

Protein-Ligand Based Pharmacophore Approach against ERK5 Involved in Breast Cancer; In-Silico Study of Flavonoids from *Blighia sapida*

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Abstract

Conclusions: Flavonoids from *B. sapida* may serve as promising inhibitors of ERK5 for breast cancer management.

Background: Breast cancer is a global public health issue that can be caused by environmental or hereditary factors. There are still a shortage of effective treatments with enhanced efficacy and acceptability against the disease, as many breast cancer drugs have serious side effects. Hence, the inhibitory potential of flavonoids from *Blighia sapida* against breast cancer target (ERK5) was investigated.

The interactions of the target protein and its co-crystallized ligand were used to develop a protein-ligand based pharmacophore hypothesis. The idea was applied to the screening of phytochemicals obtained from an online database. Following that, we used structural bioinformatics and theoretical chemistry tools to find new ERK5 inhibitors using molecular docking, molecular mechanics generalized Born surface area (MM-GBSA) and pharmacokinetics model in Schrödinger suite, density functional theory analysis (DFT) was also performed using Spartan 10.

Results: The technique discovered new lead molecules as inhibitors of ERK5 as breast cancer therapy through molecular docking and MM/GBSA calculation with Quercetin, Kaempferol and (+)-Catechin showing higher docking score than the co-crystallized ligand and the standard drug. In the phase-generated E-pharmacophore theory, the postulated pharmacophore hypothesis has a hydrogen bond acceptor, hydrogen bond donor, and aromatic ring. Interestingly, all the hits obeyed Lipinski rule of five. The results of the frontier molecular orbitals revealed that the E_{HOMO} values of the hit compounds range from -6.02 to -5.48 eV indicating that all the hit compounds will readily donate electron.

Conclusions: Flavonoids from *B. sapida* may serve as promising inhibitors of ERK5 for breast cancer management.

Background

Cancer is a condition in which some cells in the body grow out of control and spread to other parts of the body (NCI, 2007). Breast cancer is the most prevalent cancer diagnosed in women in the United States. It is the second-largest cause of death in women, following lung cancer (America Cancer Society). This disease is most common in women and has a greater rate in women in industrialized countries, although it can also affect men (Mustafa et al., 2016). Extracellular signal-regulated kinase 5 (ERK5) is a member of the mitogen-activated protein kinase (MAPK) family, which includes highly conserved enzymes found in all eukaryotic cells and is involved in a variety of biological responses such as cell survival, proliferation, migration, and differentiation (Barbara and Rovida, 2019). As ERK5 is more abundantly expressed in breast cancer cells and tumor tissue compared to normal breast cells and tissue, accumulating lines of evidence suggest a critical involvement of ERK5 in the initiation and advancement of different types of cancer in recent years (Hoang et al., 2020).

ERK5 has a substantially bigger C-terminus than other MAP kinases, and it comprises both auto-inhibitory and nuclear shuttling activities (Drew et al., 2012). This structure lends itself to the development of specific therapeutic targets that are unlikely to interact with the functioning of other MAP kinases. Because of this specificity, tailored therapies may be able to block this pathway while reducing the inhibition of other MAP kinases that are important for healthy cell survival (Drew et al., 2012). Signaling substances such as growth factors, cytokines, neurotransmitters, hormones, or various cell stressors activate MAPKs by sending signals through tyrosine kinase, G-coupled protein, or hormone receptors (Flaherty et al., 2010).

Flavonoids have been demonstrated to have many anti-cancer properties, including modulating reactive oxygen species (ROS)-scavenging enzyme activities, participating in cell cycle arrest, inducing apoptosis and autophagy, and suppressing cancer cell proliferation and invasiveness. Flavonoids have a dual role in ROS homeostasis: they operate as antioxidants in healthy cells and are potent pro-oxidants in cancer cells, inducing apoptosis and down-regulating pro-inflammatory signalling pathways (Kopustinskiene et al., 2020).

Blighia sapida is a member of the Sapindaceae family and Sapindales order, commonly known as ackee or breadfruit (Olayinka et al., 2021). It is distributed across nations around the world, including Spain, Guatemala, Panama, Mexico, Venezuela, Colombia, Costa Rica, Portugal, France, Portugal, and Surinam (Olayinka et al., 2021; Aloko et al., 2019). Traditional medicine claims that the ackee fruit has medicinal characteristics that can be used to treat or relieve symptoms such as fever, constipation, skin infections, and diarrhea. It is also said to contain phytochemicals with anti-cancer and anti-diabetic properties (Xiao et al., 2016; Omoboyowa et al., 2021). In this study, the ligand-docking technique, pharmacophore hypothesis, and quantum calculations via Density Functional Theory(DFT) were used to predict ERK5 inhibitors from *B. sapida* Phyto-compounds for breast cancer management.

Methods

All computational experiments, such as E-pharmacophore hypothesis generation, virtual screening, molecular docking, MMGBSA, and visualization, were done with Maestro Schrodinger version 11.1 software. While DFT calculations were all done with the Spartan Software. All of the methods are detailed below and illustrated as well (Supplementary Fig. 1).

PROTEIN PREPARATION

PDB ID: 5O7I was used as the 3-dimensional crystal structure of ERK5. Bond orders were assigned to the target protein (ERK5) and hydrogen atoms were added. Water molecules within 5Å distance of the ligand were eliminated from the structure of the protein-ligand complex using Protein Preparation Wizard of Schrödinger suite 2020. Prime tool was also used to patch up missing loops and side chains (Jacobson et al., 2004). ERK5 was further improved by creating tautomeric states at a neutral pH and using the OPLS3 force field to constrain minimization. For molecular docking, the prepared ERK5 was chosen.

RECEPTOR GRID GENERATION

For protein-ligand docking, receptor grid generation determines the binding orientation and size of the active site. Using the receptor grid generation module of Schrödinger Maestro 11.1 (Omoboyowa et al., 2020), the scoring coordinates of the ERK5 binding pocket were computed based on the co-crystallized ligand. The coordinates for the x, y, and z grids are 43.47, -1.06, and 11.01, respectively.

LIGAND PREPARATION

The 2D structures of the bioactive compounds from *Blighia sapida* and the reference compound were downloaded from the NCBI PubChem database, and their bioactive compounds from *Blighia sapida* were gathered from published literature. OPLS3 forcefield was used to prepare the ligands using the LigPrep of the Schrodinger suite. The Epik module was used to produce the compounds' ionization states. Per ligand, just one stereoisomer was produced (LigPrep, 2020). Additionally, the ERK5 Co-ligand (4-(2-bromanyl-6-fluoranyl-phenyl)carbonyl-~{N}-pyridin-3-yl-1~{H}-pyrrole-2-carboxamide) was identified and retrieved from PubChem, after which it was prepared similarly to the other ligands and afterward utilized as a reference drug.

