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# In silico Screening of Violacein as an epidermal growth factor receptor inhibitor

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ORIGINAL STUDY

# *In silico* Screening of Violacein as an Epidermal Growth Factor Receptor Inhibitor

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**Abstract**

**EGFR is a key player in the signalling cascades of various kinds of cancers and tyrosine kinase inhibitors block EGFR signalling. Natural products have long been used as candidates for therapy in the management of cancer. Violacein, a bacterial pigment, has been known for its numerous biological applications such as antimicrobial, antileishmanial, antiviral, as well as antitumoral. Computational studies have concluded that it may have activity against cancers like pancreatic cancer, thyroid cancer, colorectal cancer, and endometrial cancer indicating its potential application as a broad range of anti-cancerous drug. This study aimed to perform the molecular docking of violacein with the EGFR protein to ascertain its EGFR inhibitor property, which could lead to the development of a novel anti-cancer drug.**

*Keywords:* Violacein, *In silico*, EGFR kinase, Cancer

## 1. Introduction

Cancer has been a global public health concern and currently ranks second among the leading causes of death. The WHO estimates around 27 million cancer incidences by 2050, with an annual mortality rate of 17.5 million individuals [1]. Even though there are numerous treatment options for cancer, there are not any without side effects. Natural products have long been used as candidates for therapy in the management of cancer and other diseases such as diabetes, cardiovascular disease, and multiple sclerosis to name a few. Natural compounds are reported to possess unique advantages and characteristics over synthetic substances [2]. Natural pigments have proven multifaceted applications in medicines, foods, clothes, furniture, cosmetics, and other product [3].

Violacein, a bacterial pigment, is a bisindole purple-colored natural compound that is being studied for its several biological functions. Violacein [3-(1,2-dihydro- 5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1,3-dihydro-2H-indol-2-one], a purple-coloured bacterial compound, derived from proviolacein, has a molecular weight of 343.3 and the molecular formula C20H13N3O3 [4,5] (Fig. 1). Violacein is insoluble in water and decomposes at >290 °C (3). Its chemical structure was speculated as a result of a series of degradation experiments which was later ascertained by chemical synthesis [4,5]. The alkaloid is formed as a result of oxidative coupling of 1,3-dihydro-2H-indol-2-one and 1,3-dihydro-2H-pyrrol-2-one, both in the third position which is then replaced by a 5-hydroxy-1H-indol-3-yl group at the fifth position [7]. It is formed of 3 structural units including the 5-hydroxyindole, 2-pyrrolidone, and an oxindole, with the involvement of 2 indole rings hence violacein is known as a bisindole, which falls in the visible spectrum [6,8].

This secondary metabolite is linked to biofilm formation in the majority of violacein-producing bacterial strains and acts as a major defence mechanism for the strain producing it [9]. The purple

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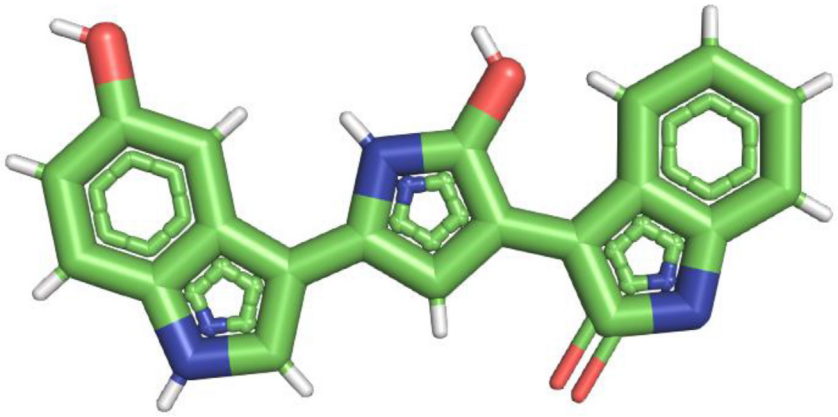

