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Article

Virtual Screening and Molecular Modelling Anticancer Molecules Targeting Fibroblast Growth Factor Receptor 4

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Abstract. Cancer, characterized by uncontrolled cell proliferation, is a leading global cause of mortality. Targeting the fibroblast growth factor receptor (FGFR), a receptor tyrosine kinase (RTK), holds promise for anticancer drug development. FGFR4, a specific subtype, regulates various cellular processes, making it a valuable target. In-silico methods were employed to screen 20 compounds against FGFR4 (PDB ID 5JKG) using AutoDock Version 4.2.6. The top three potential inhibitors, based on Gibbs energy (ΔG) and inhibition constant (Ki), were identified: epigallocatechin3-O-pcoumarate (ΔG = -10.46 kcal/mol; Ki = 21.37 nM), 6_deoxoteasterone (ΔG = -10.22 kcal/mol; Ki = 32.35 nM), and epigallocatechin3-O-caffeate (ΔG = -9.78 kcal/mol; Ki = 68.16 nM). ADMETOX analysis confirmed compliance with Lipinski's rules, indicating their safety. These compounds show promise as FGFR4 inhibitors, potentially as standalone therapy or in combination with other anticancer drugs.

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1. Introduction

Cancer, characterized by unregulated cell proliferation, is a global health challenge with a devastating impact on life expectancy worldwide [1-3].The World Health Organization (WHO) estimates that cancer is a leading cause of premature mortality in numerous countries, highlighting its global significance [3-4].

Traditionally, cancers have been classified based on their tissue or organ of origin. However, recent advancements focus on the molecular properties of cancer cells, offering opportunities for more precise diagnostics and safer, more effective treatments [5]. Among these advancements, the fibroblast

growth factor receptor (FGFR), a subtype of receptor tyrosine kinase (RTK), has emerged as a promising target for anticancer therapies. FGFR encompasses four highly homologous subtypes: FGFR1, FGFR2, FGFR3, and FGFR4 [6-7].

FGFR4, a tyrosine kinase receptor for fibroblast growth factors (FGFs), plays a pivotal role in regulating diverse cellular processes, including cell proliferation, differentiation, migration, metabolism, and bile acid production [8]. Dysregulated FGFR4 activation, often associated with amplification of its ligand FGF19, has been implicated in various solid tumors and hematologic malignancies, promoting cancer initiation and progression [9]. Genetic alterations leading to abnormal FGFR activation are linked to several cancer types, including breast, liver, lung, gastric, uterine, bladder, and rhabdomyosarcomas [10].

In the pursuit of innovative anticancer therapies, chemometrics and chemoinformatics have emerged as invaluable tools. Molecular docking, a prominent technique, predicts the binding modes of ligands to target proteins, offering insights into potential drug interactions. However, molecular dynamics (MD) simulations are often used to refine these predictions, providing a deeper understanding of binding kinetics and ligand selectivity [11-12].

This study leverages molecular docking and MD simulations to explore the anticancer potential of compounds from the Indonesian herbal database as potential FGFR4 inhibitors. Parameters such as docking scores, binding energies, root mean square displacements (RMSD), and root mean square fluctuations (RMSF) were computed to assess ligand performance [13-14]. Additionally, ADMETOX predictions were employed to evaluate the compounds' toxicity profiles. The primary goal of this research is to identify alternative agents that can effectively inhibit FGFR4 for potential anticancer applications [15-16].

2. Experimental Section

This research has been completed using a workflow to discover anticancer compounds that target FGFR4. This process consists of target protein selection, compound screening, protein and ligand preparation, molecular docking and molecular dynamics and ADMETOX analysis. An overview of the research flow can be seen in Figure 1.

