Capstone 46
African Natural Products as Potential Inhibitors of FLT3 for Acute Myeloid Leukemia

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Introduction

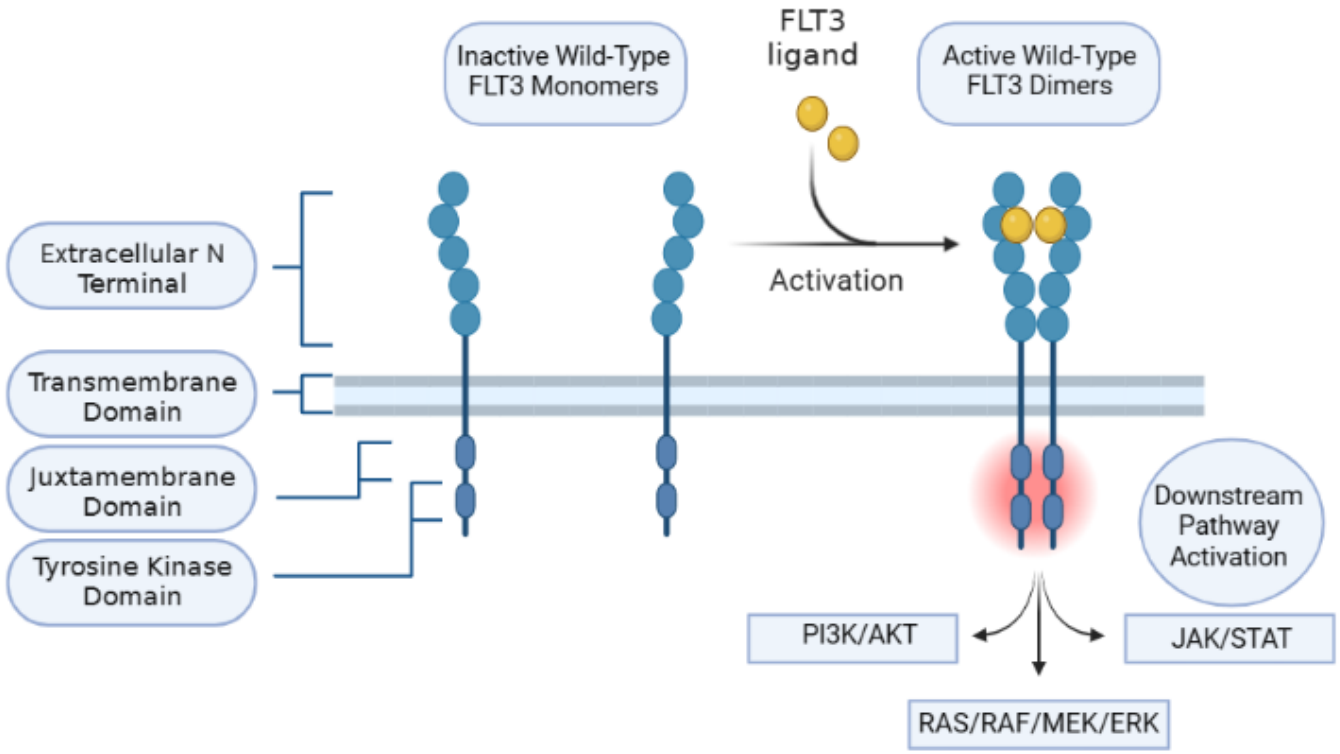
Acute Myeloid Leukemia

- Acute myeloid leukemia is a hematological malignancy characterized by infiltration and overproduction of malignant myeloid cells into the bone marrow, blood, and other tissues.
- It is considered one of the adults' most common forms of leukemia.
- Several etiologic factors contribute to the disease, including heredity, radiation, and long-term use of anti-cancer drugs.
- The majority of AML cases are due to genetic mutations.