*Fig. 1. The structure of violacein (Pubchem CID 11053).*

pigment has been proven for its numerous biological applications such as antimicrobial, antileishmanial, antiviral, as well as antitumoral [10–13]. One of the non-therapeutic applications of the pigment includes dyeing natural and synthetic fabrics along with antimicrobial polyamide fabrics [14]. The tumoricidal effects of violacein have been investigated in recent years. The pigment's efficacy evaluated on *in vitro* cancer models *viz.*, colon cancer (HT29 and HCT116), head and neck cancer (HN5), and breast cancer (MCF-7) cell lines indicated enhanced anti-cancer activity under hypoxic conditions [12]. Tumor invasion and metastatic matrix metalloproteinase were inhibited by violacein treatment in MCF-7 cells [15]. The anti-tumoral effects were reported in Ehrlich Ascites Carcinoma (EAC) and MOLT-4 leukaemia cell lines where effective regression was achieved both *in vivo* and *in vitro* [16,17]. Although the pigment has been researched for its multi-sided biological properties, studies on anticancer mechanisms are still emerging. Tentatively, violacein has been reported to inhibit cancer progression via the PI3K/AKT/mTOR pathway acting on cell proliferation, cell cycle progression, and apoptosis [13].

The main tool for structural molecular biology and computer-aided drug design is molecular docking. The goal is to figure out what the ligand's main binding modes are to a three-dimensional protein structure. As a result, the intermolecular complex produced between the two molecules is discovered [18]. Molecular docking employs a scoring system that can predict binding strength, complex energy, and binding affinity between the protein and the ligand, all of which can lead to downstream activation or inhibition [19,20]. Few studies have tried to elucidate the antineoplastic potential of violacein via computational studies and have concluded that it may have activity against cancers like pancreatic cancer, thyroid cancer, colorectal cancer, and endometrial cancer indicating its potential application as a broad range of anti-cancerous drug [21]. Notwithstanding, there is a need to identify the mechanistic basis of the observed activity(ies) and validate the same *in vitro* and *in vivo.*

The Epidermal Growth Factor Receptors (EGFR) are a wide family of receptor tyrosine kinases (TKs) seen in oesophageal, lung, breast, and head and neck cancers. EGFR is a key player in a complicated signalling cascade that controls cancer cell proliferation, survival, adhesion, migration, differentiation, and signalling. EGFR has emerged as a promising option for anti-cancer therapy due to its pivotal role in cancer incidence and progression [22]. The abnormal activity of EGFR, in particular, has been demonstrated to have a noteworthy role in the genesis and development of tumour cells, where it is implicated in a variety of cellular responses such as proliferation and cell death [23]. Tyrosine kinase inhibitors are small molecules that are ATP analogues that block EGFR signalling by competing with and binding to ATP binding sites on the intracellular catalytic kinase domain of receptor tyrosine kinases, blocking autophosphorylation and activation of numerous downstream signalling cascades [24].

Cancers develop resistance to such EGFR inhibitors as a result of the existence or occurrence of point mutations in the receptor's tyrosine kinase domain. New anti-EGFR medicines with potent therapeutic properties are desperately needed. The overexpression and activation of EFGR in malignancies are linked to a poor patient prognosis. As a result, EGFR is an essential molecular target for clinical onco-therapeutics. In recent years, several EGFR inhibitors have been discovered and clinically approved. Natural products are a great source

for the identification and development of innovative cancer therapy alternatives as the majority of anticancer medicines are of natural origin [25,26]. Using omics-directed screening, the current study aims to determine the EGFR inhibitory efficacy of violacein for its antineoplastic application and possible mechanistic intricacies associated with the same.

## 2. Materials and methods

### *2.1. Library and macromolecule preparation*

The Protein Data Bank (PDB) is a database of protein structures in biology. The structure of EGFR was retrieved in PDB format from the PDB (3W2S). With the help of version 2.4 of the PYMOL tool (Schrodinger), water molecules were removed from the 3-dimensional structure and examined for any prior attachment to ligands before being saved in.pdb format for further validation. The protein was refined and optimised using the online Galaxyweb tool (http://galaxy.seoklab.org/), after which the active sites of the proteins were determined using the online CASTp server.

