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EXPLORATION OF HYDROXYL SUBSTITUTED REGORAFENIB DERIVATIVES AS A TYROSINE KINASE INHIBITORS OF LEUKIMIA THROUGH MOLECULAR DOCKING

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Abstract
The primary cause of leukemia, a diverse group of hematological cancers in humans, is an imbalance in the expression of tyrosine kinases, which promote unchecked cell growth and survival. Despite being established multi-kinase inhibitors, Regorafenib is not widely utilized to treat leukemia. Investigating hydroxyl-substituted derivatives of Regorafenib as potential tyrosine kinase inhibitors was the aim of this study. Seven (7) derivatives (Regorafenib and R1-R7) were subjected to molecular docking in relation to a leukemia-associated tyrosine kinase target. By calculating binding affinities (ΔG) and inhibition constants (Ki), their therapeutic potential was ascertained. The highest binding affinities were found in R1 (-14.03 kcal/mol, 52.31 pM), and many derivatives (R1, and R7) outperformed the others. These results indicate that the derivatives modified with hydroxyl moiety, especially R1, are good prospects to be further experimentally validated as effective counter-tyrosine kinase inhibitors in the treatment of leukemia.

INTRODUCTION

The uncontrolled proliferation and abnormal build-up of leukocytes in the bone marrow and peripheral circulation are the hallmarks of leukaemia, a diverse group of haematological cancers.[1-3] Its rapid growth, intricate molecular foundation, and significant therapeutic obstacles make it a significant worldwide health concern.[4, 5] Despite great progress in the diagnosis and treatment of leukaemia, resistance to targeted drugs and traditional chemotherapy continues to be a major challenge, frequently resulting in poor clinical results and disease recurrence.[6-8] As a result, the creation of molecularly focused treatments has emerged as a crucial tactic for raising therapeutic effectiveness. Because of their crucial function in controlling intracellular signaling networks, protein tyrosine kinases (PTKs) have garnered special interest among the molecular pathways linked to leukemic cell proliferation, survival, differentiation, and migration.[9, 10]

Tyrosine kinases, both receptor and non-receptor, are important signaling proteins that use phosphorylation-dependent methods to control cellular functions.[11] Tyrosine kinases are appealing targets for the development of anticancer drugs because aberrant activation, overexpression, or mutation of these enzymes can interfere with normal signaling and encourage unchecked cellular growth.[12] Several receptor tyrosine kinases implicated in

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tumor growth and angiogenesis are inhibited by Regorafenib, a therapeutically significant multi-kinase inhibitor with a diaryl urea pharmacophore.[13] Regorafenib is a useful structural scaffold for the logical design of novel derivatives with possibly enhanced target interactions and biological features because of its well-established kinase-binding framework.[14] One popular method for optimizing ligand–protein interactions is structural modification of pharmacologically active scaffolds, especially by adding functional groups that can establish extra hydrogen-bonding.[15-17]

In this case, a hydroxyl substitution on the Regorafenib framework might offer a chance to modify the parent molecule's electronic and physicochemical characteristics while adding more hydrogen-bond donor and acceptor interactions with amino acid residues inside the kinase-binding pocket.[18, 19] By predicting ligand-binding orientations, detecting important intermolecular interactions, and measuring relative binding affinities, molecular docking provides a useful computational method for assessing these structural changes.[20-22] Therefore, utilizing molecular docking analysis, the current study investigates hydroxyl-substituted Regorafenib derivatives as possible tyrosine kinase inhibitors against leukemia-associated targets. Binding modalities, interaction profiles, docking scores, and the role of hydroxyl functionalization in ligand–target recognition are all given special consideration.[23, 24] The findings are intended to provide a computational basis for identifying promising Regorafenib-based candidates and guiding the future development of more effective targeted inhibitors for leukemia.[25]

In this study we have investigation on hydoxy-substitued derivatives of Regorafenib for their ability to inhibit tyrosine kinase was motivated by preliminary studies on the anti-cancer properties of Regorafenib, a standard cancer inhibitor, to demonstrate the binding conformation and mechanism of tyrosine kinase using molecular docking analysis.