Nature Reviews Disease Primers, (2016) volume 2, 16011

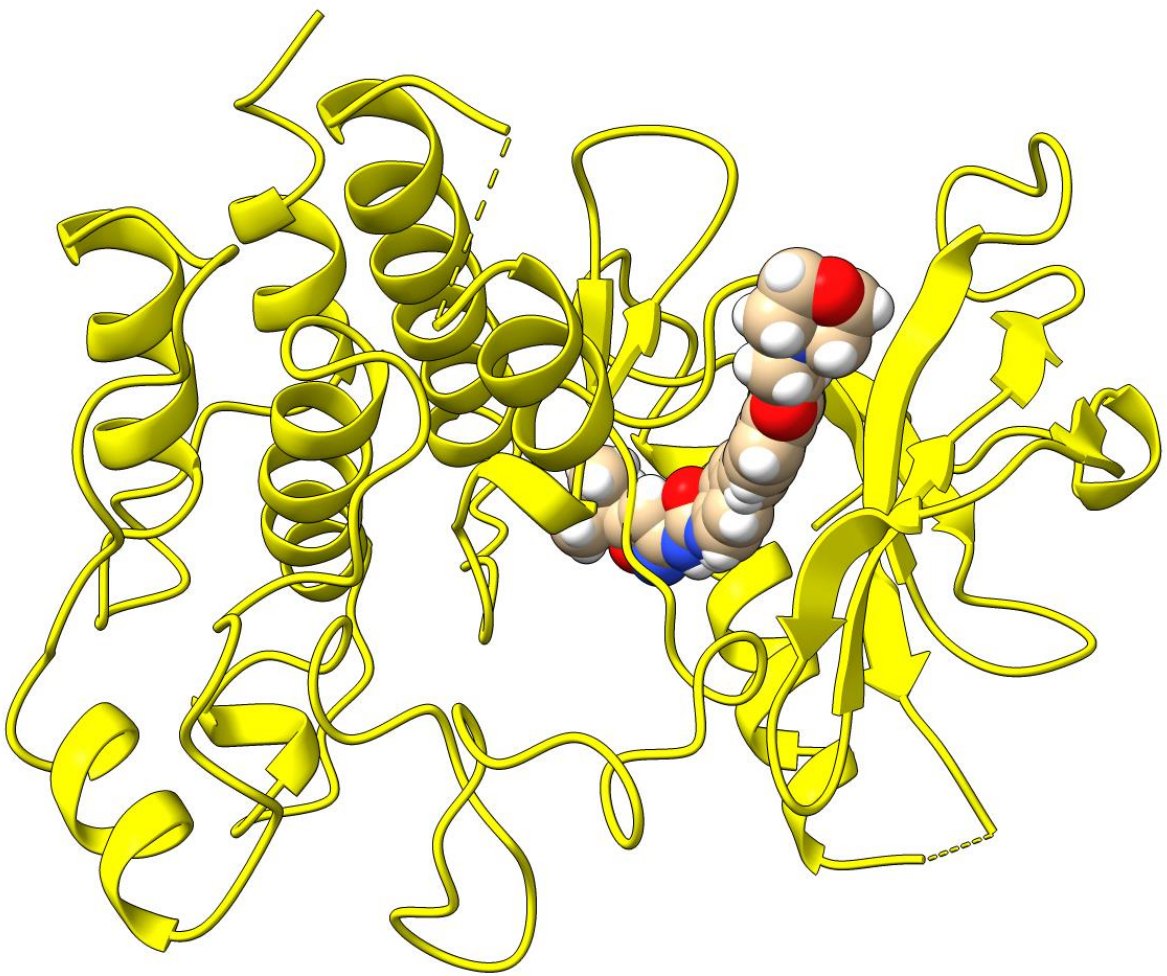
FLT3

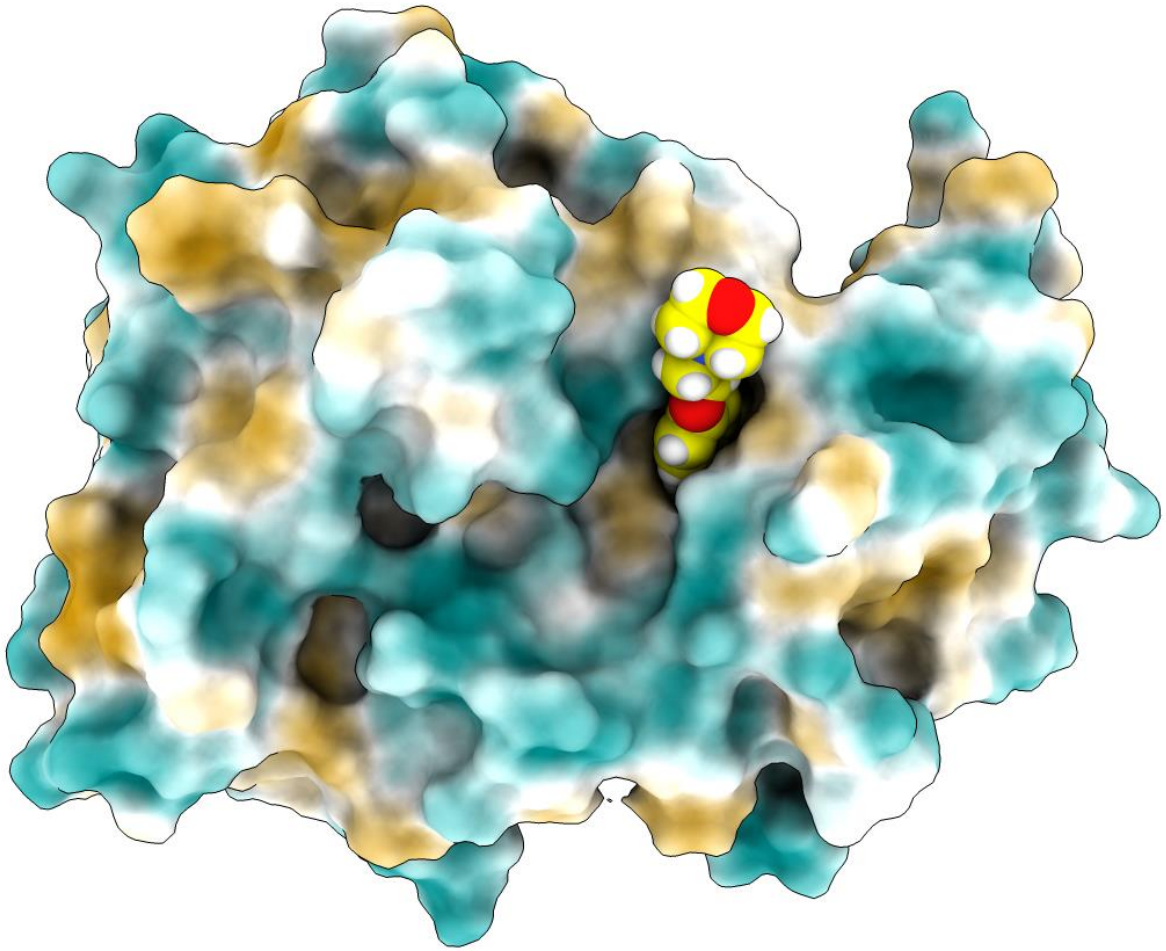
- FMS(Feline McDonough Sarcoma)-Like Tyrosine Kinase 3 is a type III receptor tyrosine kinase that helps regulate cell survival and reproduction.



Front. Oncol., (2020) Sec. Hematologic Malignancies, Volume 10, 2020

Crystal Structure of FLT3 Tyrosine Kinase

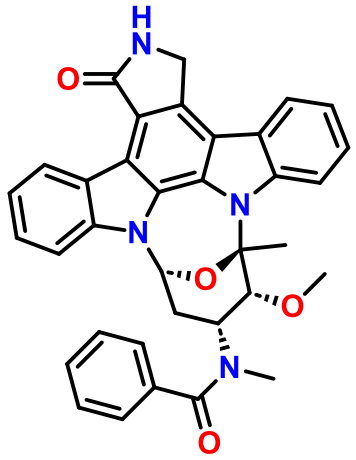




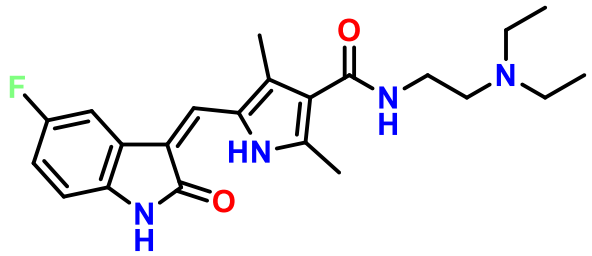
PLOS ONE, (2015) 10(4): e0121177.

First Generation FLT3 Inhibitors

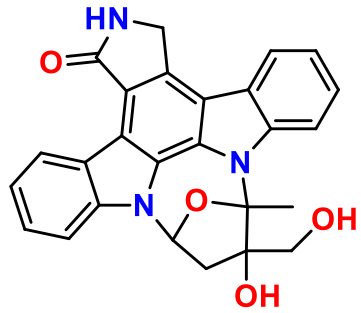
- First-generation FLT3 inhibitors such as midostaurin, sunitinib, and lestaurtinib are multikinase inhibitors that are not selective to FLT3, and high drug doses were needed to induce sustained inhibition.



Midostaurin



Sunitinib

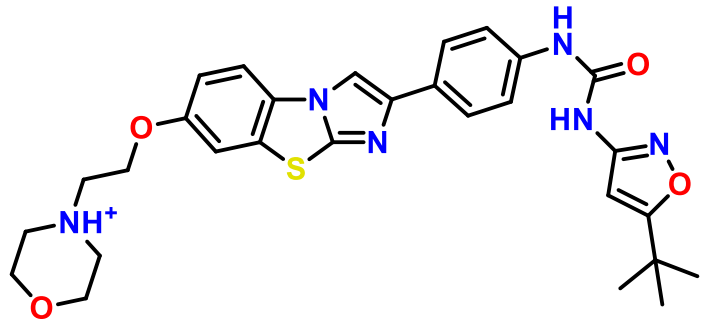


Lestaurtinib

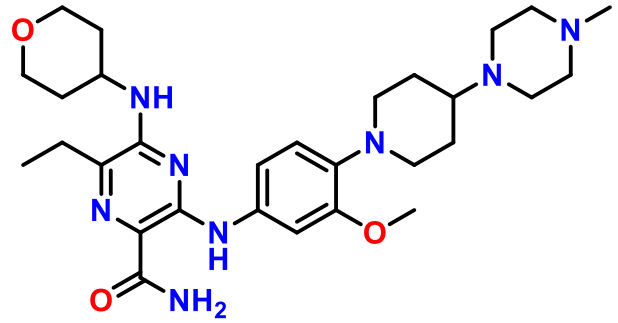
Mol Cancer Ther, (2017) 16 (6): 991–1001

Second Generation FLT3 Inhibitors

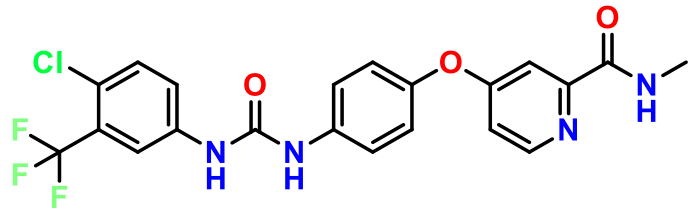
- Second-generation FLT3 inhibitors such as quizartinib and gilteritinib more selectively inhibit the enzyme and have better tolerability and more constant effects, but inhibitory responses are typically short-lived since resistant mutations emerge.