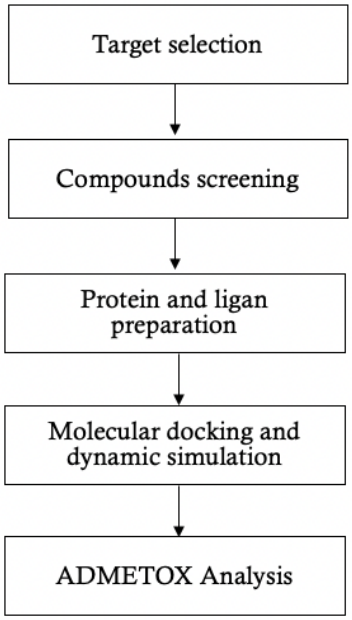


Figure 1. Research pipeline for the search for anticancer compounds that target FGFR4

2.1 Protein Selection and Preparation

The selection of the protein for this study, the crystal structure of FGFR4 with its native ligand (PDB ID 5JKG), was made based on several considerations. First, the choice of PDB ID 5JKG was due to its origin from Homo sapiens, ensuring relevance to human biology. Additionally, the resolution of this crystal structure met the necessary criteria for accurate molecular docking studies [17].

To prepare the protein for molecular docking simulations, the AutoDock 4.2.6 program was employed. The initial step involved isolating the protein from its original ligand, and subsequently, all water molecules were removed from the protein file to ensure a clean molecular environment. The resulting protein structure was then saved in pdb format. Gasteiger charges were assigned to the protein, introducing polar hydrogen atoms while removing non-polar ones to enhance accuracy [18]. Finally, three different docking grid sizes—40x40x40, 50x50x50, and 60x60x60—were utilized to specify potential binding pockets on a grid map for comprehensive analysis.

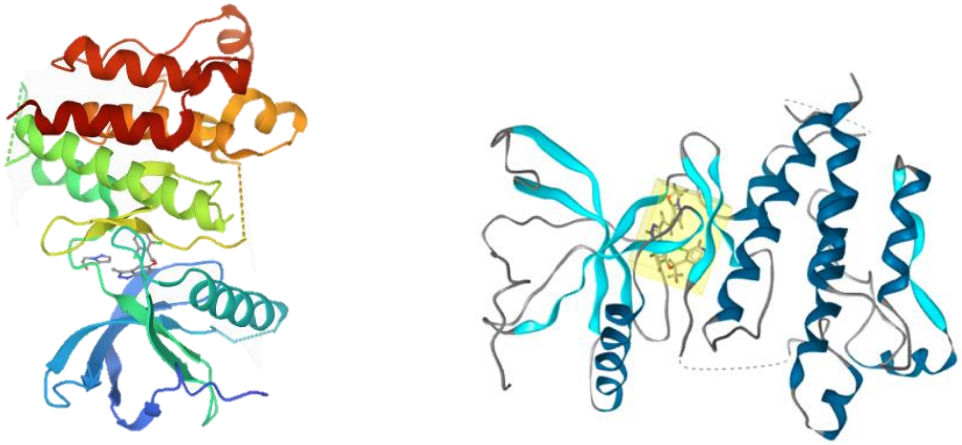


Figure 2. 3D Structure of FGFR4 kinase domain in complex with LY2874455(PDB ID: 5JKG)

2.2 Selection of Hit Compounds by Virtual Screening

The selection of hit compounds was started by determining the original ligand pharmacophore (Figure 2) using the LigandScout 4.4.5 software. Virtual screening was carried out on the herbal database (HerbaldB) with the parameter scoring function being pharmacophore fit and maximum turnover feature was 4 out of 11 pharmacophores. Virtual Screening resulted in 96 hit compounds based on the highest pharmacophore value and the next analysed hit compounds were 20 Hit compounds.

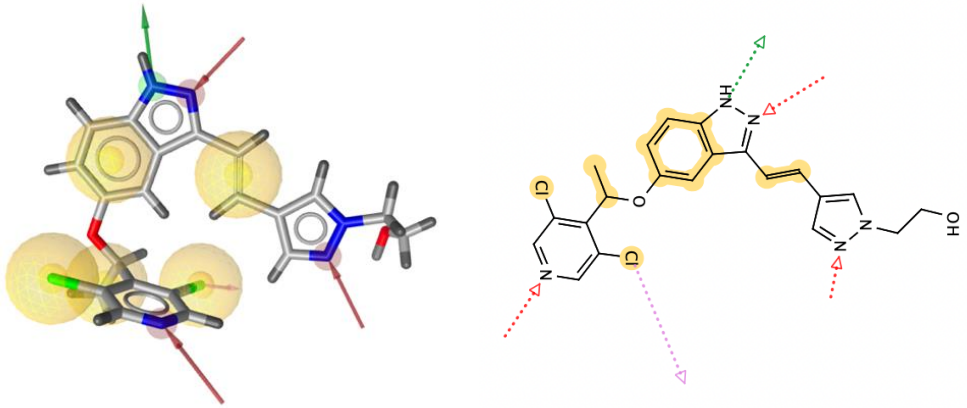


Figure 3. Native Ligand Pharmacophores. Red arrow: HBA; green arrow : HBD; circle ; yellow: hydrophobic interaction