### *2.2. Ligand optimization*

The chemical structures of the phytochemical were obtained in.sdf format from PubChem. Violacein structure (PubChem CID 11053), carboplatin (PubChem CID 426756), and Gefitinib (PubChem CID 123631) structures were thus downloaded. Using the OpenBabel programme, the obtained .sdf files were transformed into PDB files by adding hydrogen atoms. Finally, the PDB data were geometrically enhanced using the ArgusLab software version 4.0.1.

### *2.3. Protein validation*

The protein structure validation and evaluation of its amino acid residues in the allowed and favoured regions were performed using PROCHECK to obtain the Ramachandran plots. The proteins were deemed fit for molecular docking as they had a higher number of residues in the favoured region.

### *2.4. Molecular docking and interaction*

To evaluate the inhibition potential of these ligands, molecular docking was carried out against prepared proteins using the AutoDock Vina in PyRx (https://pyrx.sourceforge.io/). The prepared protein and ligand files were uploaded in their .pbd format and converted to. pdbqt format and validated for the confirmation of hydrogen molecule addition. Once confirmed, the required changes were incorporated, followed by the recognition of active sites, assignment, and generation of a grid box to cover the binding sites. Once docking was performed, results were available in multiple poses and models with various binding affinities and RMSD values, out of which, the models with the highest binding affinities were selected and saved (.pdb format) for visualization of the interaction between the ligands and the proteins.

The best-docked poses from the results attained from the docking procedure were further visualized using the discover studio software which provides the near-able binding residues, which confirmed the best docking poses of the selected ligands and their binding affinities were noted.

### *2.5. ADMET evaluation*

The structures of Violacein compound were downloaded from Pubchem tool (https://pubchem.ncbi.nlm.nih.gov/) and were converted to their canonical simplified molecular-input line-entry system (SMILES). They were submitted to the SwissADME (http://www.swissadme.ch/) and PreADMET tool to determine the metabolism, excretion, distribution, toxicity, and absorption characteristics of a compound. The organ toxicities and toxicological endpoints of the isolated compounds were predicted using PreADMET (http://biosig.unimelb.edu.au/pkcsm/prediction).

## 3. Results

One of the factors linked to cancer development is abnormal protein tyrosine kinase activity. EGFR tyrosine kinases are involved in cell proliferation, differentiation, angiogenesis, apoptosis inhibition, and cell cycle progression, among other functions [27]. As a result, it's predictable that aberrant tyrosine kinase activity can contribute to the emergence and maintenance of many malignancies, predominantly breast adenocarcinoma. Inhibition of EGFR-TK has been shown to be successful in cancer treatment [28]. However, although there are multiple efficient anticancer medications and active inhibitors against a variety of protein targets, rising resistance and a slew of adverse effects need the development of new, better treatments [29,30].

### *3.1. Active site prediction*

The active site amino acids on 3W2S were identified using online Castp tool as shown in Fig. 2.