Quizartinib



Gilteritinib

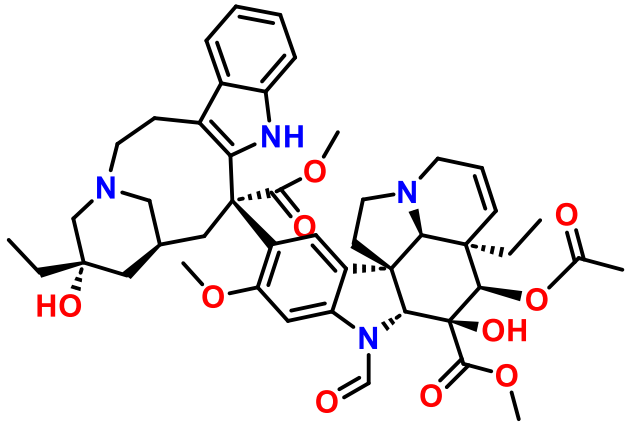


Sorafenib

Minerva Medica, (2020) 111(5):427-42

Natural Products

- It has long been recognized that natural products have the potential for the prevention or treatment of major diseases.
- Many studies highlight the anti-neoplastic effect of natural products, such as triggering apoptosis, and lowering the resistance against chemotherapies, suggesting that they have the potential to become novel interventions for AML.



Vincristine

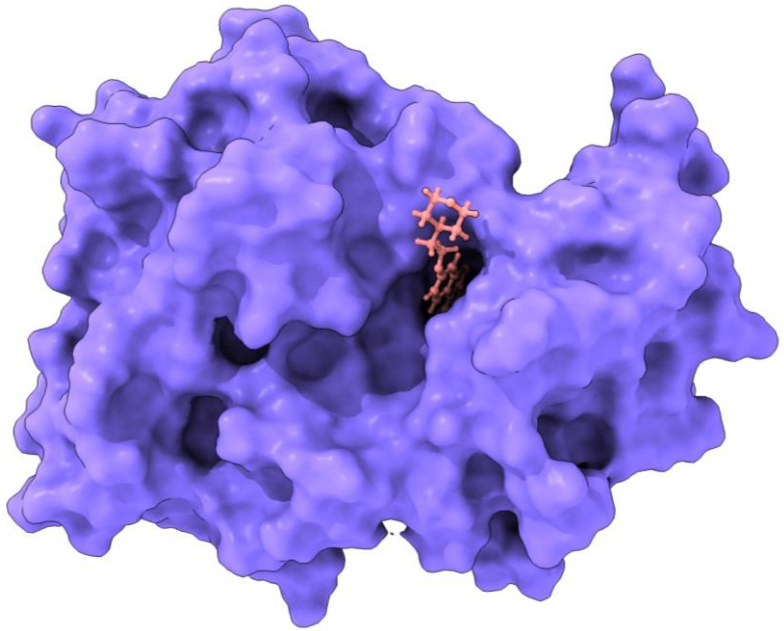
Natural Products and Bioprospecting, (2021) volume 11, 5–13

In Silico Approach

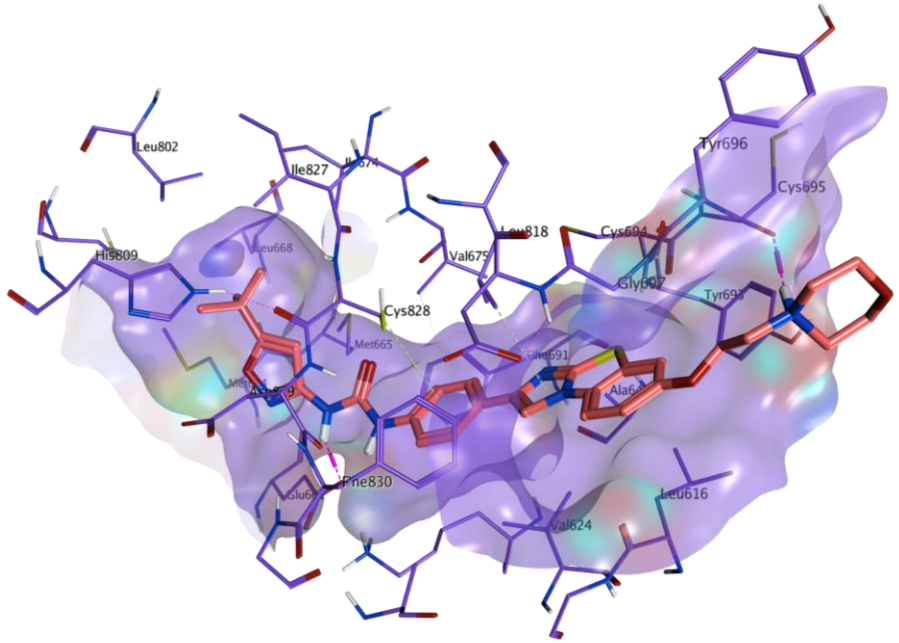
- *In Silico* originally meant "performed on computer or via computer simulation," in modern science, it refers to experimental techniques performed by computers.
- It relates to the more established terms of *in vivo* and *in vitro* studies.
- *In Silico* docking techniques are used to investigate the affinity at the molecular level of a ligand and a protein target.

Aim of Project

To find a natural product drug candidate that could inhibit the FLT3 gene via targeting the FLT3 kinase domain with the COCONUT database containing 407,270 African Natural Products.