2.3 Ligand Preparation

A total of 20 hit compounds (ligands) were determined and the 3D structure searched for on the PubChem website https://pubchem.ncbi.nlm.nih.gov/ and saved in sdf format then 3D cleaning was performed and saved in pdb format. Ligands were prepared using AutoDock 4.2.6 software with files saved in pdbqt format.

2.4 Molecular Docking, Molecular Dynamics and Interaction Studies

In the molecular docking phase, we designed ligands using the Lamarckian genetic algorithm within AutoDock 4.2.6 software, employing default parameters. Default parameters are widely accepted in the field for their reliability and comparability with prior research. The docking process involved ligand conversion, AutoGrid4, and Autodock4 execution via the command prompt. Subsequently, AutoDock 4.2.6 software analyzed results, revealing ligand conformation, affinity values, and interactions with FGFR4.

To visualize protein-ligand interactions, we used LigPlus software, facilitating a 3D view of binding interactions. For molecular dynamics, we utilized the LARMD webserver (http://chemyang.ccnu.edu.cn/ccb/server/LARMD/index.php/home/index) to observe ligand-protein behavior over time, providing insights into complex stability and dynamics.

2.5 ADMETOX Analysis

This analysis is to determine drug likeness, pharmacokinetics, and toxicity of potential compounds of FGFR4 inhibitors. Drug likeness and pharmacokinetics were analyzed using the SwissADME webserver (http://www.swissadme.ch/) while the toxicity analysis used the ProTox-II webserver (http://tox.charite.de/protox_II.).

3. Result and Discussion

3.1. Molecular docking

High FGFR4 activation is tightly linked to the amplification of its particular ligand FGF19, where it works as an oncogene promoting cancer formation and progression [19-20]. When compared to FGFR1-3, the majority of FGFR inhibitors have much lower efficacy against FGFR4 [21-22]. So the search for FGFR4 protein inhibitor is needed to prevent the expression of the protein so that it can be controlled cancer progression. The structure of the FGFR4 protein in this study was taken from the Protein Data Bank (PDB) website with PDB ID 5JKG with a structure resolution of 2.35 Å. Other information regarding this protein is that it has a identical side chains (A), sequence length 311, derived from humans (Homo sapiens), and has native ligand 2-[4-[E-2-[5-[(1R)-1-[3,5-bis(chloranyl)pyridin-4-yl]ethoxy]-1H-indazol-3-yl]ethenyl]pyrazol-1-yl]ethanol denoted by the LY2874455 ligand (Figure 2).

Table. 1 Grid Docking Validation Results

	40x40x40	50x50x50	60x60x60
Grid Center x = -40.313 y = -16. 794 z = 377.737	-8.82	-8.60	-8.59
RMSD	3,33A	1,169 A	2,52 A
Inhibition Constant	342.63 nM	496,26 nM	502.93 nM

The binding pocket on the FGFR4 protein was previously determined for its active site using a grid map approach that covers all active sites. From the three docking grids analysed, the appropriate docking grid was obtained, namely at 50x50x50 with the lowest bond energy, namely -11.59 Kcal/mol, RMSD 3.24 Å with an inhibition constant of 496,26 nM. Table 1 shows the complete validation results of the grid docking. The grid box selection was based on consideration of the low bond energy value, RMSD 2, and the relatively low inhibition constant. In this study, the best RMSD value of the three grid boxes was 1.169. It is on this basis that the 50x50x50 grid was selected for further analysis in docking molecular studies.