Methodology

The binding interaction mechanism of the modified Regorafenib as urea derivatives for both competitive and noncompetitive inhibition of the tyrosine kinase enzyme was investigated by molecular docking experiments. The crystal structures of the pertinent proteins will be obtained via the Protein Data Bank. Protein will be hypothetically prepared utilizing Auto Dock MGL tools by removing all cofactors, heteroatoms, and water molecules for computational convenience.[26] For further investigation, we will select the best docking conformer with the lowest binding energy. The docking positions of selectively altered Regorafenib as urea derivatives against the targeted tyrosine kinase inhibitors will be displayed using PyMol, Auto Dock Vina, and BIO via Discovery Studio following the analysis of the protein-ligand interaction results utilizing a very stable confirmer. The modified ligands were tailored by keeping the important pharmacophoric core of Regorafenib intact and carrying out appropriate structural changes at the appropriate sites on the scaffold.

The derivatives were constructed in a logical manner that the major structural structure was not incompatible with the ATP-binding site of the tyrosine kinases. The derivatives of the anti-leukemic drugs and their modified structure of Regorafenib are given in Figure 1

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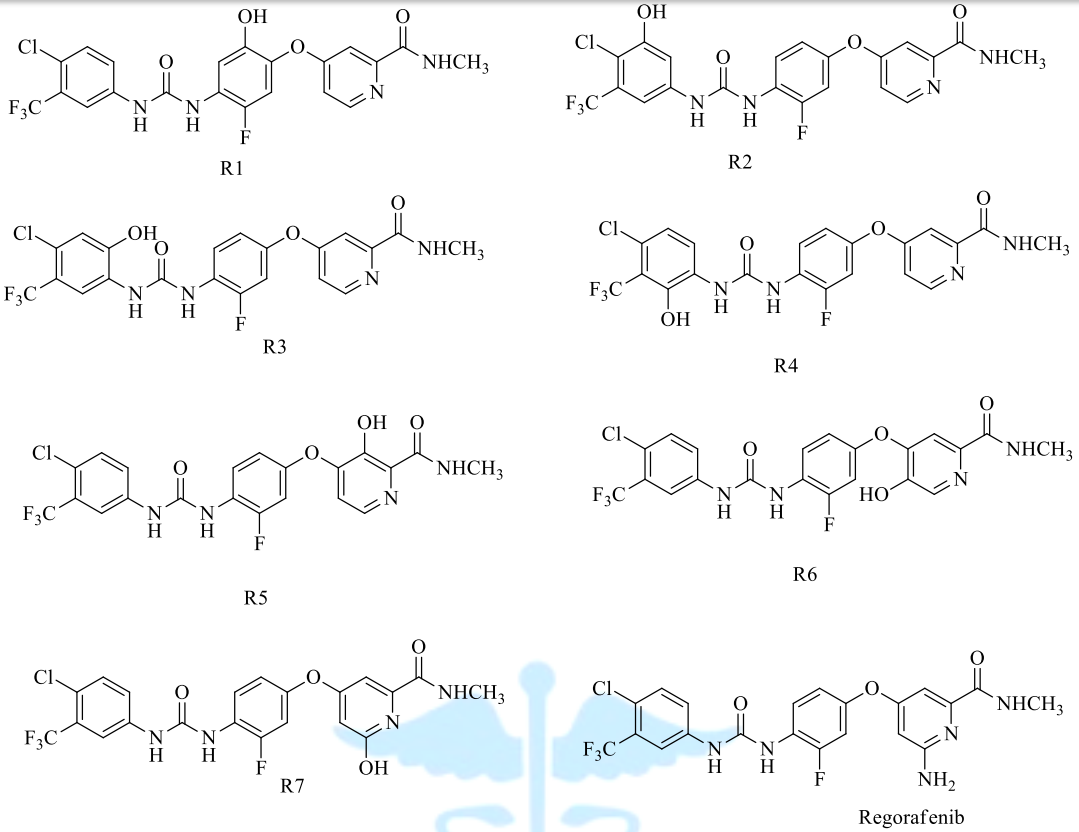