COCONUT database

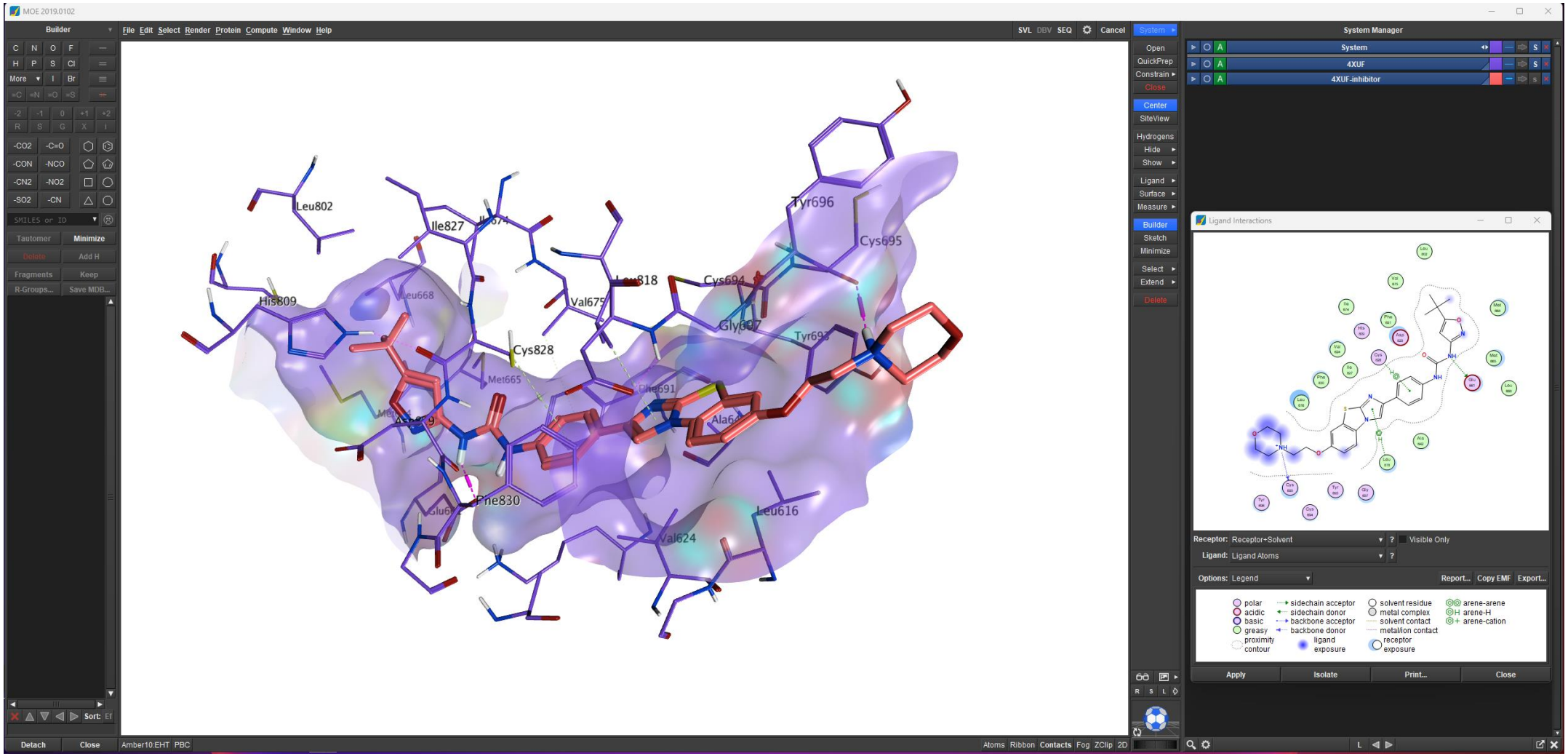


Methods and Materials

MOE

- Molecular Operating Environment (MOE) is a drug discovery software platform that integrates molecular simulation, protein structure analysis, small molecule data processing, and other comprehensive support for small molecule drug and biopharmaceutical design under a unified operating environment.

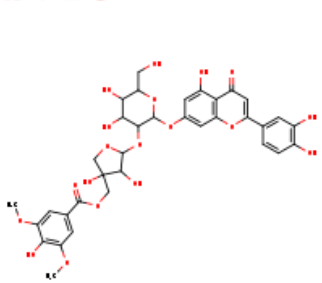
MOE

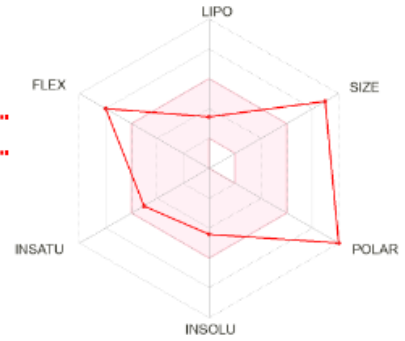

MOE 2019.0102
Builder
File Edit Select Render Protein Compute Window Help
SVL DBV SEQ Cancel
System
Open
QuickPrep
Constrain
Close
Center
SiteView
Hydrogens
Hide
Show
Ligand
Surface
Measure
Builder
Sketch
Minimize
Select
Extend
Delete
System Manager
System
4XUF
4XUF-inhibitor
Ligand Interactions
Leu802
Ile827
His809
Leu668
Val675
Leu818
Cys694
Tyr696
Cys695
Gly697
Tyr693
Cys828
Met665
Phe691
Ala621
Phe830
Leu616
Val624
Receptor: Receptor+Solvent
Visible Only
Ligand: Ligand Atoms
Options: Legend
Report...
Copy EMF
Export...
polar
acidic
basic
greasy
proximity
contour
sidechain acceptor
sidechain donor
backbone acceptor
backbone donor
ligand exposure
solvent residue
metal complex
solvent contact
metal/ion contact
receptor exposure
arene-arene
arene-H
arene-cation
Apply
Isolate
Print...
Close
Detach
Close
Amber10:EHT PBC
Atoms Ribbon Contacts Fog ZClip 2D

SwissADME

- SwissADME is a free web tool that allows access to predict and compute physicochemical properties, pharmacokinetics parameters, drug-likeness, and medicinal chemistry of molecules, to indicate the compounds that have the potential to be lead drug candidates.

Molecule 1





SMILES OCC1OC(Oc2cc(O)c3c(c2)oc(cc3=O)c2ccc(c(c2)O)O)C(C(C1O)O)OC1OCC(C1O)(O)COC(=O)c1cc(OC)c(c(c1)OC)O

Physicochemical Properties	
Formula	C35H36O19
Molecular weight	760.65 g/mol
Num. heavy atoms	54
Num. arom. heavy atoms	22
Fraction Csp3	0.37
Num. rotatable bonds	12
Num. H-bond acceptors	19
Num. H-bond donors	9
Molar Refractivity	179.23
TPSA	293.96 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	3.39
Log $P_{o/w}$ (XLOGP3)	0.55
Log $P_{o/w}$ (WLOGP)	-0.19

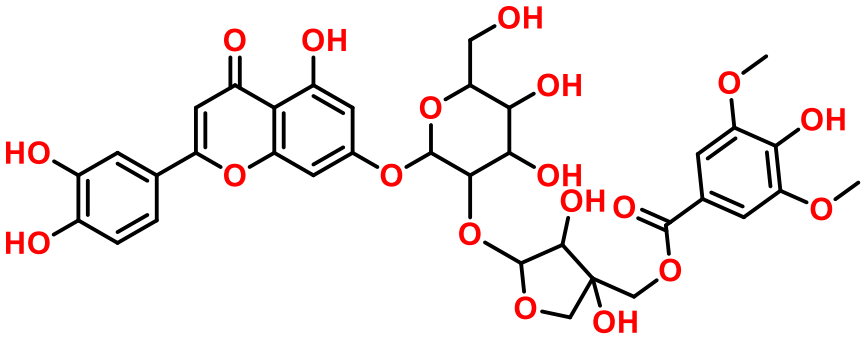
Water Solubility	
Log *S* (ESOL)	-4.41
Solubility	2.95e-02 mg/ml ; 3.87e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	-6.30
Solubility	3.86e-04 mg/ml ; 5.07e-07 mol/l
Class	Poorly soluble
Log *S* (SILICOS-IT)	-3.52
Solubility	2.28e-01 mg/ml ; 3.00e-04 mol/l
Class	Soluble

Pharmacokinetics	
GI absorption	Low
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-10.55 cm/s

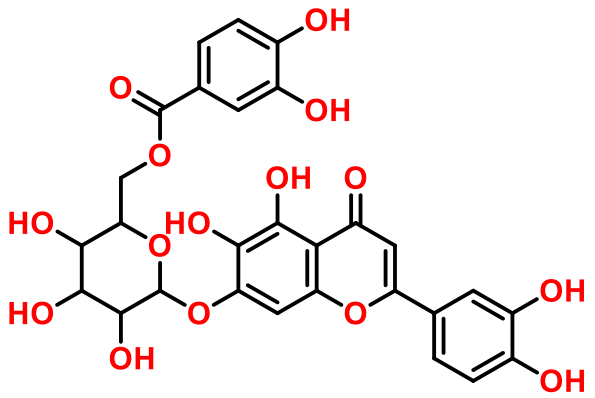
Druglikeness	
Lipinski	No; 3 violations: MW>500, NorO>10, NHorOH>5
Ghose	No; 3 violations: MW>480, MR>130, #atoms>70
Veber	No; 2 violations: Rotors>10, TPSA>140
Egan	No; 1 violation: TPSA>131.6
Muegge	No; 4 violations: MW>600, TPSA>150, H-acc>10, H-don>5