GENERATION OF E-PHARMACOPHORE MODEL

An energy-optimized pharmacophore hypothesis (E-pharmacophore) was generated using the crystal structure of ERK5 linked to the pyrrole inhibitor at a resolution of 2.1 Å. The E-pharmacophore model was created using the Phase module's Develop Pharmacophore from Protein-Ligand Complex option. The manual approach was used instead of selecting the AUTO (E-pharmacophore). The max (More) option of the 'SHOW FEATURES' option was selected. For the hypothesis settings, features that made interactions with the protein were chosen, and then a receptor-based excluded volume shell was created to mimic the receptor binding site (Fig. 1), ignoring receptor atoms whose surfaces are within 2.00 Å of the ligand surface, and limiting excluded volume shell thickness to 5.00 Å (Schrödinger TV, 2016).

E-PHARMACOPHORE BASED VIRTUAL SCREENING

E-pharmacophore-based virtual screening was performed with the previously prepared ligands done with LigPrep. A pharmacophore-based virtual screening was carried out using the phase module of the Schrodinger suite (Dixon et al., 2006; Phase, Schrödinger, 2020) to develop a subset of medicines with the requisite chemical characteristics for optimal binding to ERK5, as mapped by the E-pharmacophore model (Fig. 2). The best hits were chosen based on fitness scores.

STRUCTURE BASED VIRTUAL SCREENING

Virtual Screening with Glide via High Throughput Virtual Screening (HTVS) precision was performed using the output pharmacophore-matched hits. QikProp was used to calculate Lipinski rule violations (i.e. Rule of Five = 0) which were used to filter the input hits (QikProp, 2020; Omoboyowa et al., 2022). From the ADME-filtered results, five top-scoring compounds against the target protein were further screened with extra precision (XP) docking.

DOCKING PROCEDURE VALIDATION

A control study was used to validate the docking technique. The bound ligand in the crystal structure was re-docked to the pre-processed and prepared protein in the same grid box for this purpose (Fig. 3). The glide score for this docking was used as a benchmark against which the medication scores were measured. In the XP mode, the control docking was done.

ESTIMATION OF BINDING FREE ENERGY

The predicted binding free energies of the active ERK5 inhibitors were estimated using Maestro 11.1's prime module. The MM-GBSA technique for calculating binding energy uses the energy characteristics of the free ligand, free receptor, and receptor-ligand complex to compute binding affinity (Omoboyowa et al., 2022; Schrödinger Release, 2018; Genheden & Ryde, 2015). The MM-GBSA method was used to estimate binding energies for the 5 hit ligands chosen based on XP docking glide scores.

The rotamer search technique was used to calculate the relative free energy of the docked complexes utilizing the OPLS3 force field, VSBG solvent, and the rotamer search algorithm. The equation below was used to compute the binding free energy.

$$\Delta G^{bind} = G^{complex} - (G^{protein} + G^{Ligand})$$

1

DENSITY FUNCTIONAL THEORY ANALYSIS

Theoretical approaches for comparing the chemical and biological activity of substances have been increasingly popular in recent years. The physicochemical features of the top 5 hit bioactive compounds from *Blighia sapida* were investigated using quantum chemical calculations using DFT to anticipate molecules with noteworthy biological activity. To begin, each bioactive molecule was subjected to a conformer distribution search, with the most stable conformer being chosen for comprehensive analysis. DFT calculation using the B3LYP functional approach with 6-31G* basis sets in Spartan 10 computational software on an Intel (Core i3) computer with a 2.00GHz processor, 1TB hard drive, and 8.00GB RAM specifications (Becke, 1993; Jensen, 2001). Many parameters can be produced as a result of the calculations conducted using this method. Highest occupied molecular orbital energy (E_{HOMO}), lowest unoccupied molecular orbital energy (E_{LUMO}), energy band gaps (E_g), ionization energy (I), electron affinity (A), chemical hardness (η), chemical softness (δ), chemical potential (μ), electronegativity (χ), electronic energy, enthalpy, Gibb's free energy, and dipole moment are some of the parameters obtained from the calculations (D).

The difference between E_{LUMO} and E_{HOMO} was used to compute the energy bandgap (E_g).

$$E_g = E_{LUMO} - E_{HOMO} \quad (2)$$

Koopman's theorem (Koopmans, 1933) is used to connect electron affinity (A) and ionization potential (I) to E_{LUMO} and E_{HOMO} , as indicated in equations (3) and (4), respectively.

$$I = -E_{\text{HOMO}} \quad (3)$$

$$A = -E_{\text{LUMO}} \quad (4)$$

Parr and Pearson (Parr & Pearson, 1983) were used to calculate the electronegativity (χ) and chemical hardness (η) of the compounds.

$$\chi = \frac{I + A}{2}$$

5

$$\eta = \frac{I - A}{2}$$

6

Chemical softness (δ) is also the reciprocal of chemical hardness.

$$\delta = \frac{1}{\eta} \quad (7)$$

EVALUATION OF PHARMACOKINETICS PROPERTIES

Absorption, Distribution, metabolism, and excretion (ADME) properties of lead compounds were analyzed. The processes such as absorption, distribution, metabolism, and excretion are important in the development of drugs.

The Pharmacokinetics and drug-likeness study was conducted with the aid of the QikProp module prior used to filter compounds (QikProp, 2020).

Results

Table 1

Fitness Score and Interacting amino acids of lead compounds from *Blighia sapida* and control ligand.

Compound Name	PubChem ID	Fitness Score	H-Bond Residues	Interacting active site hydrophobic amino acids
Quercetin	5280343	1.865	MET 140, ASP 138	ILE 61, MET 140, LEU 139, LEU 137, ALA 82, LEU 189, ILE 115, TYR 66, VAL 69
Kaempferol	5280863	1.498	LYS 84, ASN 187, ASP 138	TYR 66, VAL 69, ALA 82, MET 140, LEU 139, LEU 137, ILE 115, LEU 189
(+)-catechin	9064	1.705	LYS 84, ASP 138, ASN 187	VAL 69, TYR 66, ALA 82, MET 140, LEU 139, LEU 137, ILE 115, LEU 189,
Actaealactone	11537736	1.809	ASP 138, GLU 102. OTHER INTERACTIONS: TYR 66(PI-PI STACKING), LYS 84(SALT BRIDGE)	MET 140, LEU 139, LEU 137, ILE 115, ALA 82, PHE 201, TYR 66, LEU 189, VAL 69
Co-crystallized ligand	118959080	2.428	MET 140, ASP 138. OTHER INTERACTIONS: TYR 66(PI-PI STACKING)	LEU 189, MET 140, LEU 139,
Alloathyriol	44575387	1.52	ASP 138, ASP 200. OTHER INTERACTIONS: LYS 84(SALT BRIDGE)	VAL 69, TYR 66, LEU 106, PHE 201, ALA 82, ILE 115, LEU 189, LEU 137, LEU 139, MET 140, ILE 61

Molecular Docking, Drug-like Properties, And Interaction Profiling Of Erk5-ligand Complexes

For molecular docking, computational approaches are frequently employed to predict the ligand-receptor complex structure; this is commonly accomplished by sampling ligand conformations in the protein's active site and ranking the conformations.