Figure 1. Structural formulae of Regorafenib and its modified derivatives (R1-R7)

3. Results and Discussion

Molecular docking study was utilized to examine binding mechanism of active drugs **(R1-R7)** within the active site of Tyrosine kinase in contrast to Regorafenib. Regorafenib interaction with Tyrosine Kinase

3.1. Regorafenib interaction with Tyrosine Kinase: The compound Regorafenib as an inhibitor as opposed to tyrosine kinase having a docking energy of -12.69 kcal/mol and 499.89pM (Pico molar) inhibition constant expressed in Table 1 The Regorafenib formed three hydrogen bonds with carbonyl oxygen and amino group of amino acid PHE-53(CO...NH, 2.4Å) as shown in the Table 2 Moreover, the interactions Conventional Hydrogen Bond, Halogen (Fluorine), Pi-Cation, Pi-Sigma and Pi-Alkyl were found between said compound Regorafenib and active unit PHE-53 of receptor tyrosine kinase depicted in Figure 2.

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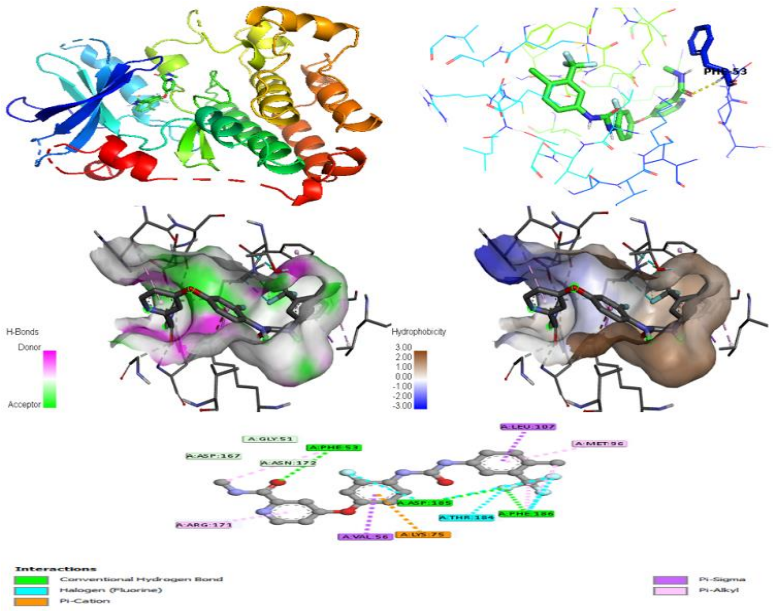


Figure 2. Molecular pose of Regorafenib with Tyrosine kinase (A) Complex, (B) 3D Interactions, (C) Hydrogen bonding, (D) Hydrophobic Interactions (E) 2D interactions

3.2. R1 interaction with Tyrosine Kinase: The docking results of compound R1 against tyrosine kinase showed binding energies of -14.03 kcal/mol and an inhibition constant of 52.31 pM (Pico molar), as shown in Table 1. The compound R1 bounded well with tyrosine kinase active site. Compound R1 showed interactions with the residue ASP-185, ARG-171 and LYS-75 of tyrosine kinase active site .The urea and hetero aromatic region of compound R1 form three Hydrogen bonds with the residue ASP-185 (CO...HN, 1.7Å), (CO...HN, 2.4Å), ARG-171 (NH....OC, 2.1Å), (NH...OC, 2.1Å) and LYS-75 (CO....HN, 2.2Å), (CO...NH, 2.4Å) of enzyme as show in Table 2. The said compound also incorporated in π-alkyl, π -sigma and weak Vander Waal interactions with and ALA-866 residues of R1 compound given in figure. Additionally the compound R1 also show different type of interactions with the residues amino acids ASP-185, ARG-171 and LYS-75 of tyrosine kinase shows Conventional Hydrogen Bond, Halogen (Fluorine), Pi-Sigma and Pi-Alkyl as shown in the Figure 3.