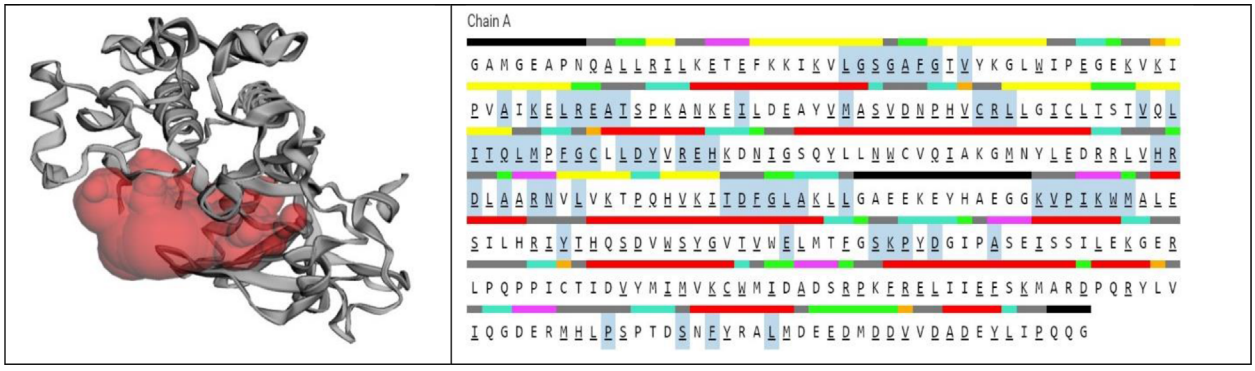


*Fig. 2. The sequence depicts residues forming the binding pocket along with the binding pocket of 3W2S.*

### 3.2. Protein structure validation

The protein was evaluated using Rampage. After initial validation and refinement of the protein, revealed more than 90% of residues in the allowed region, indicating that the protein is stable enough to proceed with further molecular docking experiments. Fig. 3 depicts the percentage of residues in the favoured (93.8%) and allowed (5.5%) regions.

### 3.3. Molecular docking interaction

The molecular interaction studies performed through molecular docking of violacein against EGFR proteins exhibited strong binding affinities and molecular interactions.

Highest binding affinity (−10.9 kcal/mol) was observed with EGFR tyrosine kinase, (3W2S), which displayed 3 hydrogen bonds with amino acid residues

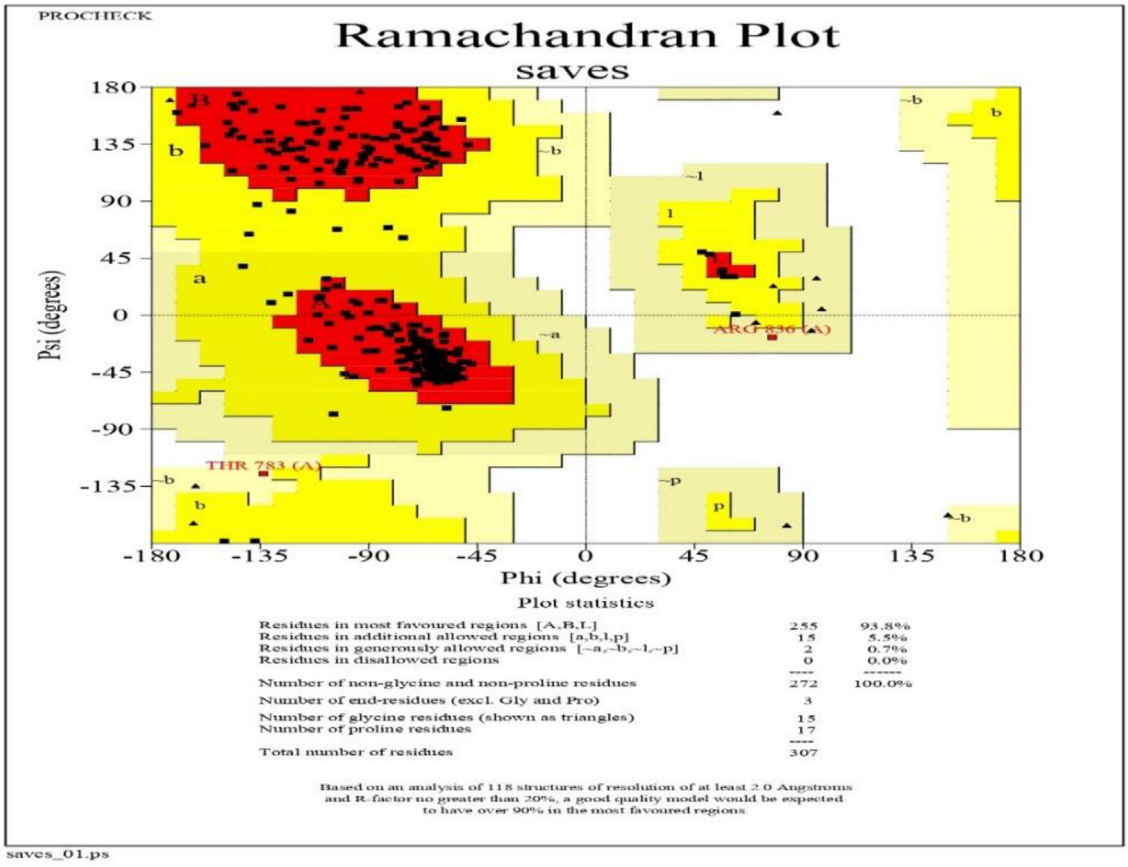


*Fig. 3. Ramachandran plot analysis of selected 3D protein model by PROCHECK serve.*

