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In Silico Study of Compounds Identified in *Curcuma aeruginosa* Roxb Rhizome as BRAF V600E Inhibitors in Melanoma Cancer

Ririn Suharsanti*, Muhammad Ryan Radix Rahardhian, Lia Kusmita

Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang; Central Java, 50192, Indonesia
*Corresponding author: Ririn Suharsanti | Email: ririnsuharsanti@stifar.ac.id

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Abstract: *Curcuma aeruginosa* Roxb rhizome contains secondary metabolite compounds and plays a role in various activities such as antioxidant, antibacterial, anthelmintic, antiandrogenic, antinociceptive, and anticancer. Anticancer activity that has been reported in *Curcuma aeruginosa* Roxb rhizome is limited to breast and cervical cancer. The purpose of this study was to explore the potential of *Curcuma aeruginosa* Roxb rhizome in melanoma cancer through the mechanism of inhibiting the BRAF V600E. The 96% ethanol extract of *Curcuma aeruginosa* Roxb rhizome was separated to produce n-hexane (HF), ethyl acetate (EAF), and ethanol (EF) fractions. The GC-MS results showed that there were 31 compounds from the three fractions. The docking validation process was carried out on the native ligand N-(3-{[5-(4-chlorophenyl) -1H-pyrrolo [2,3b]pyridin3yl] carbonyl}2,4difluorophenyl) propane-1-sulfonamide. All compounds were prepared as ligands for molecular docking with the BRAF V600E receptor (PDB ID: 3OG7). Docking validation on native ligand showed RMSD 1.03Å. The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68 kcal/mol); Cyclooctenone (-6,23 kcal/mol); and vemuravnib is still better as K+ (-11.11 kcal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. These results indicate that further structural development is needed for better activity.

Keywords: *Curcuma aeruginosa*; GC-MS ; In Silico, BRAF V600E inhibitor, Vemuravenib

1. INTRODUCTION

Over the past 50 years, melanoma incidence has steadily climbed globally. Melanoma is more prevalent in lower latitudes and among white-skinned individuals. Melanoma is the most common cancer in teenagers and young adults, but it is often more prevalent in the elderly population. In 2020, melanoma of the skin is expected to account for 1.7% of all cancer diagnoses worldwide, with an estimated 325,000 new cases [1]. Vemurafenib and dabravenib are BRAF mutation-inhibiting chemotherapy drugs to treat melanoma [2], [3]. Approximately 50% of cutaneous melanoma patients have active BRAF V600 mutations, so selective inhibitors were developed. Vemurafenib is responsive in 50% of patients with BRAF V600 mutations and is longer progression-free than dacarbazine (DTIC). In previous research, the reticuline compound in soursop leaves was proven to have the potential to treat cancer through the BRAF V600E inhibitor mechanism in silico [4]. In addition, in silico evaluation of several 4-(quinolin-2-yl)pyrimidin-2-amine derivatives as potent V600E-BRAF inhibitors was carried out [5]. There are several active compounds as BRAF V 300E inhibitors, which provide an opportunity for other natural ingredients to have the same activity.

Traditionally, the *C. aeruginose* rhizome has been used medicinally to treat stomach ache, obesity and rheumatism, asthma and cough, scurvy and mental disorders [6]. Essential oil content has been identified from the results of the distillation of *C. aeruginosa* Roxb. rhizomes such as curzerenone (24.6%), 1,scineole (ll.O%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%), curcumenol (5.6%) and filranogermenone (5.5%) [7]. Other identified compounds include champor (29,39%) dan germacrone (21,21%) [8], monoterpen (21,47%) berupa β-pinen dan 1,8 cineol [9], 1,8-

cineol (22.65%) dan germacrone (17.70%) [10], tropolene (18,1%) dan eucalyptol (17,9%) [11], β-pinene (21.9%), neocurdione (16.1%) and curcumol (15.2%) [12]. Meanwhile, the compounds that were successfully separated from the black turmeric extract using chromatography include germacrone, zederone, dehydrocurdione, curcumenol, zedoarondiol dan isocurcumenol [13]; dehydrocurdione, curcumenol, dan germacrone [14]; Pyrocurzerenone, Dehydrochromolaenin, Curzeone, Linderazulene, Curzerenone, 8, 12 - Epoxy - 1 (10), 4(15), 7, 11 -germacratetraen-6-one [15]; aeruginon and curcumenone [16]; dan flavon [17]. *C aeruginosa* isolates that have quite potential in various activities are germacrone as antiandrogenic [13], hair growth promoter [14], antinociceptive [18], and anticancer [16]. Anticancer activity that has been reported in *C. aeruginosa* Roxb rhizome is limited to breast cancer (MCF-7 and T-47D) and cervical cancer (Ca Ski and HeLa S3) [19], [20]. There have been no reports of *C. aeruni*osa being tested for BRAF V600E inhibitory activity as an anti-melanoma cancer in silico so that it is worthy of being processed.