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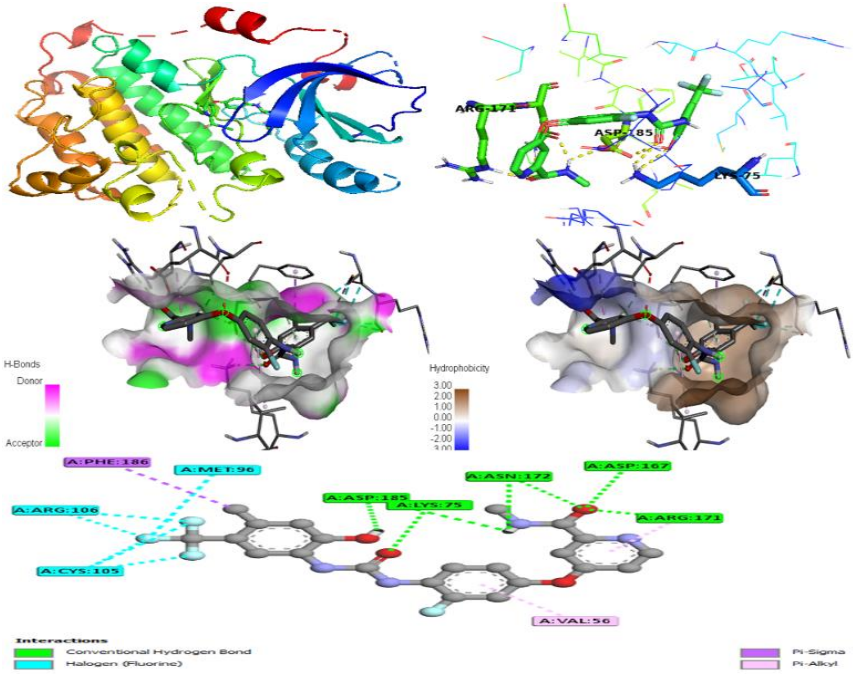


Figure Error! No text of specified style in document.**. Molecular pose of R1 with Tyrosine kinase (A) Complex, (B) 3D Interactions, (C) Hydrogen bonding, (D) Hydrophobic Interactions (E) 2D interactions**

3.3. R2 interaction with Tyrosine Kinase: R2 was the chosen drug that had docking pose that best inhibited tyrosine kinase with binding energies of -13.83 kcal/mol and an inhibition constant of 112.56pM, as indicated in Table 1 The selected chemical R2 was allowed to dock deep inside the center core that is created by tyrosine kinase active residues. The mentioned chemical R2 mechanism of interaction with the tyrosine kinase enzyme revealed interaction with ALA-52 of tyrosine kinase active site. The NH-group of urea region of the ligand make hydrogen bond with ALA-52 (NH….OC, 2.1Å) Table 2. Compound R2 make weak Vander Waal, π-sigma and π-alkyl interactions with ALA-52 amino acid. Additionally, the selected ligand R2 also incorporated in Conventional Hydrogen Bond, Halogen (Fluorine), Pi-Sulphur, Pi-Lone Pair and Pi-Alkyl with ALA-52 residue of tyrosine kinase portrayed in Figure 4.