Table 2
Drug Likeness properties of lead compounds and control ligand

Entry Name	mol MW	Hbond Acceptors	Hbond Donors	ALogP	Polar Surface Area	Rule of Five
Quercetin	302.24	7	5	2.531	131.36	0
Kaempferol	286.24	6	4	2.7984	111.13	0
(+)-Catechin	290.272	6	5	1.9202	110.38	0
Actaealactone	358.304	7	4	0.9343	147.35	0
Co-crystallized Ligand	388.195	4	1	2.7951	71.95	0
Alloathyriol	274.229	5	2	1.7616	102.96	0

The interaction of the ligands inside the binding pocket of Extracellular signal-regulated kinase 5 (ERK5), MM-GBSA(Table 1), and their pharmacokinetic profile are all part of this computational analysis (Table 6). ERK5 is a protein kinase with a transcriptional transactivation domain and a nuclear localization signal(Cook et al., 2020). Because ERK5 inhibition has therapeutic potential in cancer and inflammation, ERK5 kinase inhibitors have been developed(Carmell et al., 2021).

From Quercetin to Alloathyriol, bioactive compounds from *Blighia sapida* demonstrated a favorable binding affinity and optimally saturated the active site of ERK5, with binding energies of -9.257kcal/mol and 7.847kcal/mol, respectively. A stronger binding is associated with lower binding energy. The lead compounds in Table 1 bind securely inside the active region of ERK5 while creating primary amino acid interactions with the hydrophobic amino acid residues MET 140, LEU 189, LEU 139, TYR 66, LEU 137, ALA 82, ILE 115, and VAL 69, according to the docking technique (Table 1; Fig. 5). These amino acid residues are critical for anticipating the ERK5 binding site and catalytic mechanism. Through H-bond formation with the hydroxyl group, the docked molecules interact with MET 140, ASP 138, ASP 200, GLU 102, ASN 187, and LYS 84 in the ERK5 binding pocket.

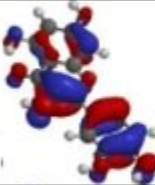
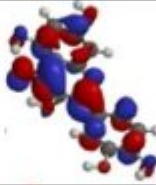
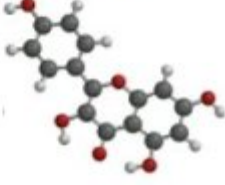
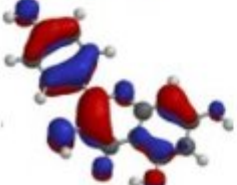
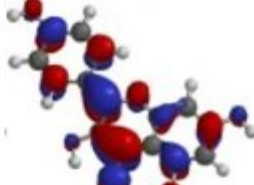
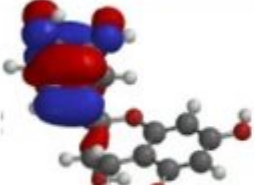
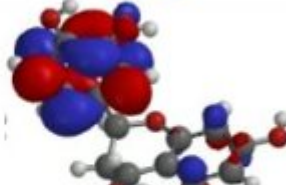
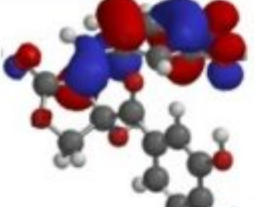
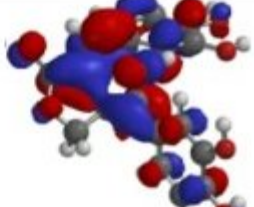
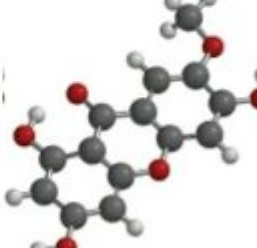
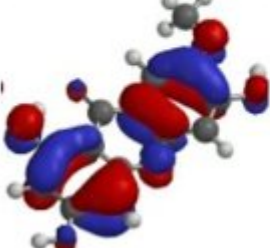
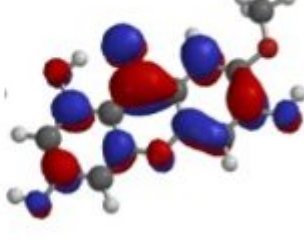
Inter and intramolecular interactions such as hydrogen bonding, pi-pi stacking, pi-cation, and salt bridge occur as a result of the ligand-ERK5 complexes. The best bioactive molecule of *Blighia sapida* was quercetin, which had maximum binding energy of 9.257kcal/mol. Hydrogen bonds are formed between it and the hydrophobic and negatively charged amino acids MET 140 and ASP 138. With binding energy of 8.788kcal/mol, kaempferol is thought to interact mostly with negative-charged, positive-charged, and polar amino acids: LYS 84, ASN 187, and ASP 138. (+)-Catechin is found to completely occupy the ERK5 binding site with a binding energy of 8.384kcal/mol while interacting with negative, positive, and polar amino acids: LYS 84, ASP 138, and ASN 187 via hydrogen bonds. While establishing a hydrogen bonding connection with negatively charged amino acid, ASP 138, actaealactone, and alloathyriol had binding energies of 7.974 and 7.847kcal/mol, respectively(Fig. 5). They both had additional interactions, including a salt bridge connection with LYS 84, a positively charged amino acid(Fig. 6). In addition,

Actaealactone and TYR 66 developed a PI-PI stacking interaction. The top four bioactive compounds of *Blighia sapida* have higher binding energy than the pyrrole inhibitor (4-(2-bromanyl-6-fluoranyl-phenyl)carbonyl-N-pyridin-3-yl-1H-pyrrole-2-carboxamide) that was utilized as a positive control for the ligands. The binding energy of the pyrrole inhibitor was 7.864kcal/mol, indicating that *Blighia sapida* bioactive molecules have a strong potential to bind ERK5 for the treatment of various cancers, particularly breast cancer.

The MM-GBSA module, which is integrated with the Schrodinger suite's prime program, was used to compute the ΔG_{bind} for ERK5-lead ligand complexes. Following the docking analysis, the ΔG_{bind} was used to calculate the binding energy for the screened compounds using advanced mechanics. MM-GBSA technique is a reliable post docking method for estimating the binding position of docked complexes, according to several researchers. Quercetin, Kaempferol, (+)-Catechin, Actaealactone, and Alloathyriol had binding energies of 36.10, 53.04, 44.67, 29.59, and 24.16 kcal/mol, respectively, according to the MM-GBSA output (Fig. 4).