2. MATERIALS AND METHODS

2.1. Chemical

Ethanol, methanol, n-hexane, and ethyl acetate as solvents from Smart Lab, Indonesia. All reagents used for the research were of analytical grade.

2.2. Plant Collection

C. aeruginosa Roxb dried rhizome from the Center for Research and Development of Traditional Medicinal Plants and Medicines Tawangmangu, Central Java, harvested in February 2020.

2.3. Instrumentation

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm.

2.4. Software and Hardware

The Protein Databank (PDB, www.rcsb.org) provided PDB ID: 3OG7 for download [4], [21]. The natural chemical's 3D structure files were obtained from PubChem (www.pubchem.ncbi.nlm.nih.gov). Ligands made with chemdraw 3D 15.0 for molecular docking. AutoDock Tool 1.5.6 Sep_17_14 employed the molecular docking procedure for in-silico screening, and Biovia Discovery Studio V21.1.0.2.20298 was used to view the results. Using a Lenovo laptop running Windows 10 with a Core i3 CPU, 4 GB of RAM, 64-bit operating system, and an x-64 processor, pharmacokinetics and toxicity prediction are performed. The online SMILES Translator (https://cactus.nci.nih.gov) was used to translate the compound into SMILES format. To forecast pharmacokinetics and chemical toxicity, the SMILES-formatted molecule was processed using the pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) [22].

2.5. Extraction and Fractination

One kg of the powdered material was macerated for three days at a ratio of 1:5 using 70% ethanol. Ethanol extract (EE) was obtained by combining the filtrates and drying them out using a revolving vacuum evaporator set at 60 °C and 100 rpm. Then, using solvents ranging from non-polar (n-hexane), semi-polar (ethyl acetate), and polar (ethanol), the ethanol extract (EE) was separated by sequential fractination to provide n-hexane (FH), ethyl acetate (EAF), and ethanol (EF). By turning the vacuum evaporator at 60 °C and 100 rpm, respectively, fractions were concentrated [23], [24].

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GCMS analysis was carried out in GCMS (Shimadzu QP 2010 SE) and mass spectrophotometer. The columns used are Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) and Carbowax (Polyethylene glycol), thickness 0.25um, length: 30.0m, inside diameter: 0.25mm. The mobile phase used is helium and was adjusted to a column velocity flow of 0.74 mL/min. Other GC-

MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 42,3 kPa; and 1 µl injector in split mode with a split ratio of 153.0 with injection temperature of 300 °C. The temperature was raised to 320 °C at the rate of 10 °C/min and held for 5 min. The total elution was 24 min.

2.7. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Protein and ligand preparation is the initial stage of molecular docking. AutoDock Tools-1.5.6 was used to carry out 3D interaction, docking, and binding investigations. The Protein Data Bank provided the target proteins for download (PDB ID: 3OG7) [4], [21]. The Biovia Discovery Studio visualizer program is used to extract native ligands and water molecules from 3D structures to create protein files (.pdb). Chemdraw 3D 15.0 was used to produce the test ligand file (.sdf), which was retrieved from PubChem, for molecular docking. The ligand contributes charge and torsion after the receptor adds charge prior to molecular docking. The grid box's dimensions and coordinates were established. X: 2.643, Y: -2.28, Z: -19.403, and spacing are the grid box coordinates, and the grid box size is 44 × 40 × 40Å, spacing 0.375. Molecular docking parameters include interacting amino acid residues and binding affinity (kcal/mol). Interaction 2D and 3D between ligand and protein were visualized using Biovia Discover Studio visualizer. The following chemical properties were predicted and explained: polar surface activity (PSA), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), the number of atom-to-atom bonds that can rotate (Torson), the logarithm of the coefficient octanol/water partition (Log P), and molecular weight (MW). These were conducted utilizing Lipinski's rule of five, a set of guidelines that aids in distinguishing between molecules that resemble drugs and those that do not, using the pkCMS web tool application [5], [21], [25]. This approach might forecast the higher probability of success or failure because of drug penetration and absorption. Following the 3D drawing of the chemical structure using Chemdraw 3D 15.0 and its saving in a particular format (.pdb), the online SMILES Translator (https://cactus.nci.nih.gov) was used to convert it to SMILES format. The pkCMS online tool (https://biosig.lab.uq.edu.au/pkcsm) was used to process the SMILES formatted compound in order to forecast chemical toxicity and pharmacokinetics [22].