LEU788, MET793, and ASP855 as shown in Fig. 4 supporting pi-sigma and pi-alkyl bonds in the interaction when visualized.

In comparison to violacein interaction over the standard EGFR inhibitors, binding of standard drug Gefitinib, which is a known inhibitor of EGFR via action on the PI3K/Akt/mTOR pathway revealed binding affinity of −8.4 kcal/mol for Gefitinib (Fig. 5).

### 3.4. ADMET profiling

The Kp value in cm/s indicated the skin absorption and the Kp value (−6.45) depicted lower skin permeability of violacein compound. Furthermore, the studies predicted that the compound doesn't show Blood–brain barrier (BBB) permeation but they did show high gastrointestinal (GI) absorption. Out of the cytochromes, violacein inhibited only CYP1A2.

The findings of Pre-ADMET analysis are shown in Table 1 and the ADMET prediction in Fig. 6. The

*Table 1. Pre-ADMET analysis of violacein compound.*

| Model Name | Predicted Value |
|---|---|
| hERG I inhibitor | No |
| hERG II inhibitor | Yes |
| Oral Rat Acute Toxicity (LD50) | 2.413 mol/kg |
| Oral Rat Chronic Toxicity (LOAEL) | 1.258 log mg/kg_bw/day |
| *T.Pyriformis* toxicity | 0.285 ug/L |
| Minnow toxicity | 2.084 mM |
| AMES toxicity | No |
| Max. tolerated dose (human) | 0.728 log mg/kg/day |
| Hepatotoxicity | Yes |
| Skin Sensitisation | No |