DENSITY FUNCTIONAL THEORY ANALYSIS

Table 3: The optimized structure, HOMO and LUMO of *Blighia sapida* lead compounds.

Compounds	Optimized Structure	HOMO	LUMO
Quercetin			
Kaempferol			
(+)-catechin			
Actaealactone			
Alloathyriol			

Thermochemical analysis

Thermodynamic characteristics are important factors in determining the spontaneity and chemical stability of a chemical reaction. Gibbs free energy is a thermodynamic parameter used to characterize the interaction between ligands and receptors. It shows the likelihood of biomolecular processes taking place. A positive free energy value suggests that binding will not occur until additional external energy is added, whereas a negative free energy value indicates that binding will occur spontaneously. The degree of the negative free energy determines the extent of the ligand's interaction with the receptor. Enthalpy is also a measure of a thermodynamic system's total energy. When the ligand attaches to the receptor, the binding enthalpy indicates the energy change in the system. The computed Gibb's free energy for the investigated compounds is shown in Table 4. Because all of the molecules have a negative free energy,

they can attach to the target receptor without requiring any external energy. The top hit chemical, Quercetin, as well as Acetalactone, had the highest free energy (-1103.987 and 1295.839Hartree, respectively), implying that Acetalactone will interact more than the other compounds investigated. The dipole moment reveals the polarity of a compound as well as the distribution of electrons inside it(Fleming, 1977). It improves the receptor protein's binding affinity, non-bonded interactions, and hydrogen bond formation. Acetalactone(7.00debye) also has the largest dipole moment, as demonstrated in Table 4.

Table 4

Molecular weight, electronic energy, enthalpy, Gibb’s free energy and Dipole moment values of *Blighia sapida* Lead compounds obtained via DFT at the B3LYP/6-31G* level.

Compounds	Molecular Weight	Electronic Energy	Enthalpy	Gibbs Free Energy	Dipole Moment
Quercetin	302.238	-1104.18	-1103.929	-1103.987	0.22
Kaempferol	286.239	-1028.96	-1028.717	-1028.773	1.54
(+) – Catechin	290.271	-1031.33	-1031.042	-1031.101	3.56
Acetalactone	358.302	-1296.12	-1295.77	-1295.839	7.00
Alloathyriol	274.228	-990.86	-990.62	-990.679	2.14

Frontier molecular orbital (FMOs)

The most important orbitals in the molecule are the FMOs, HOMO, and LUMO. They are important in optical, electric, and UV-Vis spectral chemistry, as well as quantum chemistry. The FMOs describe how a molecule interacts with other molecules and provide information on electron transport in a molecule, as well as a molecule's chemical reactivity and stability. The HOMO energy defines the molecule's propensity to donate electrons; greater E_{HOMO} values imply a stronger inclination for the molecule to donate electrons(44). The E_{LUMO} determines a molecule's ability to receive an electron; a lower E_{LUMO} number improves the likelihood of taking electrons. Therefore, higher values of E_{HOMO} and lower values of E_{LUMO} are responsible for the low stability and high reactivity of a molecule. The E_{HOMO} values for the compounds tested rise in order as shown in Table 5: Quercetin > Kaempferol > (+) – Catechin > Alloathyriol > Acetalactone. The greatest value of E_{HOMO} is found in quercetin (5.48eV), indicating that it has a higher tendency to donate an electron to the target receptor than other substances. In addition, the calculated E_{LUMO} values are provided in Table 5. Quercetin has the lowest E_{LUMO} value, indicating that it has a lower ability to receive electrons than the other compounds investigated. Furthermore, as shown in Table 3, both the E_{HOMO} and E_{LUMO} are completely dispersed across the molecule structure, implying considerable HOMO-LUMO overlapping, resulting in robust charge transfer behavior. The band gap energy between the E_{HOMO} and E_{LUMO} is crucial for forecasting a molecule's chemical reactivity. The chemical reactivity and stability of a molecule are reflected in the band gap energy levels. The molecule becomes tougher, more stable, and less reactive as the band gap energy increases. High reactivity and low stability

are associated with a narrowing of the energy band gap. The energy band gap values are as follows: Quercetin < Kaempferol < Alloathyriol < Acetalactone < (+) – Catechin. Among the isolated chemicals, quercetin has the smallest band gap, indicating that it is more reactive toward the target receptor than the others.

Global reactivity descriptors

Table 5
Global reactivity descriptors of *Blighia sapida* lead compounds obtained via DFT.

Compounds	E _{HOMO} (eV)	E _{LUMO} (eV)	E _g (eV)	I(eV)	A(eV)	η (eV)	δ (eV ⁻¹)	χ (eV)	μ(eV ⁻¹)
Quercetin	-5.48	-1.84	3.64	5.48	1.84	1.82	0.549451	3.66	-3.66
Kaempferol	-5.53	-1.81	3.72	5.53	1.81	1.86	0.537634	3.67	-3.67
(+) -Catechin	-5.63	-0.08	5.71	5.63	-0.08	2.855	0.350263	2.775	-2.775
Acetalactone	-6.02	-1.78	4.24	6.02	1.78	2.12	0.471698	3.9	-3.9
Alloathyriol	-5.74	-1.55	4.19	5.74	1.55	2.095	0.477327	3.645	-3.645
(E _g), energy band gaps; (E _{HOMO}), highest occupied molecular orbital energy; (E _{LUMO}), lowest unoccupied molecular orbital energy; (I), ionization energy; (δ), chemical softness; A, electron affinity; η, chemical hardness; μ, chemical potential; χ, electronegativity.									

To gain a thorough understanding of the chemical stability and reactivity of bioactive chemicals toward the target receptor, global reactivity descriptors (GRD) were developed. Ionization energy, electron affinity, chemical hardness, chemical softness, chemical potential, and electronegativity are the GRDs that are calculated. The ionization energy (I) of a molecule describes its chemical reactivity and stability. It's the amount of energy it takes to remove an electron from a molecule. High ionization energy suggests chemical inertness and stability, whereas low ionization energy indicates high reactivity and chemical inertness(Chakraborty et al., 2020). The ionization energy of quercetin (5.48 eV) is the lowest, making it the most reactive chemical toward the target receptor, ERK5The energy released when an electron is added to a neutral molecule is known as electron affinity (A). A molecule with a high electron affinity is more likely than one with a low electron affinity to receive electrons easily(Geerlings & Proft, 2002). The most reactive chemicals include Kaempferol and Quercetin, which have the highest electron affinity.