3. RESULTS AND DISCUSSION

3.1. Fraction Compounds

According to the GC-MS data, HF was primarily composed of sesquiterpenes (63.1%) and diterpenes (5.26%), with 31.58% of it being unknown. substances. The EF was made up of sesquiterpenes (68.42%) and others (31.58%), whereas the EAF was made up of sesquiterpenes (42.86%), diterpenes (21.43%), steroids (7.14%), and others (28.57%). Saturated fatty acids were present when IF was identified. Curcumenol and epicurzerenone were the primary constituents of HF and EF, whereas curcumenol and 2,4-Dispironorbornylcyclobuta-1,3-dione (ketene dimers) were the primary constituents of EAF. All compounds detected from the *C. aeruginosa* fraction are shown in Figure 1.

3.2. Molecular Docking Studies, Pharmacokinetics, and Toxicity Prediction of Chemical Constituents

Redocking, or confirming the docking technique between the receptor and the native ligand, is the first step in molecular docking. RMSD 1.03A is the outcome of the validation procedure. Since the RMSD value is ≤ 2A the docking procedure can be deemed acceptable, and the RMSD obtained satisfies the validation acceptance criterion [22]. To show the stance before and after docking, native ligands are shown in two distinct colors. Native ligan in the redocking process with the BRAF V600E resistor is shown in Figure 2. Vemuravenib, a commercial medication, was utilized as a control ligand against the BRAF V600E receptor (PDB ID: 3OG7), while all substances discovered by GC-MS (Figure 1) were used as test ligands. ligand preparation is the initial stage of molecular docking.