Results and Discussion

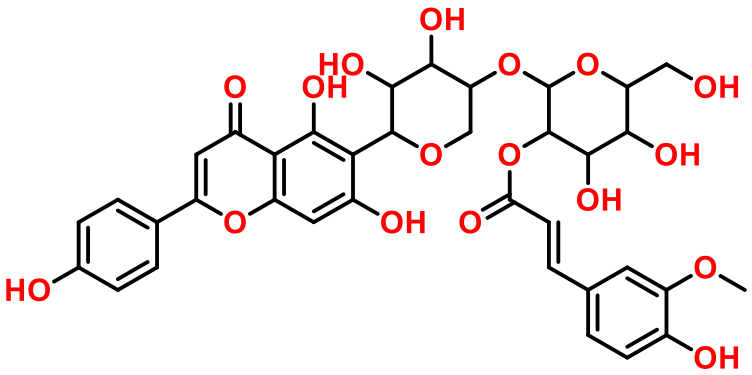
Docking Results



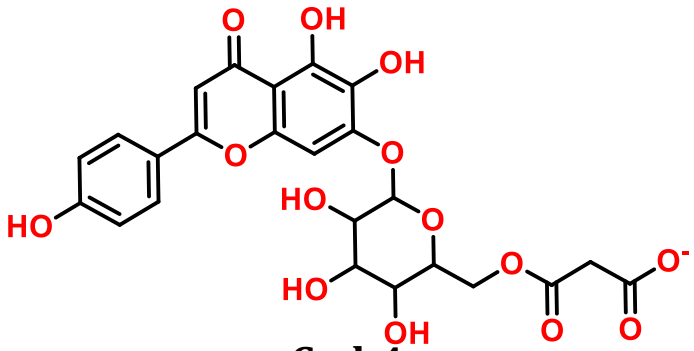
Cpd-1
Docking Score: -18.052 kcal/mol



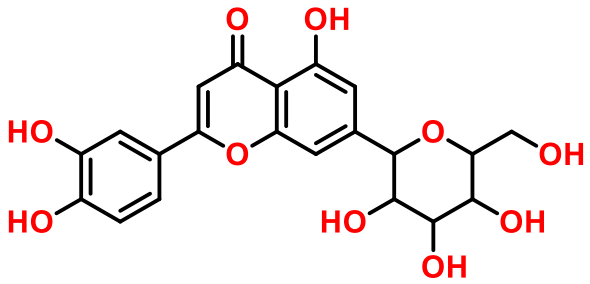
Cpd-2
Docking Score: -17.884 kcal/mol



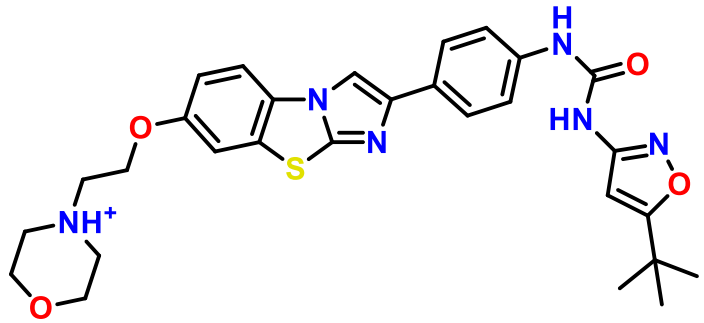
Cpd-3
Docking Score: -16.767 kcal/mol



Cpd-4
Docking Score: -16.470 kcal/mol

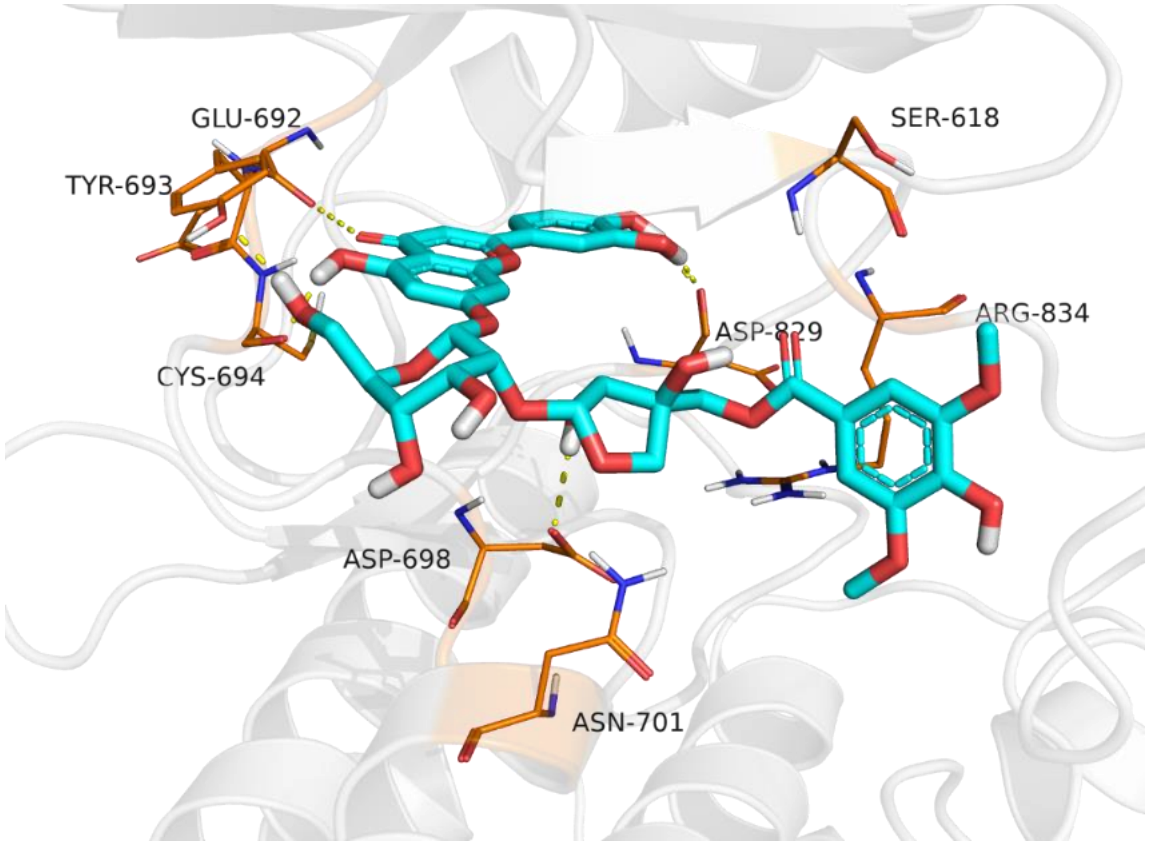


Cpd-5
Docking Score: -16.326 kcal/mol

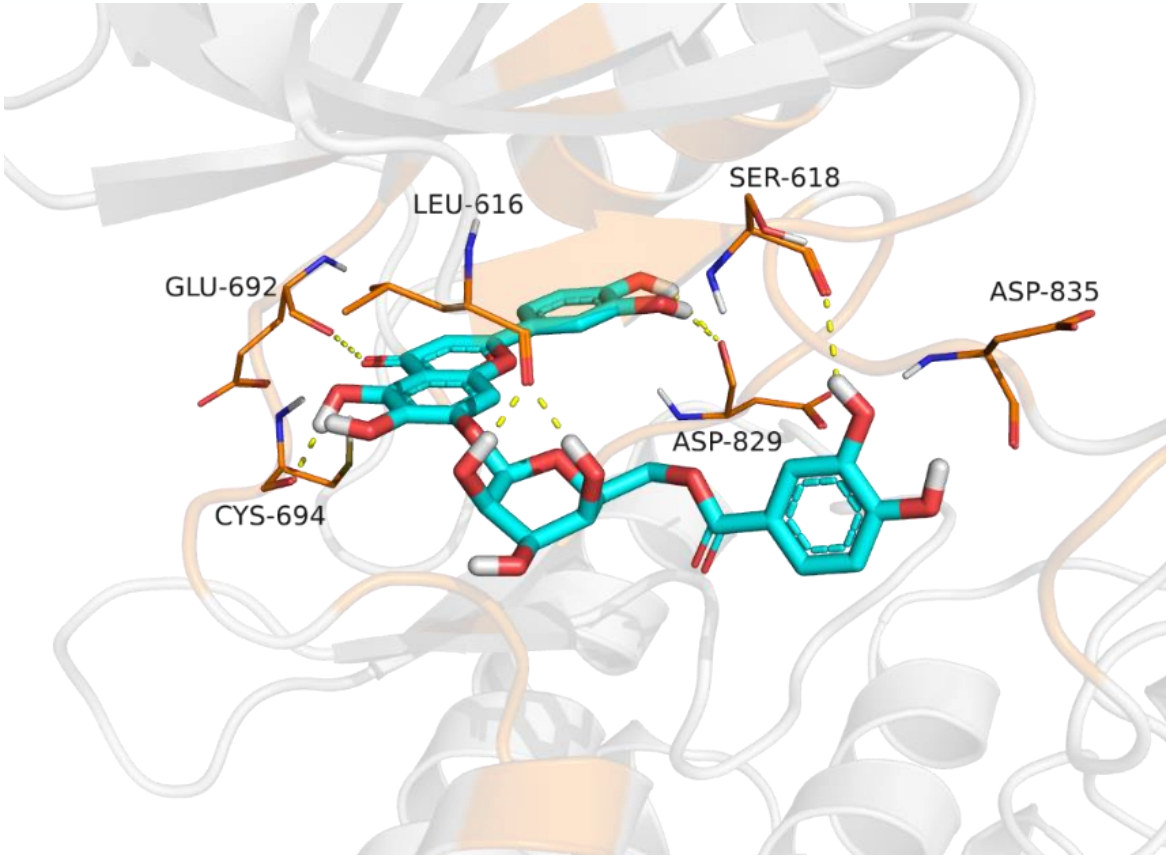


Quizartinib
Docking score = -11.616kcal/mol

Docking Results

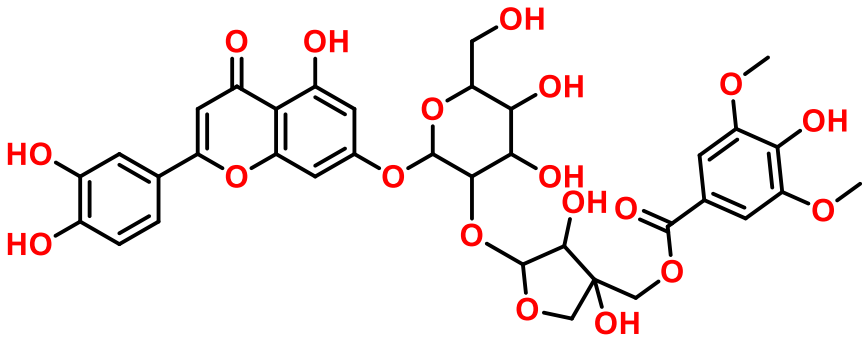


Docking Score: **-18.052** kcal/mol
H-bond Residues: Asp829, Asp829, Asn701, Asp698, Leu616, Leu616, Cys694, and Cys694



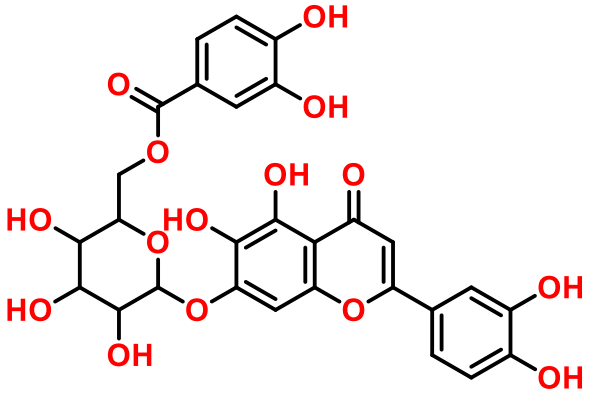
Docking Score: **-17.884** kcal/mol
H-bond Residues: Arg834, Asp829, Asp829, Leu616, Leu616, Tyr694, Cys694, and Ser618

Physicochemical Properties