Understanding the reactivity of a chemical system requires an understanding of chemical hardness and softness. Chemical hardness describes a molecule's resistance to electron cloud deformation (Mortier et al., 1985). The band gap energy of a hard molecule is enormous, whereas the band gap energy of a soft molecule is tiny. The soft molecule will polarize more quickly and easily than the hard molecule (Obot et al., 2015). Catechin has the greatest hardness value (2.86eV) in Table 5, suggesting that it is the hardest molecule. The softest chemical is quercetin, which has the lowest softness value (0.54eV). The ability of a molecule to draw electron electrons toward itself is known as electronegativity. Table 5 shows that acetalactone is the most electronegative of all the chemicals (3.9eV).

Evaluation of ADMET and druglikeness properties of lead compounds

Table 6
Pharmacokinetics profile of lead compounds of *Blighia sapida*

Model	Quercetin	Kaempferol	(+)-Catechin	Actaealactone	Alloathyriol
QPlogHERG	-4.875	-5.054	-4.897	-4.223	-4.476
QPPCaco	19.934	54.93	49.958	10.054	218.753
QPlogBB	-2.305	-1.824	-1.95	-2.673	-1.186
QPPMDCK	7.185	21.49	19.395	3.429	95.701
QPlogKhsa	-0.354	-0.201	-0.427	-0.597	-0.257
% Oral Absorption	52.196	64.083	59.884	42.501	76.2
QPlog ^{Khsa} : Binding to human serum albumin (– 1.5 to + 1.5)					
QPlog ^{HERG} : IC ₅₀ value for blockage of HERG K + channels (below – 5)					
QPP ^{MDCK} : Apparent Madin-Darby canine kidney cell permeability in nm/sec.					
QPlog ^{BB} : Brain/blood partition coefficient (– 3.0 to 1.2)					
QPP ^{Caco} : Apparent Caco-2 cell permeability in nm/sec (< 25 poor, > 500 great)					

The pharmacokinetics of phytochemicals is important to know before their development into new pharmaceutical drugs. Using the QikProp module of Schrodinger, pharmacokinetic properties of the lead compounds were predicted using binding to human serum albumin (QPlogkhsa), Madin-Darby canine kidney cell permeability (QPP^{MDCK}), blood/brain partition coefficient (QPlog^{BB}), IC₅₀ value for the blockage of HERG K + channels (QPlog^{HERG}) and Caco-2 cell permeability (QPP^{Caco}). All of the lead compounds fell into the range of values for QPlog^{KHSA}, therefore they should all be able to bind to human serum albumin. All of the lead compounds except Kaempferol have been predicted to have good values for QPlog^{HERG}. Again, all of the lead compounds fell within the range of expected values for Brain/Blood partition coefficient. The results also showed that only Quercetin and Actaealactone have poor Caco-2 cell permeability.

Discussion

The family of mitogen-activated protein kinases, which includes highly conserved enzymes, includes ERK5. The expression of this enzyme in cell migration, cell proliferation, and cell differentiation is being taken into consideration. But because ERK5 is a crucial signaling protein for all of the functions of cancer

cells Therefore, it makes sense to assume that taking inhibitory measures on ERK5 is an effective pharmaceutical strategy for treating cancer cells.(Barbara S. and Elisabetta R. 2019). A collection of phytoligands extracted from *Blighia sapida* were used in this study to target this signaling molecule in an effort to demonstrate the compounds' inhibitory potential as well as other pharmacologic factors that support the use of this plant as a potential cancer treatment.

With Quercetin, (Anintha S. et al., 2021). Kaempferol, and (+)-Catechin displaying higher docking score than the co-cystalized ligand and the standard medication, our technique found new lead compounds as inhibitors of ERK5 as breast cancer therapy with good conformation and reduced deleterious impact. (Muhammad I. et Al 2019). The suggested pharmacophore hypothesis has an aromatic ring, a hydrogen bond acceptor, and a hydrogen bond donor in the phase-generated E-pharmacophore theory. It's interesting to note that when *Blighia sapida*'s drug likeness was evaluated using Lipinski's rule of five, all of the hits obeyed. According to the results of the frontier molecular orbitals, all of the hit compounds are ready to transfer electrons because their E_{HOMO} values range from - 6.02 to -5.48 eV.

Conclusions

In this study, bioactive compounds of *Blighia sapida* were screened using a combination of E-pharmacophore and structure-based virtual screening followed by binding energy estimation to find promising new phytochemicals to inhibit Extracellular signal-regulated kinase 5 (ERK5), a target protein involved in Breast cancer.

These lead compounds had a lower binding affinity for the protein than the pyrrole inhibitor co-crystallized with the protein and also have the required drug-likeness properties according to docking data(Table 2, Table 6).

According to the results of molecular docking, lead compounds generated the same H-bond interactions with MET 140 and ASP 138 as the co-crystallized ligand. The lead compounds' drug-likeness was also predicted, and their pharmacokinetic and ADME features were shown to be in line with known chemically and physiologically active substances.

In silico, the proposed set of ligands showed good inhibitory capability against ERK5, and so can be employed for further scientific studies, with the results being expanded to experimental confirmation.

List Of Abbreviations

ERK5

Extracellular signal-regulated kinase 5

ADMET

Absorption, Distribution, Metabolism, Excretion and Toxicity.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not applicable

Availability of data and materials

Not Applicable

Competing Interests

All authors certify that they have no affiliations with or involvement in any organization with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Authors' Contributions

BDS, IOO, JFA and NHA curated, interpreted the data, wrote the first draft of the manuscript. **BDS and EAO** Wrote the methodology. **ODA** Supervised and Proofread the manuscript. All authors read and approved the final manuscript.