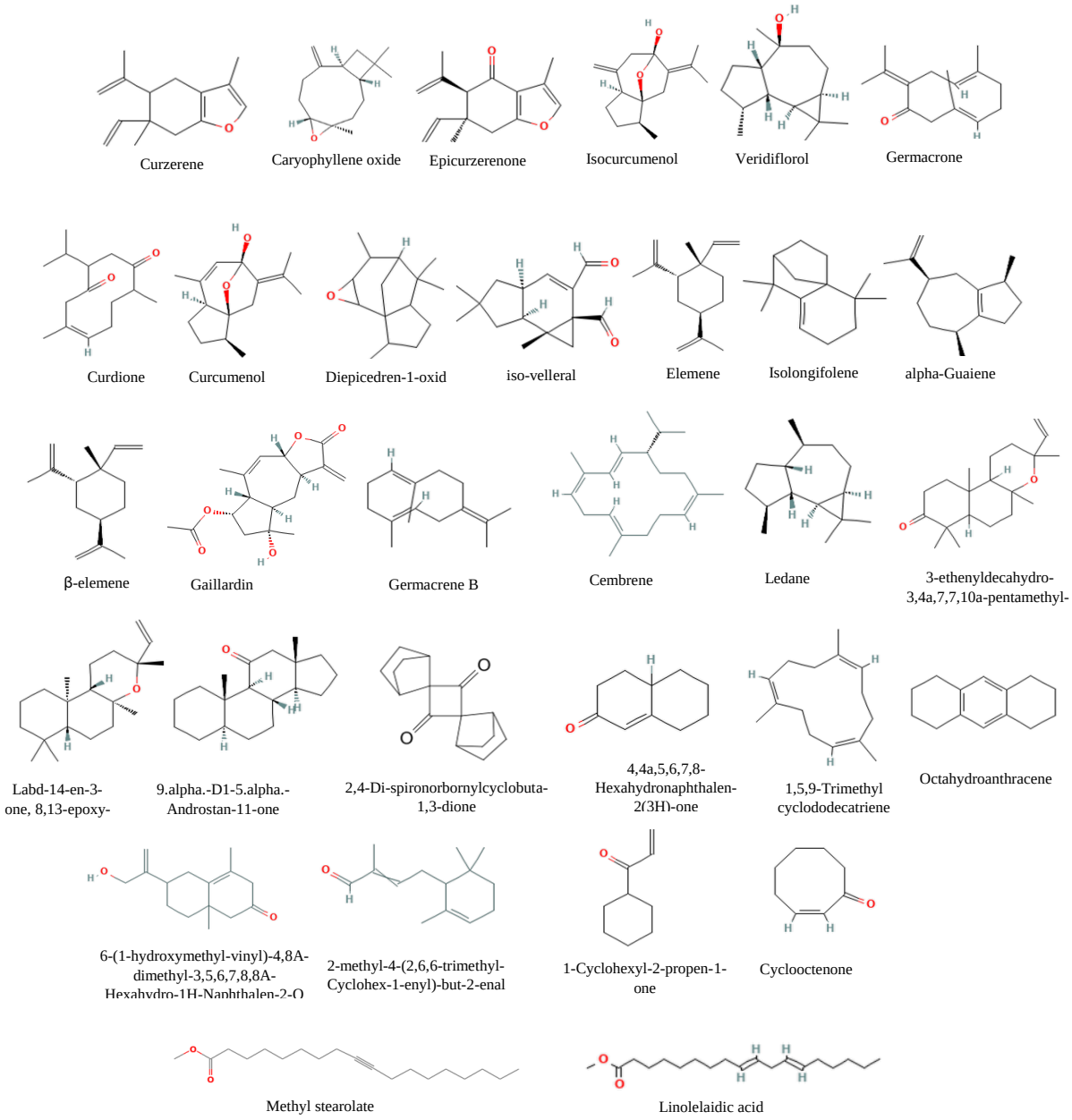

Figure 1. Compound Name and Structure Identification of *C. aeruginosa* Fractions Using GC-MS

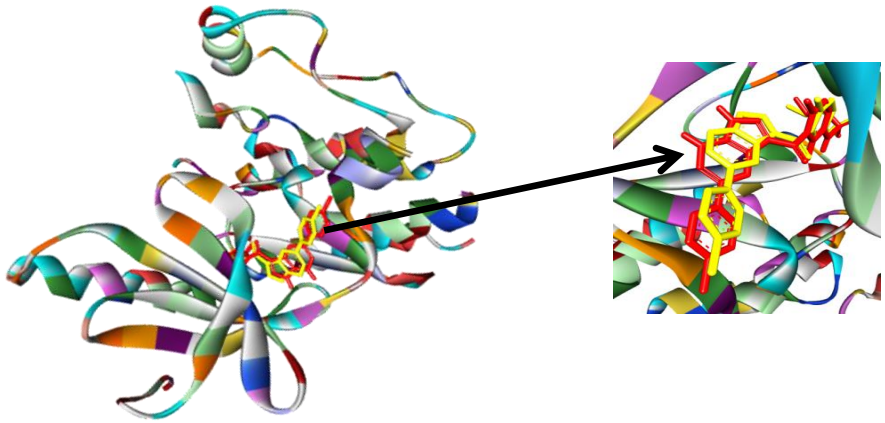

Figure 2. 3D diagram for the interaction of native ligand with BRAF-V600E receptor (yellow =before and red = after redocking)

The smallest binding affinity are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). The smaller binding affinity value, the affinity between the receptor and ligand was higher and the vice versa, the greater the binding affinity value, the affinity between receptors and the ligands is getting lower [5], [22]. All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594 [22]. The amino acid residues that were shown to interact with ligands displays in Table 1 and illustrates the interaction between ligands and the BRAF-V600E receptor using 2D visualization displays in Figure 2.

Table 1. Molecular interactions present in the selected complex ligands and BRAF V600E receptor and the amino acids involved.