Table 2. Molecular Docking Interaction of Hit Compound with protein FGFR4 Result

No	Ligand	Binding Energy (kcal/mol)	Ki (nM)/uM
1	Native Ligan	-8.54	546.05 nM
2	Epigallocatechin 3-O-P-coumarate	-10.46	21.37 nM
3	6_Deoxoteasterone	-10.22	32.35 nM
4	Epigallocatechin3-O-caffeate	-9.78	68.16 nM
5	Agnuside	-8.61	485.48 nM
6	Fragransol C	-8.56	528.57 nM
7	Fisetin4'-glucoside	-8.46	634.12 nM
8	FragransolD	-8.20	981.01 nM
9	Andrograpanin	-8.15	1.06 uM
10	Cartamone	-7.81	1.87 uM
11	Aureusidin	-7.75	2.09 uM
12	Pteryxin	-7.51	3.11 uM
13	Khusol	-7.25	4.88 uM
14	Isovalencenol	-6.99	7.47 uM
15	Angolensin	-6.97	7.74 uM
16	Kaempferol3	-6.92	8.47 uM
17	Squalene	-6.83	9.91 uM
18	PaniculidineB	-6.10	33.93 uM
19	Nerolidol	-5.97	41.83 uM
20	Dodecatrien	-4.96	229.51 uM
21	2-Methyl-6-methylene-2-7-octadien-1-ol	-4.85	278.93 uM

Based on the results of the docking simulation above (Table 2), of the 20 compounds that have the potential as FGFR4 inhibitors, the three best compounds with the lowest bond energy values were selected, namely Epigallocatechin 3-OP-coumarate, 6_Deoxoteasterone, and Epigallocatechin3-O-caffeate with binding energy values. -10.46, - 10.22 and -9.78 kcal/mol and the inhibition constants (Ki) were 21.37, 32.35 and 68.16 nM, respectively. The three best compounds have bond energies that are greater than the bond energies of the original ligands. This is possible because the topology of the structure of Epigallocatechin 3-O-P-coumarate, 6_Deoxoteasterone, and Epigallocatechin3-O-caffeate compounds has a stronger bond to the binding site of the FGFR4 receptor than the original ligand.