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Figures

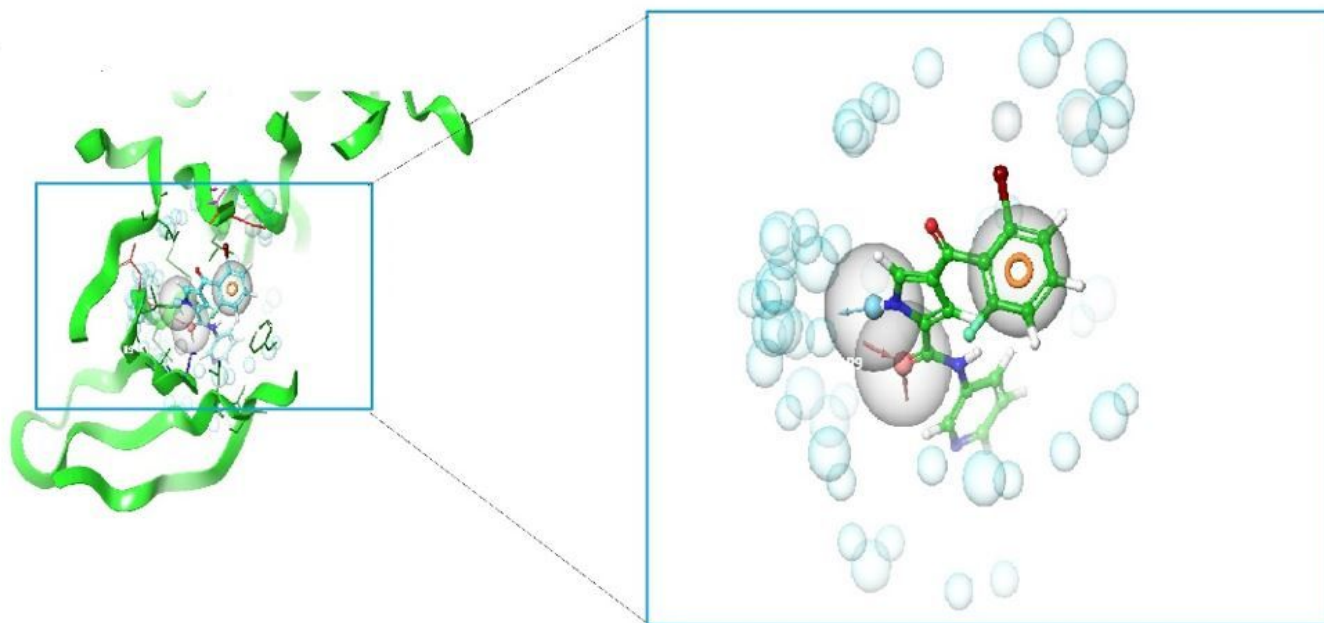


Figure 1

E-pharmacophore model for ERK5 complex mapped to the its co-crystallized ligand(**4-(2-bromanyl-6-fluoranyl-phenyl)carbonyl-~(N)-pyridin-3-yl-1~(H)-pyrrole-2-carboxamide**).

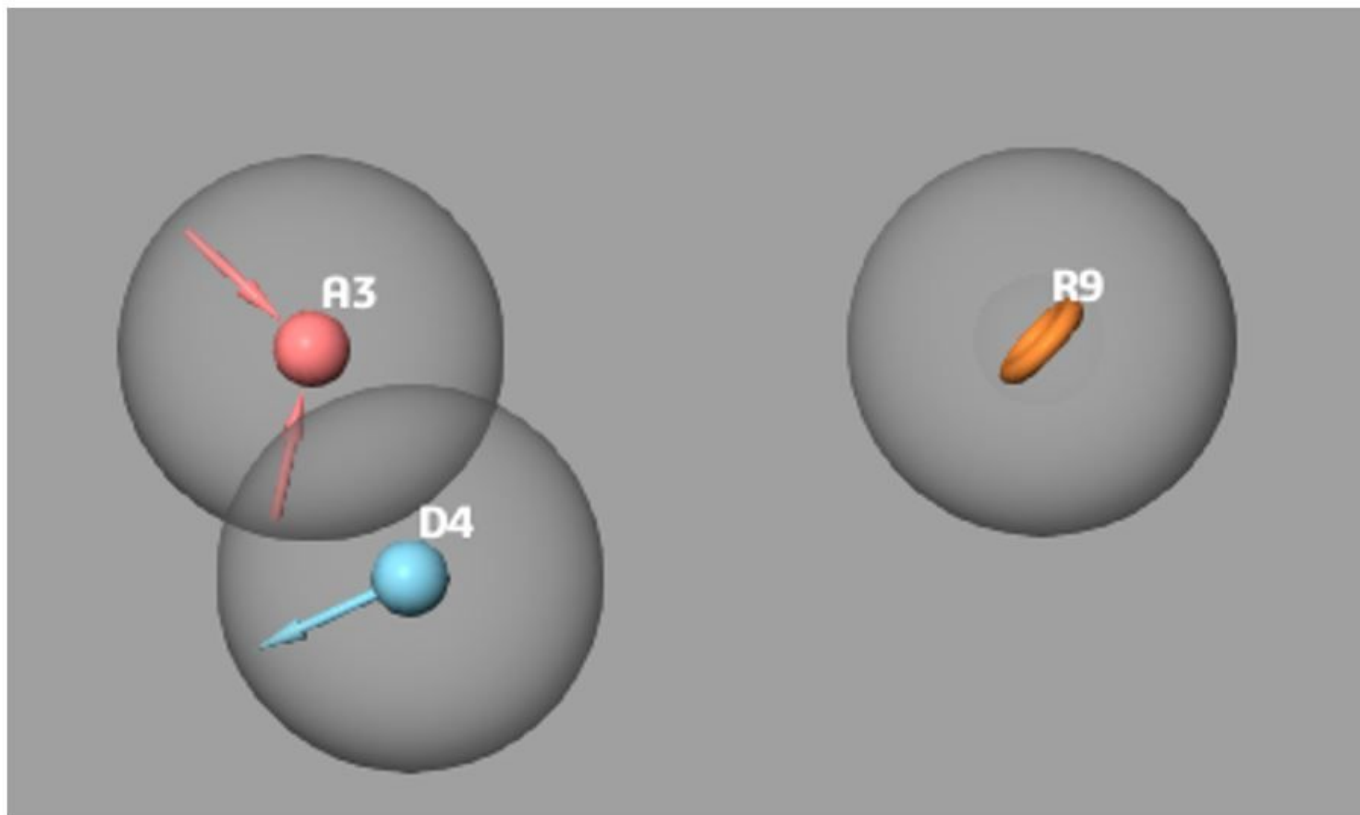


Figure 2

shows the hydrogen bond acceptor (A3: red), hydrogen bond donor (D4: blue), and aromatic ring (R9: orange) in the phase-generated E-pharmacophore theory