Complex	Binding Energy (kcal/mol)	Inhibition Constant/Ki (µM)	Amino acid residues
1-Cyclohexyl-2-propen-1-one	-6.68	12.63	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one	-6.89	8.96	Arg 575, Asp 576, Leu 577, Lys 578, Ser 616, Trp 619, Met 620, Ala 621, Val 626, Tyr 633, Ser 637, Asp 638, Ala 641
Cyclooctenone	-6.23	27.21	Arg 575, Asp 576, Leu 577, Trp 619, Met 620, Ala 621, Val 624, Tyr 633, Ser 637, Asp 638, Ala 641
Vemuravenib	-11.11	7.42	Ile-463, Val-471, Ala- 481, Lys-483, Leu-505, Leu-514, Phe-516, Ile-527, Thr-529, **Gln-530**, Trp-531, **Cys-532**, Ser-535, Ser-536, His-539, Phe-583, **Asp-594**, Asp-593, Phe-595, Gly-596

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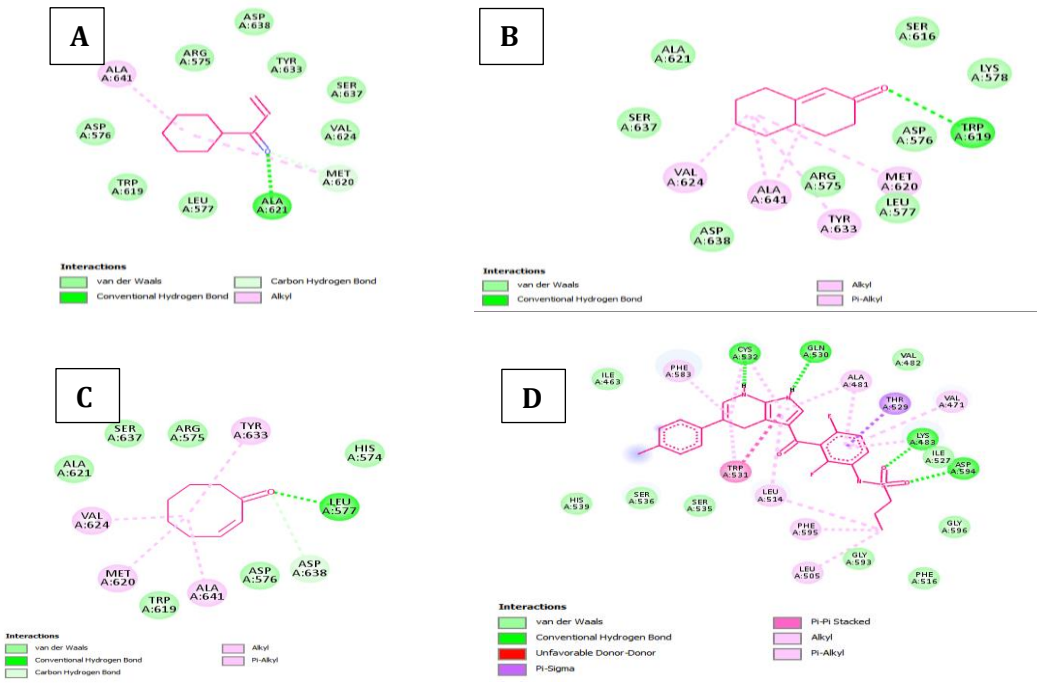

Figure 3. 2D Visualization of Complex Interactions Between Ligands 1-Cyclohexyl-2-propen-1-one **(A)**, 4,4a,5,6,7,8-Hexahydrona phthalen-2(3H)-one **(B)**, Cyclooctenone **(C)**, and Vemuravenib **(D)** with BRAF V600E receptor

The likelihood that a molecule has the same or superior activity than BRAF V600E increases with the number of amino acid similarities between the reference chemical and crucial amino acid. GLN A:530, CYS A:532, ASP A:594, and THR A:529 are important amino acids linked to the BRAF V600E receptor. The reticuline compound has the same hydrogen-bonded amino acids (GLN 530 and ASP 549) as the reference compound Vemurafenib/key amino acids [22]. Conversely, compounds that had better binding scores than vemurafe-nib and a decent MolDock score (≥− 158.139) and Rerank score (≥− 118.607) were recognized as possible hits [5].