Cpd-1

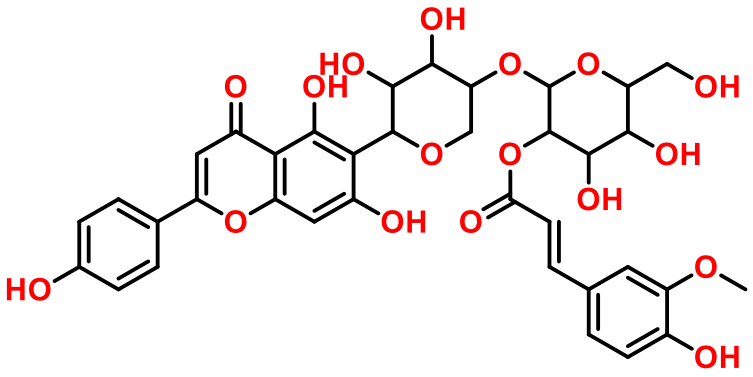
Molecular Weight: 760.65 Da
Rotatable Bonds: 12
H-bond Acceptors: 19
H-bond Donors: 9
TPSA: 293.96 Å
cLogP: 0.12
Solubility: Moderately Soluble



Cpd-2

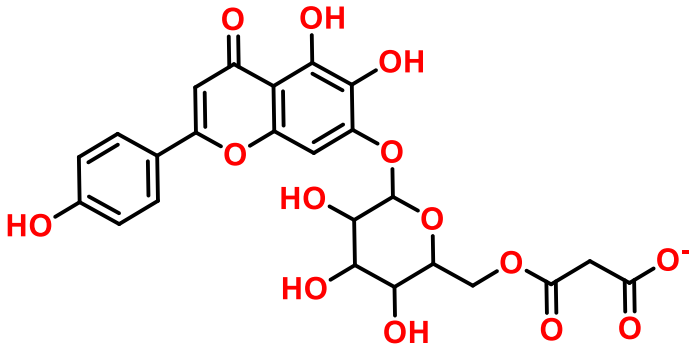
Molecular Weight: 600.48 Da
Rotatable Bonds: 7
H-bond Acceptors: 15
H-bond Donors: 9
TPSA: 257.04 Å
cLogP: 0.26
Solubility: Moderately Soluble

Physicochemical Properties



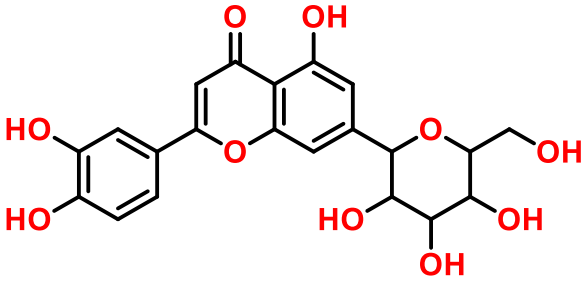
Cpd-3

Molecular Weight: 740.66 Da
Rotatable Bonds: 10
H-bond Acceptors: 17
H-bond Donors: 9
TPSA: 275.50 Å
CLog P: 0.54
Solubility: Moderately Soluble



Cpd-4

Molecular Weight: 533.42 Da
Rotatable Bonds: 8
H-bond Acceptors: 14
H-bond Donors: 6
TPSA: 236.48 Å
CLog P: -0.56
Solubility: Soluble



Cpd-5

Molecular Weight: 432.38 Da
Rotatable Bonds: 3
H-bond Acceptors: 10
H-bond Donors: 7
TPSA: 181.05 Å
CLog P: -0.12
Solubility: Soluble