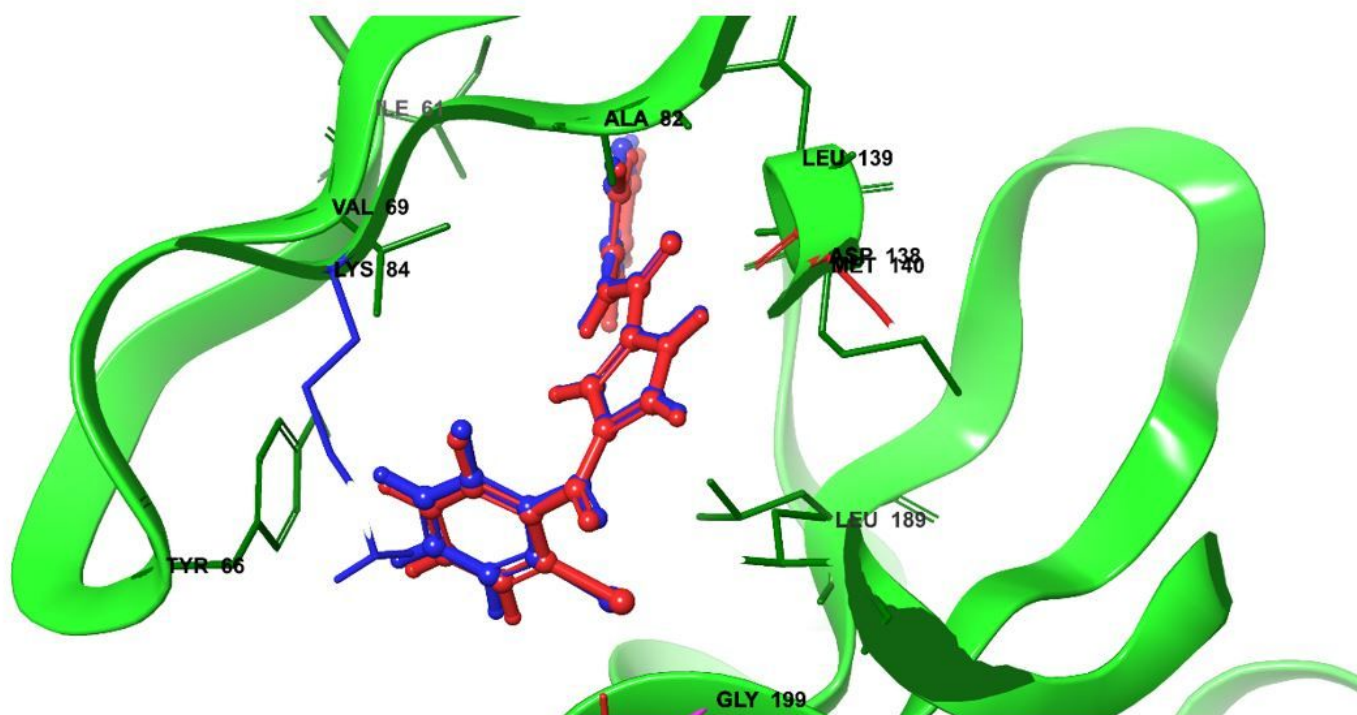


Figure 3

Superimposed on the crystal structure(red) is the docked pose of the pyrrole inhibitor (blue).

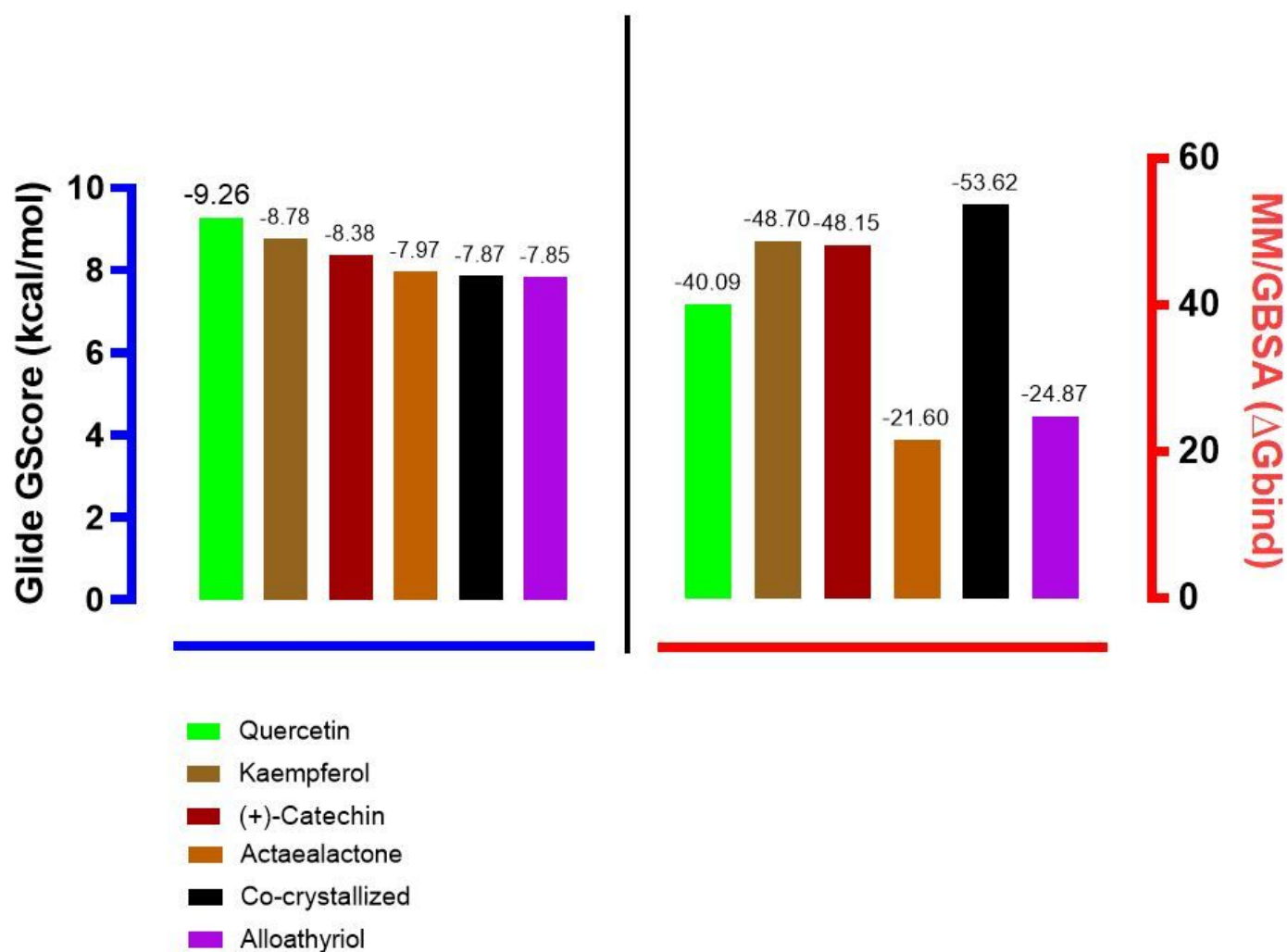


Figure 4

Graphical representation of the molecular Glide GScore and Prime/MM-GBSA binding energy (ΔG_{bind}) of *Blighia sapida* lead compounds and the control ligand. The left frame (blue) signifies the docking score, while the right frame (red) shows the MM-GBSA binding energy.