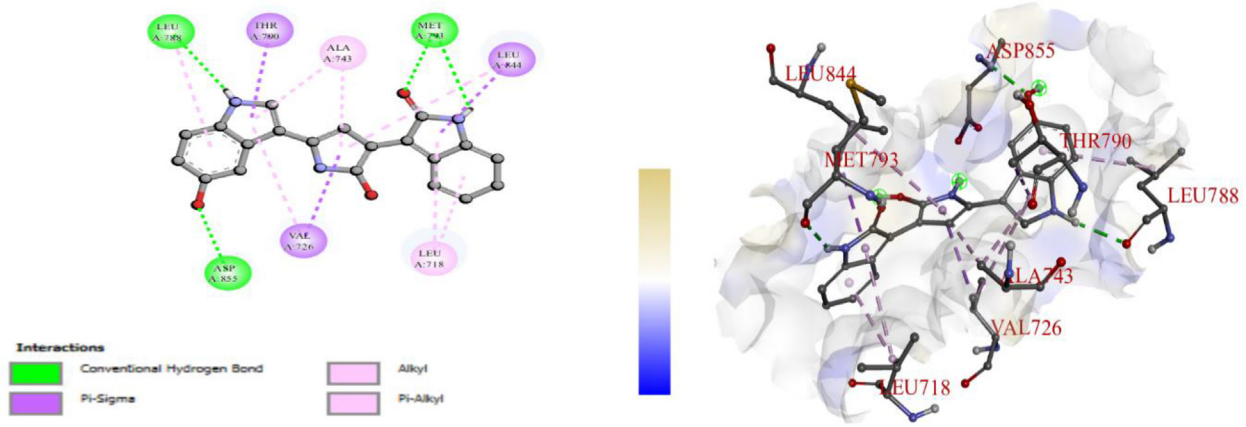


*Fig. 4. Interaction of violacein with 3W2S.*

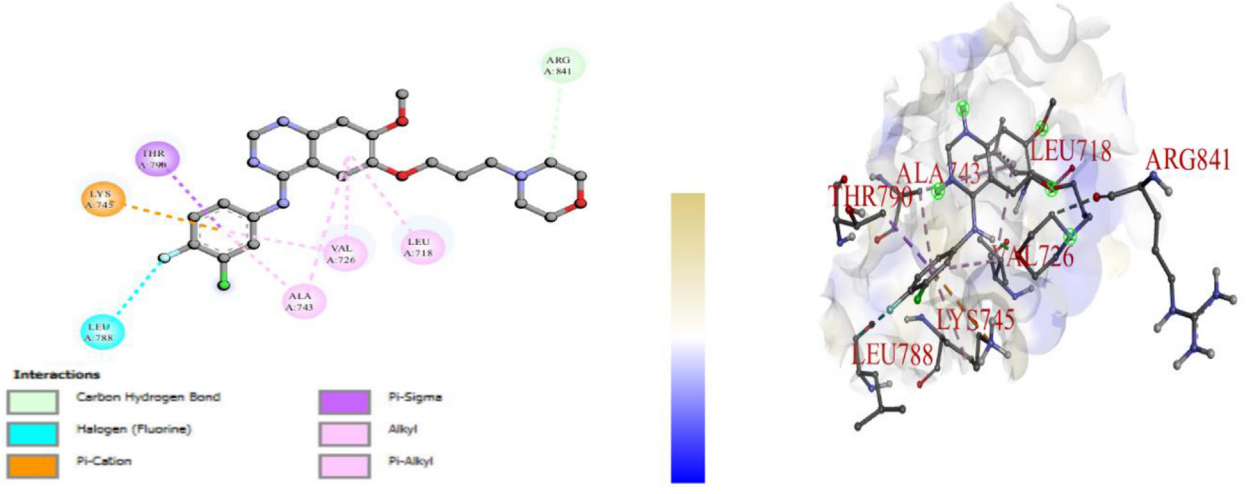


*Fig. 5. Interaction of Gefitinib with 3W2S.*

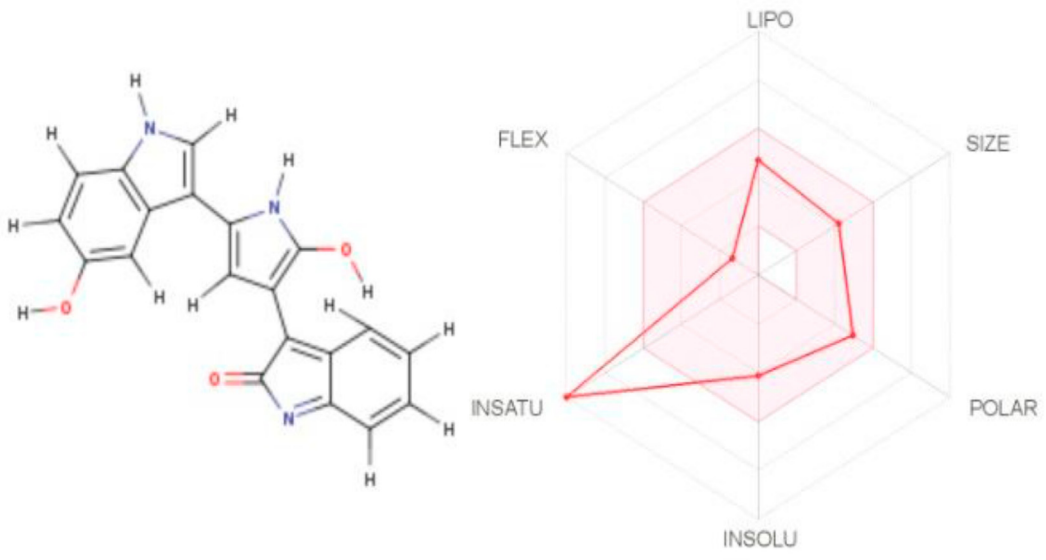


*Fig. 6. ADMET prediction of violacein compound.*

pink region determines the optimal range for each property (Size: MW between 343.34 g/mol; lipophilicity: XLOGP3 2.74; polarity: TPSA between 101.47 $Å^2$; solubility: log S not higher than 6). AMES toxicity, Skin Sensitisation, and hERG potassium channel inhibitor were the few parameters used to predict the toxicity of violacein. The results confirm that the violacein molecule is devoid of risk of toxicity.

