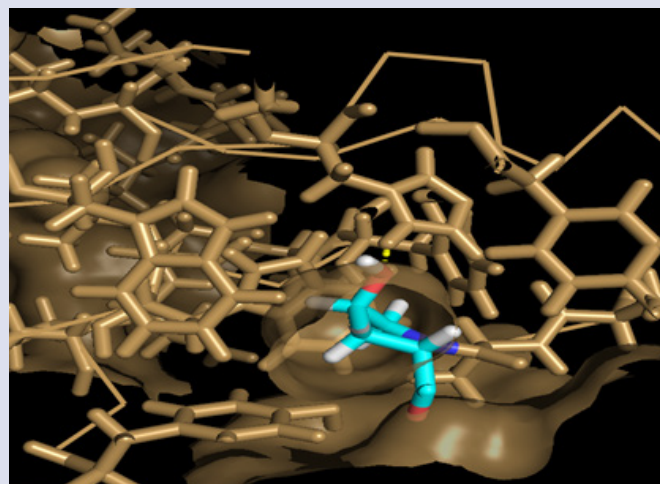
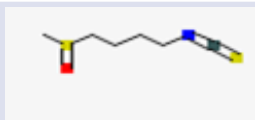


Figure 3: Molecular dynamics simulation of HIF with HTS 0115 molecule.

Table 1: List of phytochemicals from Brassicaceae

Allyl isothiocyanate		Apigenin	
Benzyl isothiocyanate		Butyl isothiocyanate	
Diallyl sulphide		Gallic acid	
Flavanol		Glucobriferin	
Hydroxy cinnamic acid		Indole 3 carbinol	
Kaempferol		Neochlorogenic acid	
Phenylethyl isothiocyanate		Propyl isothiocyanate	
Progoitrin		Quercetin	

Sulforaphane

**Table 2: Docked result of 10 molecules with HIF receptor**

Site number	Molecule	PMF	Dock score
1	HTS 01115	69.26	21.26
2	RDR 03478	-1.15	26.17
3	JFD 03560	23.9	34.47
4	BTB 02602	-38.65	21.64
5	KM 06492	20.34	32.68
6	S 01184	32.04	47.27
7	JFD 03350	0	0
8	JFD 03564	0	0
9	BTB 14837	0	0
10	RDR 01650	0	0

that molecule HTS 0115 (C₁₅H₂₁BrN₂O₃) has ANTI ANGIOGENIC property and further be optimised as an angiogenic inhibitor using *in vitro* and *in vivo* studies.

CONCLUSION

Natural product research recently focuses on optimising the phytoconstituents as druggable leads in different signalling mechanism associated with cancer. Targeting intervening mechanism such as angiogenesis associated with cancer may repurpose existing leads for new therapeutic indications. In our study we devised pharmacophore mapping from Brassicaceae and their molecular interaction to explore suitable HIF inhibitors. Among the drug like compounds reported from database, HTS 0115 (C₁₅H₂₁BrN₂O₃) with best dock score was validated using simulation studies. It shares the same pharmacophoric features of Progoitrin a biochemical form of glucosinolate. Hence it may be optimised in future as an effective natural lead in tumor angiogenesis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

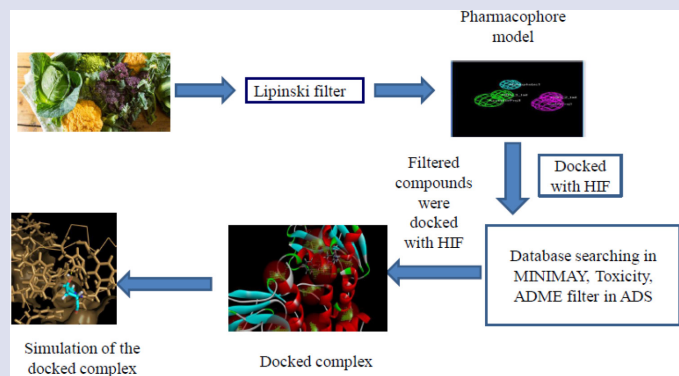
ADS: Accelrys discovery Studio; **ITC:** isothiocyanates; **PMF:** potential mean force; **HIF:** Hypoxia inducible factor; **ALA:** Alanine; **THR:** Threonine; **TRP:** Tryptophan.

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GRAPHICAL ABSTRACT



SUMMARY

- A pharmacophore model was built using phytochemicals of Brassicaceae family as an effective HIF (hypoxia inducible factor) inhibitor using pharmacophore based ligand mapping. The model was used as a key molecule and compared with compounds in database, similar compounds were filtered and docked with the receptor. Among the different compounds, the 1st molecule which shares the pharmacophoric features of Progoitrin was bound with the HIF receptor. The docked complex was found to be stable throughout the entire simulation and annealing process.