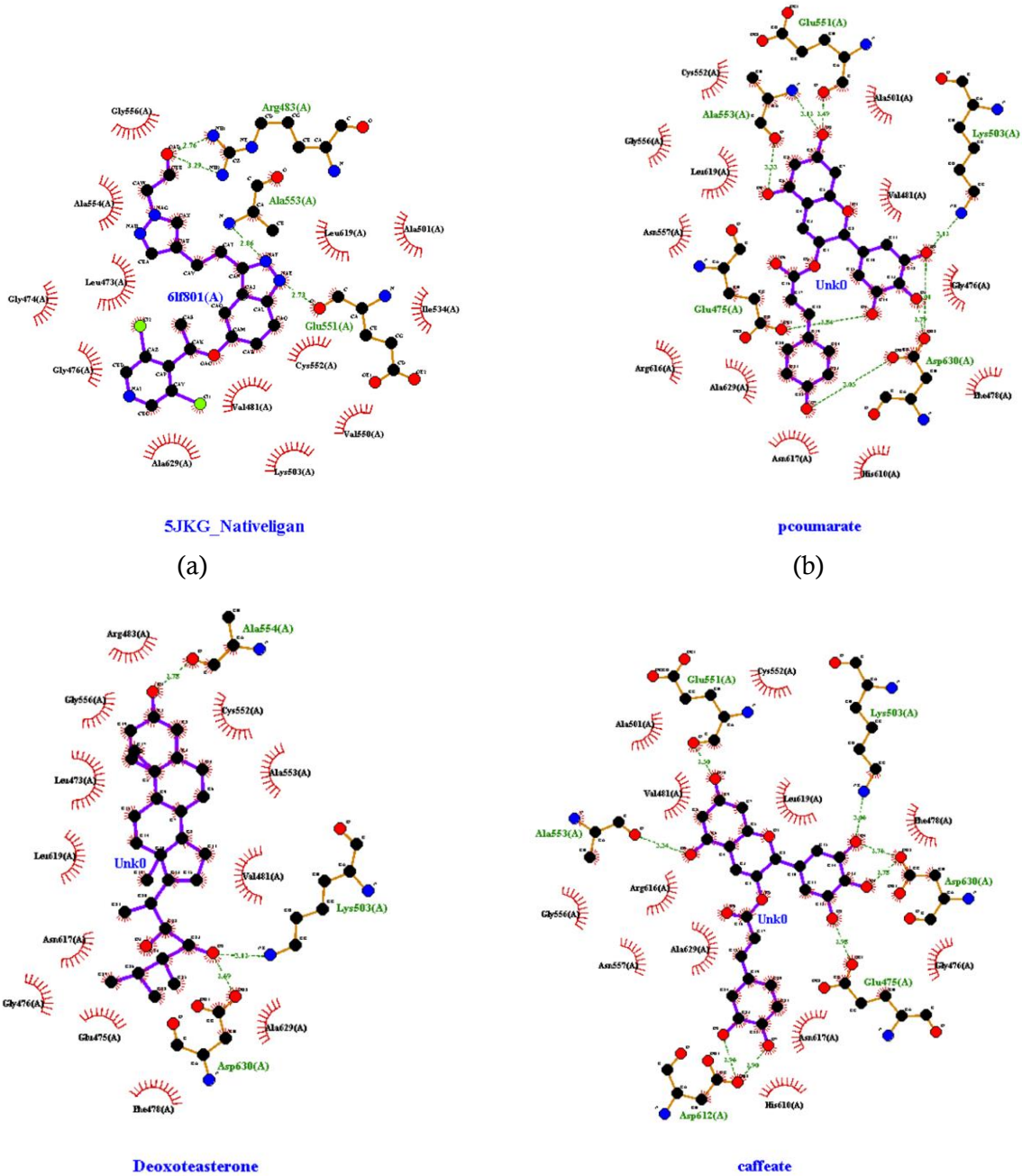


Figure 5. Interaction between FGFR4 protein receptor with the best compounds. (a) native ligand (b) Epigallocatechin3_O_pcoumarate (c) 6_Deoxoteasterone (d) Epigallocatechin3_O_caffeate

Figure 4 shows the best conformation of the compound (Epigallocatechin 3-O-P-coumarate) in the FGFR4 cavity.

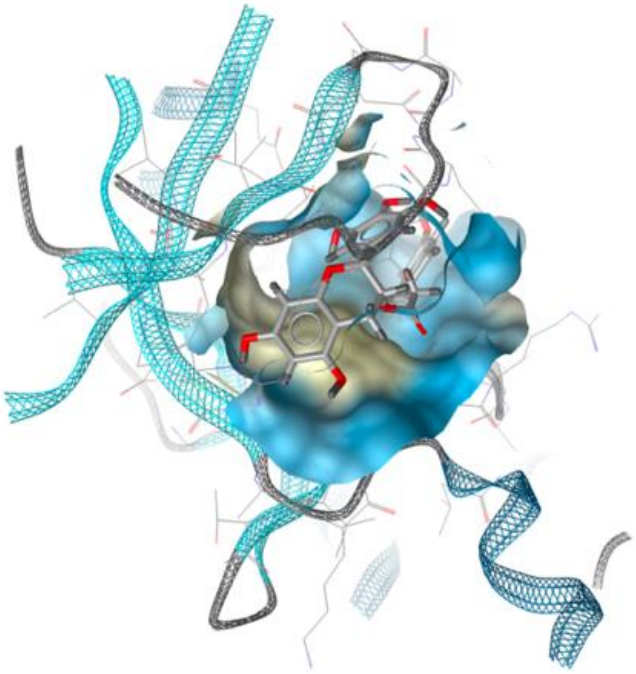


Figure 4. Interaction Epigallocatechin3_O_pcoumarate with FGFR4

The inhibition constant was utilized to calculate the binding energy in the docking conformation, whereas the binding energy revealed a stable connection between the ligand and the FGFR4 residue [23-24]. In this situation, the bond energy is the free energy (G), which is proportional to the degree of spontaneity of the reaction. If G<0 or a negative value, the reaction will move spontaneously toward the product, indicating that the ligand-protein interaction will be more consistent, and the protein complex created will be more stable. The bond energy (G) and inhibition constant (Ki) produced by the docking simulation are bolstered by additional data, such as the orientation and position of the ligand that functions as a protein inhibitor through the docking simulation [25-26].