Pharmacokinetics

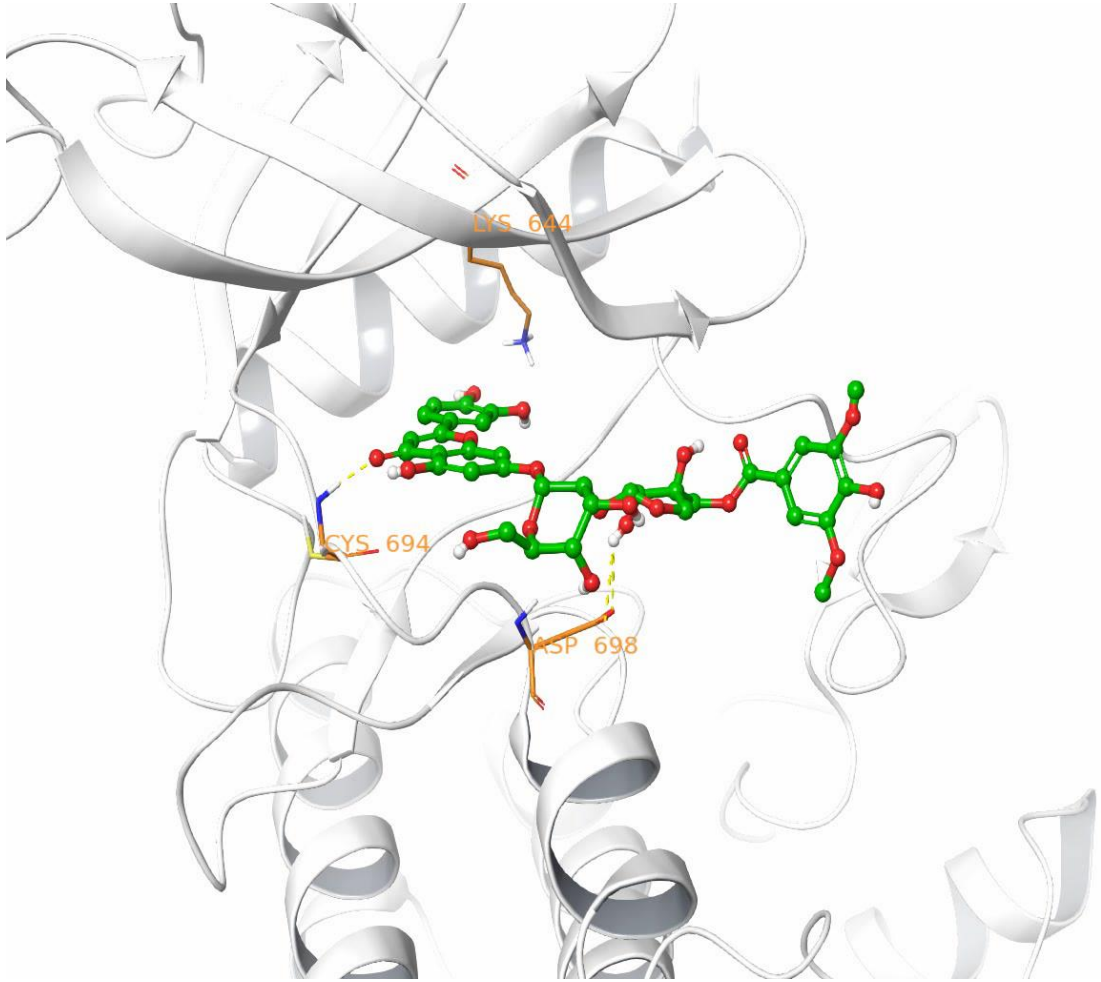
Compound	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Cpd-1	Low	No	Yes	No				
Cpd-2			No					
Cpd-3			Yes					
Cpd-4			Yes					
Cpd-5			No					

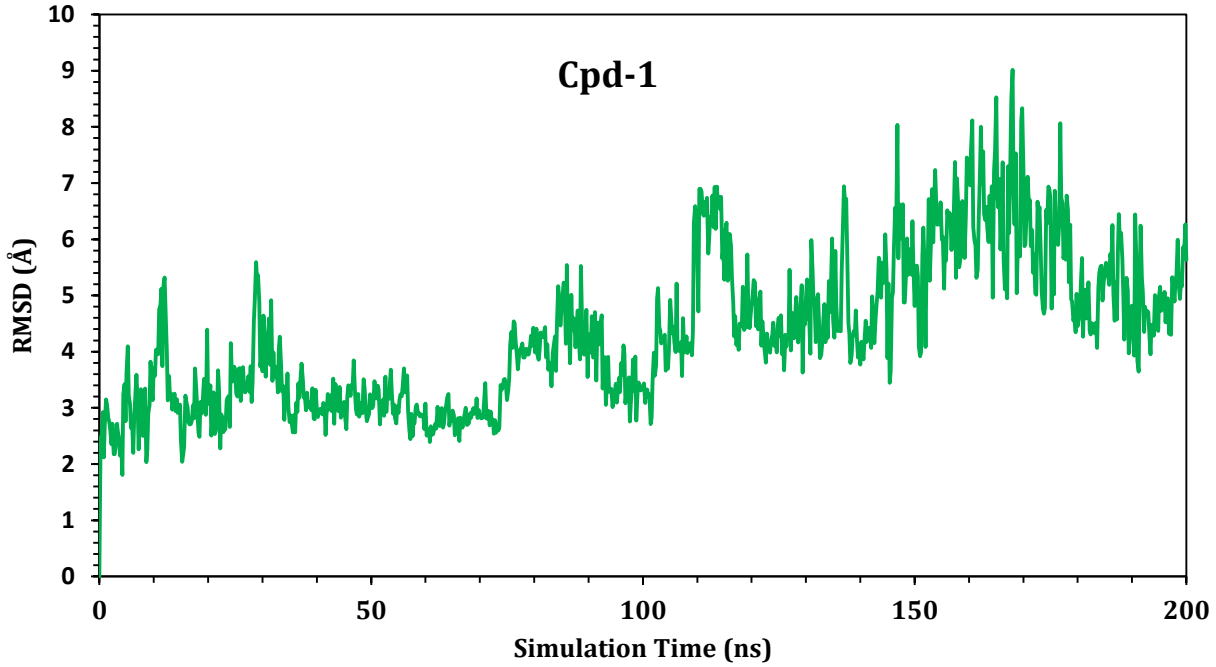
GI: Gastrointestinal
BBB: blood-brain barrier
Pgp: Permeability glycoprotein

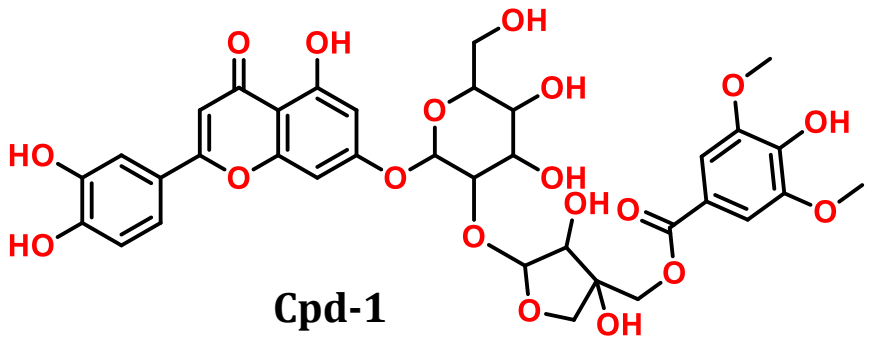
Molecular Dynamics

- Molecular dynamic simulations are a computational study that predicts the movement of every atom in a molecule over time.
- Molecular dynamics can evaluate the movement of water, ions, small and macromolecules, or even complex systems, which is extremally helpful in reproducing the behavior of chemical and biological environments
- Molecular dynamics simulations are used to study the stability of the drugs inside the active site of the protein.