## 4. Discussion

Docking studies are considered a helpful method in connecting structurally diverse items in an ordered manner. Molecular docking employs a scoring system that can predict binding strength, complex energy, and binding affinity between the protein and the ligand, all of which can lead to enzyme activation or inhibition. It is the most frequent method used in drug design because of its capacity to predict, the degree of precision, and conformation of small molecule ligands with the target binding site [19,20,31].

Several computational studies performing molecular docking of natural compounds against EGFR concluded natural compounds to have higher binding affinities against EGFR as compared to FDA-approved standard therapeutic drugs [32,33]. Violacein, a pigment obtained from *Chromobacterium violaceum* is gaining popularity due to its significant biological activity and pharmacological promise, and a comparison of the molecular docking interaction between violacein and the standard prescribed EGFR inhibitor, Gefitinib, revealed a higher binding affinity of violacein against the kinase with a higher number of conventional hydrogen bonds formed between the latter and the receptor protein [21]. This is in agreement with other computational studies of violacein that identified the compound as a potential cancer therapeutic agent [21].

Gefitinib is a first-generation EGFR inhibitor approved for clinical use by the Food and Drug Administration, made from anilinoquinazoline that was discovered in 1996 [34,35]. It is an orally bioavailable, EGFR inhibitor of slow molecular weight that inhibits only tyrosine kinase activity and not serine–threonine kinase activity [36]. It is thought to upregulate the CDK inhibitor p27 and downregulate the transcription factor c-fos, resulting in CDK activity inhibition and cell-cycle arrest in the G1 phase [37]. Results of this study indicate that violacein shows an effective binding towards EGFR. It could also be speculated that violacein demonstrated inhibitory action on EGFR via similar pathways, although more studies are required to validate this theory.

Any potential drug could become ruined due to its limited metabolism, excretion, distribution, toxicity, and absorption characteristics. Understanding pharmacokinetics, or the fate of a molecule inside the body, is critical throughout the development of innovative medicine. Theoretically, individual variables known as ADMET variables are often used to determine these factors, and computer models are typically employed in the place of experimental methods. Various Log P and S prediction tools, such

as ILOGP, XLOGP3, WLOGP, ESOL, and SILICOS-IT, were used to compile the results on lipophilicity and hydrophilicity of the specified ligand molecule in this study. The logarithm of the ratio of the concentration of drug ingredient between two solvents in an un-ionised form is used to determine the lipophilicity of a molecule.

For druggable chemicals, the Lipinski rule prescribes a maximum of 5. At the pre-clinical stage of drug development, Lipinski's rule of five states that any chemical that violates more than two criteria is impermeable or poorly absorbed [38]. The lower the log P values, the greater the lipophilicity of the chemical substance. It is important to evaluate the skin's permeability i.e., to determine the potential form for transdermal drug delivery. Using SwissADME, the druggable characteristics and drug-likeness of the selected antiproliferative agent, violacein, were predicted using a pharmacokinetic profile and was determined to be orally bioavailable, indicating that the dye could be a potential anticancer agent acting via the inhibition of EGFR kinase.

## 5. Conclusion

The present study demonstrates the *in silico* molecular docking of violacein against EGFR kinase. Violacein seems to be a promising anticancer therapeutic candidate with higher binding affinities towards EGFR kinase as compared to the Gefitinib positive control. Further *in vitro* and *in vivo* investigations into its antineoplastic properties in various types of cancers as well as the molecular pathways involved, could lead to the development of an affordable, novel EGFR-inhibiting, an orally-bioavailable anticancer agent.

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Nil.

## Conflict of interest

There are no conflicts of interest.

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