The structure of the three best compounds is comparable to that of the original ligand, which impacts the interaction between the amino acid residues of the protein FGFR4 and these compounds. Table 3 shows an overview of the interactions of compounds with amino acid residues. According to Table 3, at least 8 of the 16 kinds of amino acids that interact with these molecules are the same as the original ligand, Epigallocatechin 3-OP-coumarate, 6_Deoxoteasterone, and Epigallocatechin3-O-caffeate. Gly 556, Gly 476, Ala 629, Ala 553, Leu 619, Cys 552, Val 481, and Lys 503 were the amino acid residues. Hydrogen bond interactions were found in the majority of amino acid residues. Furthermore, the top three compounds have more hydrogen bonds than the original ligands. As a result, the top three compound linkages may have a stronger connection to the FGFR4 protein's active site than the native ligand.

Table 3. Molecule Interaction with Amino Acid Residue

Amino Acid	Native Ligand	Epigallocatechin3_O_pcoumarate	6_Deoxoteasteroe	Epigallocatechin3_O_caffeate
Gly 556	v	v	v	v
Ala 554	v	x	V	x
Gly 474	v	x	x	x
Leu 473	v	x	v	x
Gly 476	v	v	v	v
Ala 629	v	v	v	v
Arg 484	v	x	x	x
Ala 553	v	v	v	v
Leu 619	v	v	v	v
Ala 501	v	v	x	v
Glu 551	v	v	x	v
Ile 534	v	x	x	x
Cys 552	v	v	v	v
Val 550	v	x	x	x
Cys 552	v	x	x	x
Val 550	v	x	x	x
Val 481	v	v	v	v
Lys 503	v	v	v	V
Asn 557	x	v	x	v
Arg 616	x	v	x	v
Asn 617	x	v	v	v
His 610	x	v	x	v
Phe 478	x	v	v	v
Glu 475	x	v	v	v
Asp 630	x	v	v	v
Asp 612	x	x	x	v
Asp 483	x	x	v	x

Lipinski's five criteria predict the resemblance of medication compounds (drug likeness) [27].These criteria define molecular features that are significant for drug pharmacokinetics in the body. They pertain to drug orally similarity and offer information regarding ligand-drug similarity. A good medicinal molecule has a molecular weight of less than 500 g/mol, a hydrogen bond acceptor (HBA) of less than 10, a hydrogen donor bond (HBD) of less than 5, and a log P value of less than 59, according to Lipinski's guidelines. If the log P value is more than one, it shows lipophilicity, which is connected to a medicinal compound's capacity to enter biological membranes.

Table 4. ADMETOX analysis prediction

Ligans	Druglikeness				Pharmacokinetics					Toxicities		
					Inhibitor CYP							
	MW (g/mol)	HBA	HBD	Log P	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	AMES	Carcinogenesis	AOT
Epigallocatechin3-O Pcoumarate	452.41	9	6	2.11	No	No	No	No	No	-	-	IV
6_Deoxoteasterone	434.69	3	3	5.08	No	No	No	No	No	-	-	IV
Epigallocatechin3-O-caffeate	468.41	10	7	1.71	No	No	No	No	No	-	-	IV

To create potential therapeutic molecules, pharmacokinetic study of these compounds against cytochrome P450 (CYP) protein inhibitors is also required. This protein is a metabolic enzyme in the liver that serves as a biomarker for determining the response impact of an anticancer medication. The human CYP enzymes CYP 1A2, CYP 2C family, CYP 2D6, and CYP 3A4 are key in drug metabolism, thus this study looked at whether these chemicals that may be used as pharmaceuticals had an inhibiting impact on these enzymes.

The enzyme was not inhibited by any of the three substances tested. Furthermore, the toxicity of a medication molecule is investigated to determine if it is harmful or not, ensuring that it is safe to consume in the future. According to Table 4, the three therapeutic compounds selected were not harmful in terms of AMES, carcinogenesis, and AOT (acute toxicity) characteristics. The three selected substances fall into category 4 based on the findings of the AOT value study. Acute toxicity values vary from 1-6, with the greater the degree of toxicity, the safer (non-toxic). The globally harmonized chemical labeling categorization system (GHS) is used to establish toxicity class. The global harmonized chemical labeling categorization system (GHS) is used to define the toxicity class. The LD50 value is expressed in [mg/kg]. If consumed, this substance is classified as Class IV (300 LD50 2000) [28-29].