Molecular Dynamics

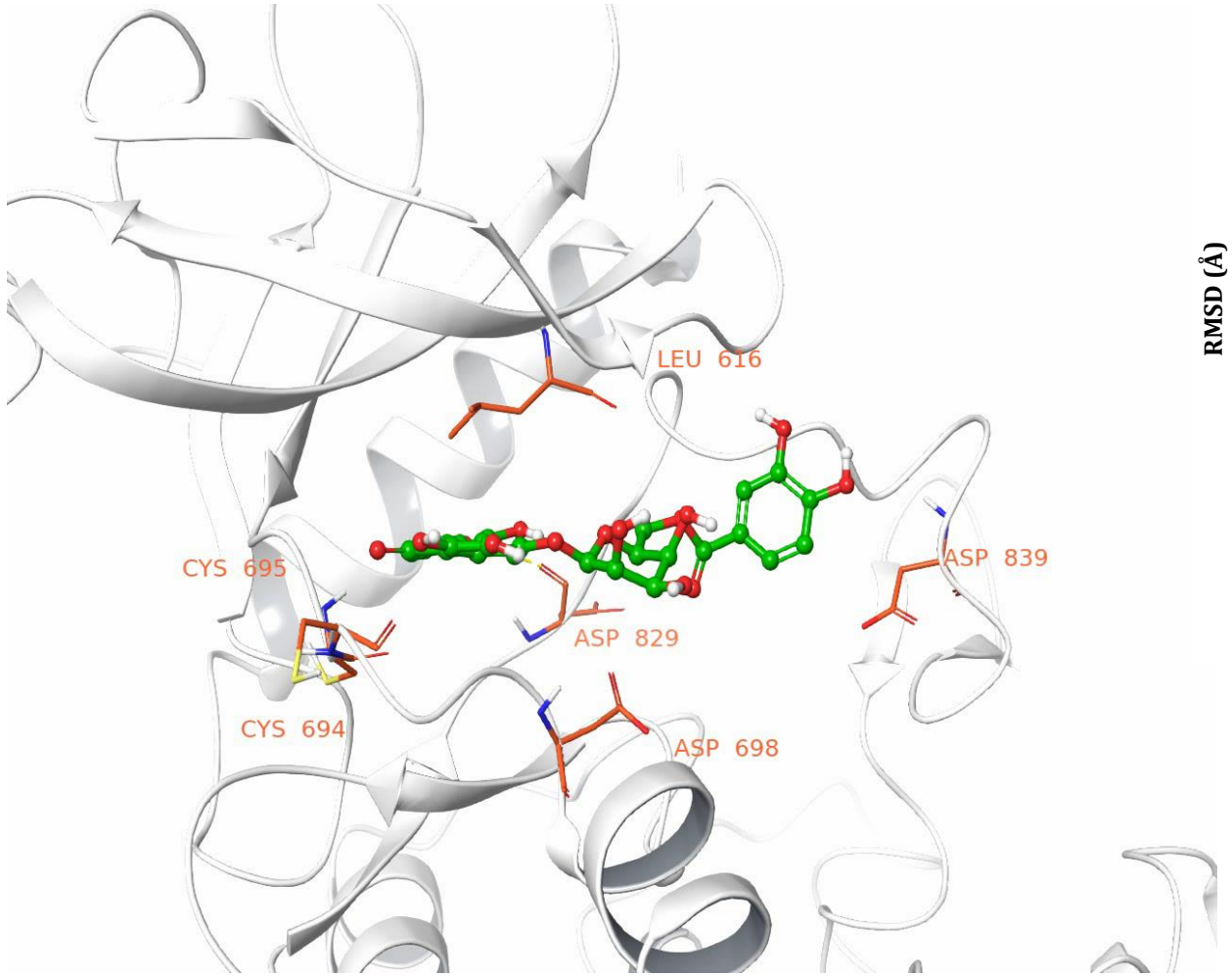


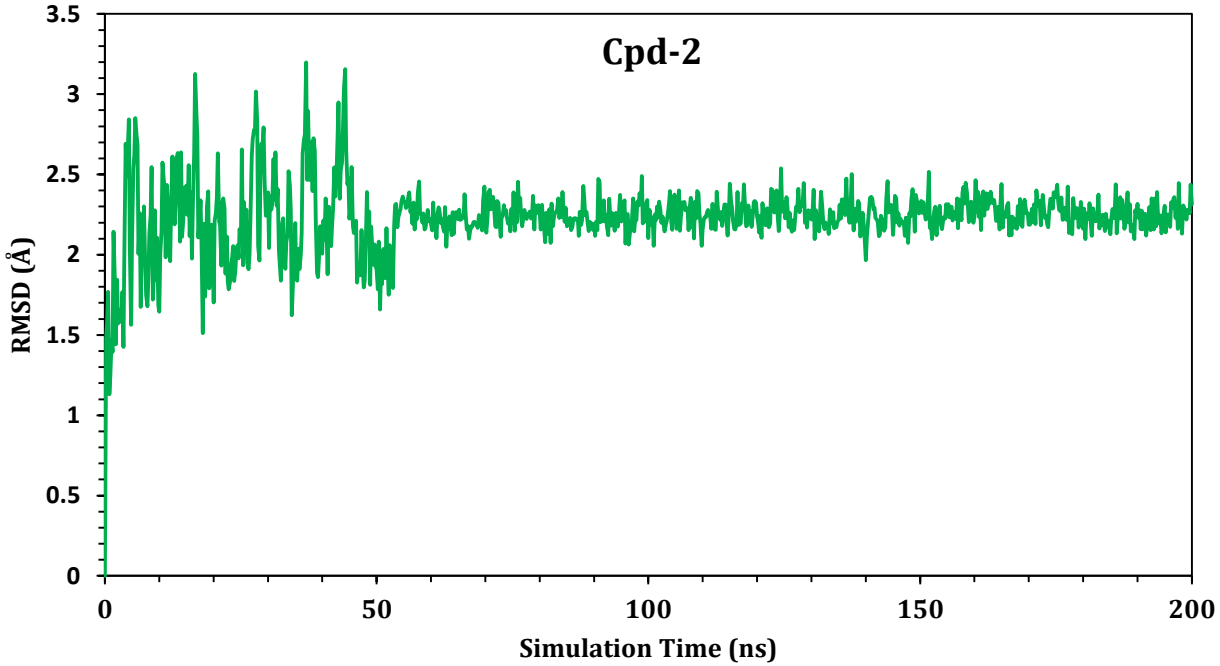


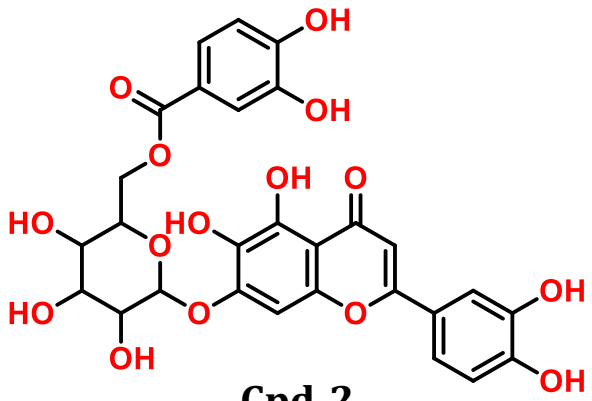


Cpd-1

Molecular Dynamics







Cpd-2

Conclusion

Conclusion

- In this study, we screened a COCONUT database of African Natural Products to identify potential inhibitors against the FLT3 kinase domain (PDB ID: 4XUF).
- Top ten compounds were superior in docking score compared to the reference drug Quizartinib (Docking Score -11.616kcal/mol).
- Cpd-1 showed a docking score of (-18.052 kcal/mol), and Cpd-2 showed a docking score of (-17.884 kcal/mol).
- MDs reveal that Cpd-2 is more stable than Cpd-1 inside the active site.
- Most compounds showed a lower GI absorption, with no BBB penetration nor Cytochrome P450 isoenzyme interactions.

Future work and recommendation

- The other eight compounds should be subject to molecular dynamic simulations.
- Further structure-activity relationship studies should be conducted to overcome GI absorption issues.
- We highly recommend that these compounds be tested *in vitro.*
- The selectivity of the frontier compounds should be tested toward unmutated kinase domains.

Thank You