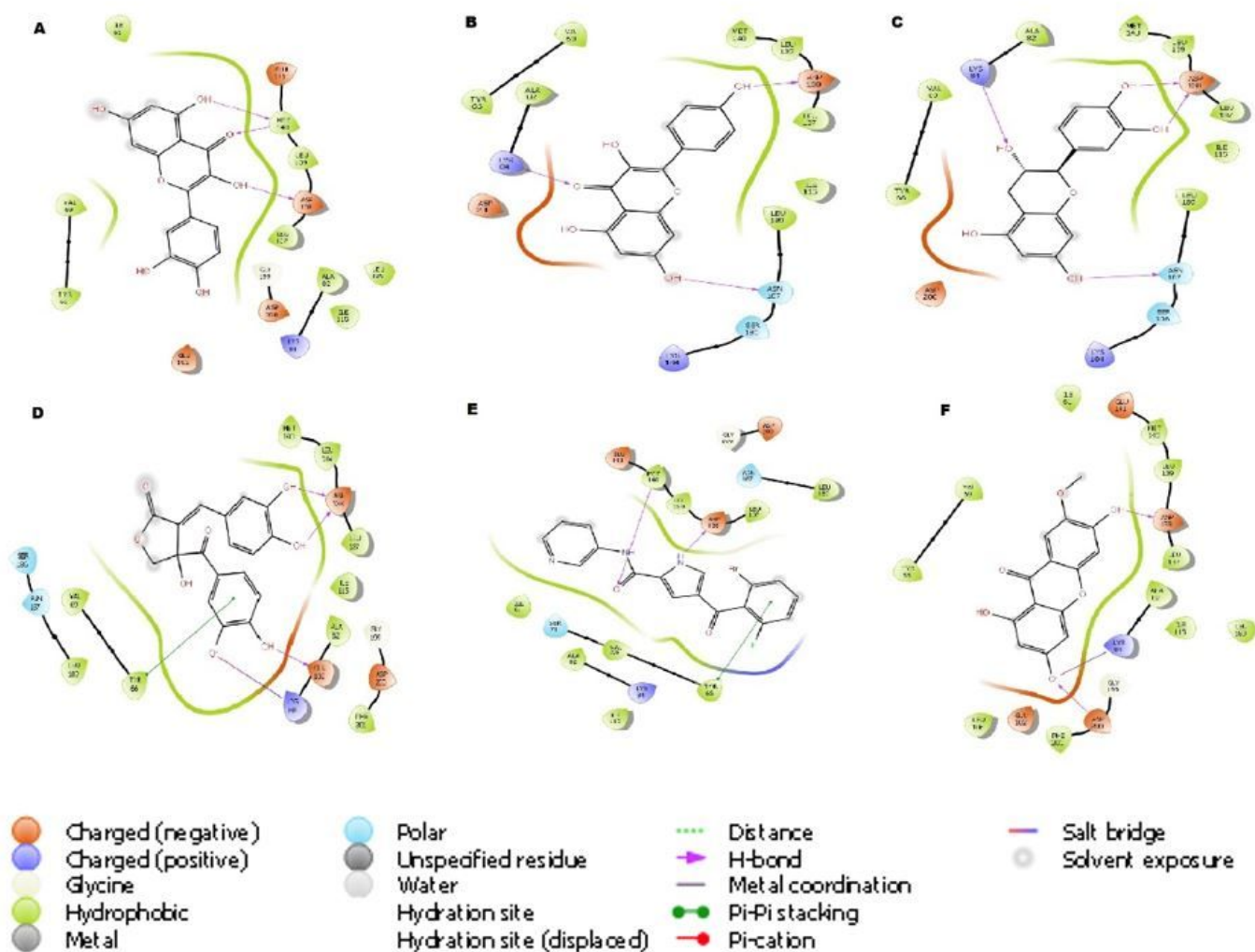


Figure 5

2D molecular contacts profiling of lead compounds with amino acid residues (4.00Å) at the active site of ERK5: (a) Quercetin (b) kaempferol, (c) (+) - Catechin (d) Actaelactone (e) Co-crystallized Ligand and (f) Alloathyriol

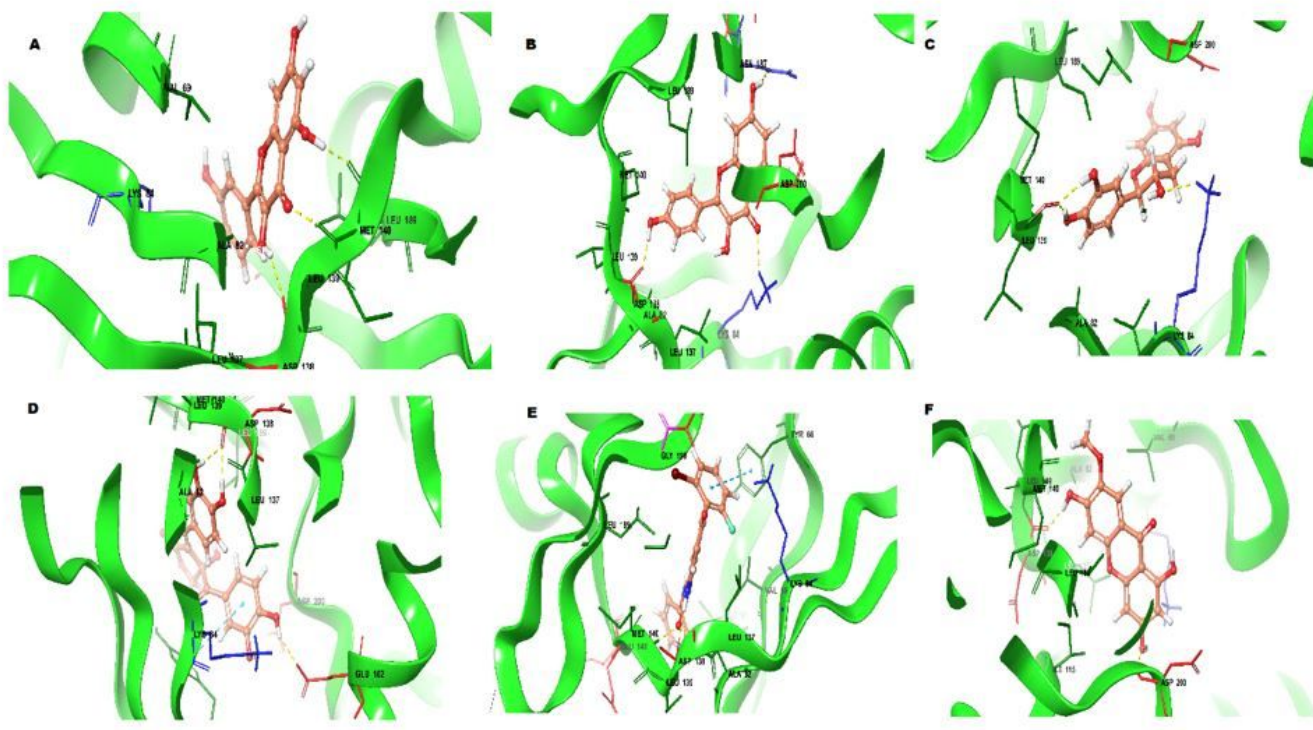


Figure 6

3D molecular contacts profiling of lead compounds with amino acid residues at the active site of ERK5: (a) Quercetin (b) kaempferol, (c) (+) – Catechin (d) Actaelactone (e) Co-crystallized Ligand and (f) Alloathyriol

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