On a one-ns time scale, the molecular dynamic simulation findings of the three drugs and chosen receptors (FGFR4) were examined. Understanding the dynamic behavior and stability of molecules is the goal of molecular dynamic simulation. The stability and dynamic behavior of all three molecules are excellent [30]. The next step is to figure out why the amino acid residues in the FGFR4 complex and the ligand reported in the RMSF plot analysis fluctuate so much. The RMSD and RMSF plot of the FGFR4 complex with the three best ligands is shown in Figure 5.

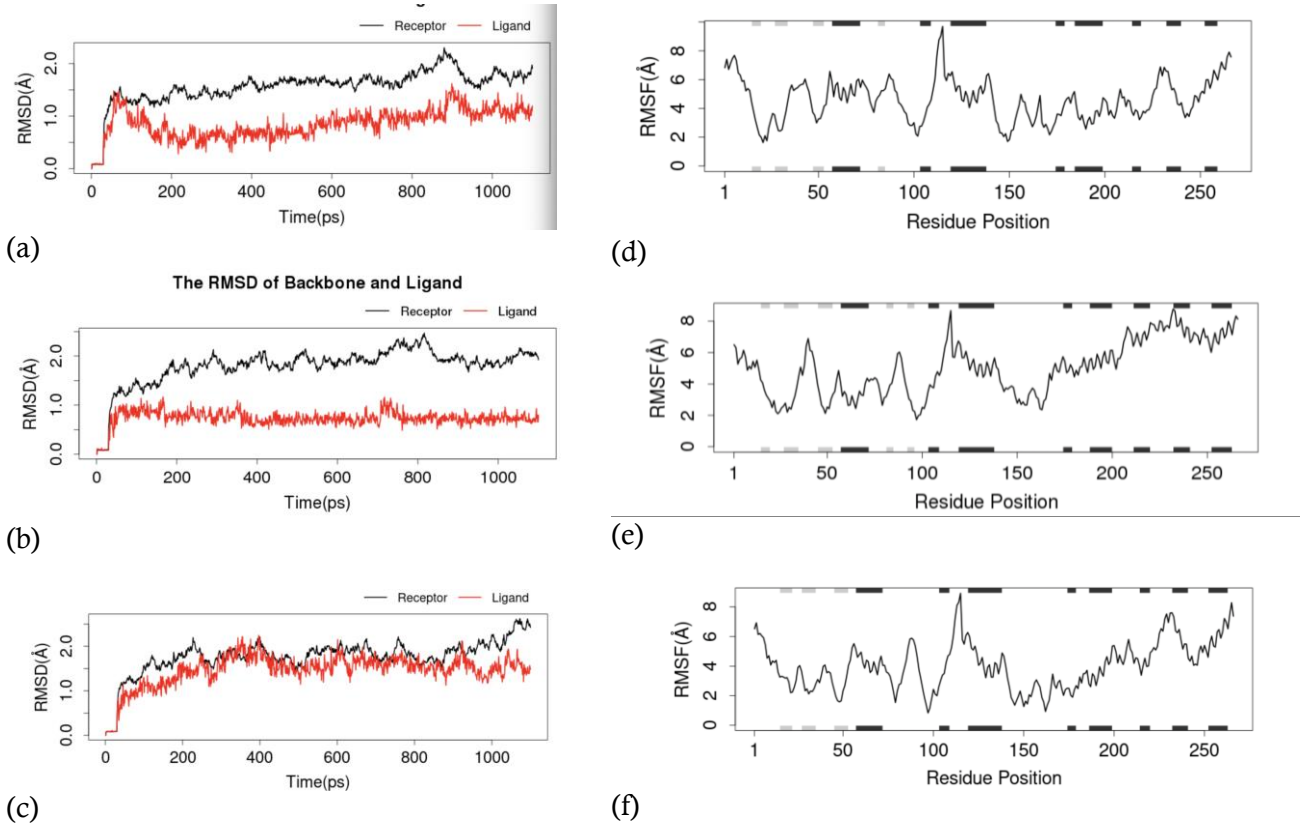
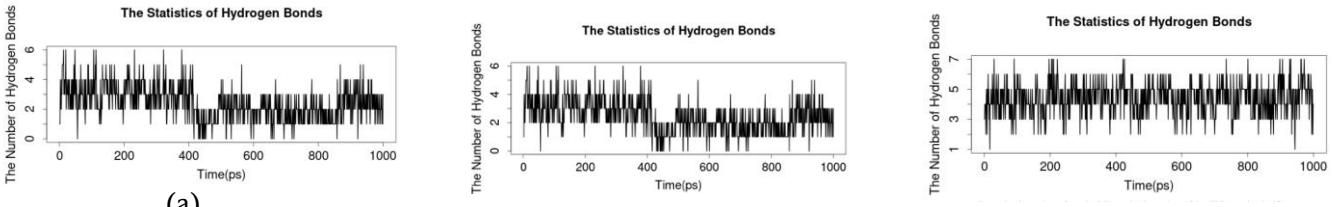