Out of all the compounds, the three identified compounds found by GC-MS were found to have the smallest binding affinities. The pkCMS online tool was used to further investigate these compound's pharmacokinetic and toxicity characteristics (ADMET). The Lipinski test uses passive diffusion to ascertain whether a substance in cell membranes is hydrophobic or hydrophilic. According to Lipinski's guidelines, a ligand must have a molecular weight of less than 500 Da and a LogP value of less than 5. molar refractivity between 40 and 130, donor hydrogen bonds < 5, and acceptor hydrogen bonds < 10. Cell membranes are more readily penetrated by ligands with molecular weights less than 500 Da than by those with molecular weights greater than 500 Da. The polarity of the ligand in fat, oil, and non-polar solvents is correlated with the logP value. Ligands that are widely dispersed throughout the body and have a log P value greater than 5 will interact more readily via the lipid bilayer layer of cell membranes. As a result, the ligand becomes more hazardous and its sensitivity to binding to the target molecule decreases. Because they are more broadly distributed throughout the body and are kept in lipid membranes for longer, excessively hydrophobic compounds typically have a high level of toxicity. The ligand is hydrophobic and has a tendency to dissolve in water when the log P value is less. Since the ligand cannot cross the lipid bilayer membrane, its Log P value cannot be negative. The biological activity of a ligand or medicine is correlated with the amount of hydrogen bonds in the donor and acceptor. The amount of energy needed for absorption increases with the strength of the hydrogen bond [25]. Table 2 displays the outcomes of the molecular docking studies, which showed that the three compounds satisfied Lipinski's guidelines.

Table 2. Ligand's Lipinski Rules of Five

Complex	Molecular Weight	Log P	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	Polar surface activity (PSA)
1-Cyclohexyl-2-propen-1-one	138.21	2.3218	0	1	62.125
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	150.221	2.4659	0	1	67.484
Cyclooctenone	124.183	2.0758	0	1	55.760

When evaluating the pharmacokinetics of novel pharmacological compounds, ADMET estimates are essential. If a compound's anticipated value is more than 0.09, it has significant Caco-2 permeability. Human colorectal cancer epithelial cells are known as Caco-2 cells. To estimate oral drug absorption, Caco-2 cell monolayers are frequently employed as an in vitro model of the human intestinal mucosa. The volume needed for a drug to be uniformly distributed and produce the same concentration as in blood plasma is known as the Steady State Volume of Distribution (VDss). Excretion in log (ml/min/kg) is predicted by total clearance (CLtot). Hepatic clearance (liver metabolism and biliary clearance) and renal clearance (renal excretion) are the two primary parts of drug clearance. AMES toxicity is frequently used to evaluate a compound's capacity to cause mutagenesis using bacteria. Positive findings suggest that the substance is mutagenic and carcinogenic [22]. If a compound's predictive value is greater than 0.90, it is deemed to have high Caco-2 permeability [26]. It

has good permeability because the test results showed a value greater than 0.90. When the volume of distribution (VDss) is less than 0.71 L/kg (log VDss<-0.15), it is deemed low; when it is greater than 2.81 L/kg (log VDss>0.45), it is deemed excessive [27]. In Table 3, all compounds are in the range volume distribution requirements so that it can be predicted that all these compounds can be distributed evenly to provide the same concentration as in blood plasma. Based on Table 3, all ligands have good pharmacokinetic parameter.

Table 3. Pharmacokinetics (ADMET) Parameters of Ligands

Complex	Absorption Caco2 permeability	DistributionVDss (human)	Metabolism (CYP2D6 substrate)	Excretion (Total Clearance)	AMES toxicity	Hepato toxicity
1-Cyclohexyl-2-propen-1-one	1.085	0.148	No	0.221	No	No
4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one	1.501	0.344	No	0.112	No	No
Cyclooctenone	1.487	0.136	No	0.213	No	No

4. CONCLUSION

The best potential components exploration of *C. aeruginosa* fraction with the smallest binding affinity and meet the pharmacokinetic requirements are 4,4a,5,6,7,8-Hexahydronaphthalen-2(3H)-one (-6,89 kcal/mol); 1-Cyclohexyl-2-propen-1-one (-6,68kcal/mol); Cyclooctenone (-6,23kcal/mol); and vemuravnib is still better as K+ (-10.593,68kal/mol). All three compounds do not bind to key amino acid residues of BRAF V600E such as vemuravenib at GLN A:530, CYS A:532; ASP A:594. All three compounds have good pharmacokinetic parameter. These results indicate that further structural development is needed for better activity.

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Conflicts of interest: The authors declare no conflict of interest

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