Figure 6. RMSD and RMSF curves (a) RMSD of FGFR4 complex with epigalocathecin 3-O-P-coumarate ligand (b) RMSD of FGFR4 complex with 6_Deoxoteasterone ligand (c) RMSF of epighalocatechin 3-O-caffeate ligand (d) RSMF epigalocathecin 3-O-P-coumarate ligand (e) RSMF of 6_Deoxoteasterone ligand (f) RMSF of epighalocatechin 3-O-caffeate ligand.

The RMSD curves of the FGFR4 complex with the epigalocathecin 3-O-P-coumarate and 6 Deoxoteasterone ligands (Figures 5a and 5b) revealed that the epigalocathecin 3-O-P-coumarate and 6 Deoxoteasterone ligands had a lower RMSD than the FGFR4 protein but remained stable on a 1 ns time. Meanwhile, the RMSD FGFR4 complex with the epighalocatechin 3-O-caffeate ligand (Fig. 5c) had virtually the same RMSD as the protein, with relatively stable levels on a timeframe of about 0.9 ns, albeit there were swings close to 1 ns. The dynamic mobility in the loop area is shown by the RMSD deviation of the FGFR4 complex with the ligand. To calculate the deviation in the RMSF study, each amino acid was counted at each location on a 1 ns time scale.

There are up to 8 variations of the amino acid at positions 100-150. In addition to this location, the additional amino acids, especially 1-6, are irregular. In dynamic situations, the existence of this deviation allows the loss of hydrogen bond interactions at a certain time scale. Figure 6 illustrates the number of hydrogen bonds involved in the stability of the compound. The number of hydrogen bond contacts between epigalocatechin 3-OP-coumarate, 6 Deoxoteasterone, and FGFR4 protein varied from 0 to 6 (Fig. 6a,6b), while the number of hydrogen bond interactions between epighalocatechin 3-O-caffeate and FGFR4 protein ranged from 1-7 (Figure 6c).



(a) (b) (c)

Figure 7. Interaction of hydrogen bonds between FGFR4 protein (a) epigalocathecin 3-O-P-coumarate (b) 6_Deoxoteasterone (c) epighalocatechin 3-O-caffeate

Epigalocathecin 3-O-P-coumarate, 6_Deoxoteasterone and epighalocatechin 3-O-caffeate are active compounds derived from herbal plants. Some of the three compounds have been tested for their biological activities such as antibacterial. Such as epigallocatechin 3-O-p-coumarate which has activity as an inhibitor of Saccharomyces cerevisiae alcohol dehydrogenase and antioxidant activity assessed as DPPH radical scavenging activity [31-32]. The activity of these three compounds as anticancer has not been found. So that further research is needed in the laboratory to prove that the compounds Epigalocathecin 3-O-P-coumarate, 6_Deoxoteasterone and epighalocatechin 3-O-caffeate act as FGFR4 inhibitors and their relationship with cancer pathways.

4. Conclusion

Virtual screening approaches and molecular modeling can be used to find possible compounds as putative inhibitors of the FGFR4 protein. The three best compounds, epigallocatechin3-O-pcoumarate, 6 deoxoteasterone, and epigallocatechin3-O-caffeate, were chosen based on binding energy, inhibition constants, druglikeness analyses, pharmacokinetics, and toxicity, and have topological structures that are comparable to the original ligand. These three compounds are also known to have more inhibitory activity than the original ligands, suggesting that they might be more effective inhibitors. With a time scale of 1 ns, epigallocatechin3-O-pcoumarate, 6 deoxoteasterone, and epigallocatechin 3-O-caffeate show high stability. Furthermore, three substances have the potential to act as FGFR4 protein inhibitors via cancer pathways.

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