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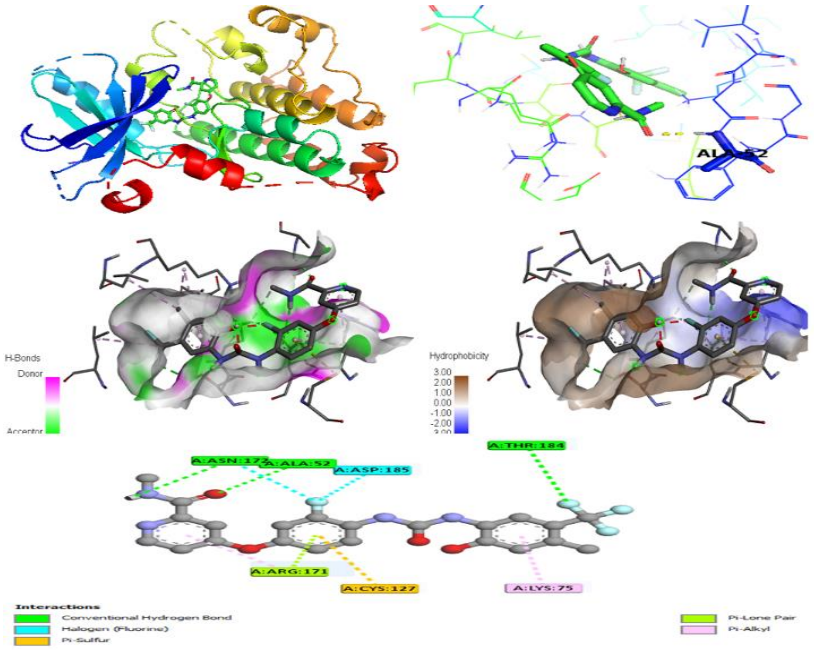


Figure 4. Molecular pose of R2 with Tyrosine kinase (A) Complex, (B) 3D Interactions, (C) Hydrogen bonding, (D) Hydrophobic Interactions (E) 2D interactions

3.4. R3 interaction with Tyrosine Kinase:

The compound R3 was found to interact with tyrosine kinase with an inhibition constant of 26.72 µM (Micro molar) and binding energy of -6.24 kcal/mol as shown in Table 1. The compound R3 show no interactions and having no hydrogen bonding but still it shows some interactions like Conventional Hydrogen Bonding, Halogen (Fluorine), Pi-Anion, Pi-Sigma and Pi-Alkyl with tyrosine kinase as shown in the Figure 5.

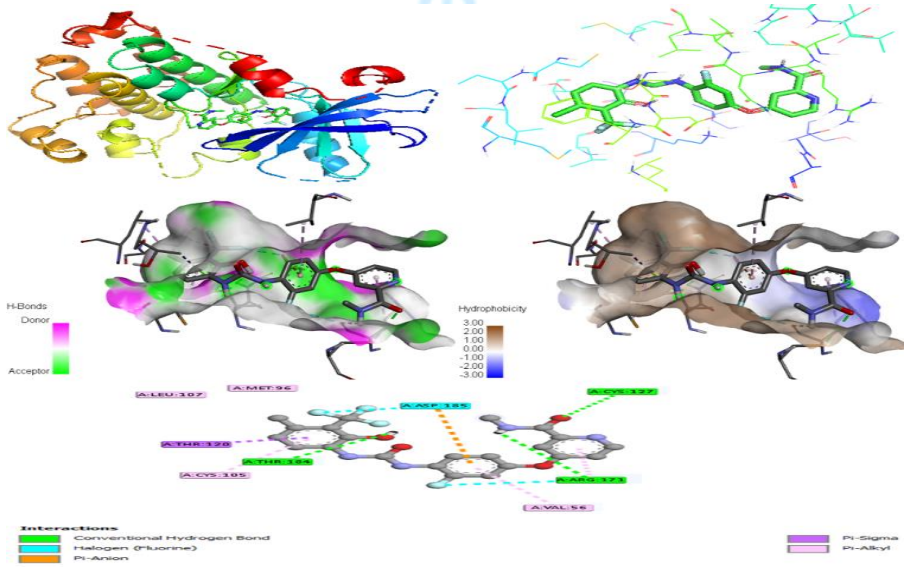


Figure 5. Molecular pose of R3 with Tyrosine kinase (A) Complex, (B) 3D Interactions, (C) Hydrogen bonding, (D) Hydrophobic Interactions (E) 2D interactions

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3.5. R4 interaction with Tyrosine Kinase:

Based on the docking parameter, it was found that the title chemical R4 bound to tyrosine kinase with an inhibition constant 177.70nM (Nano Molar), and a binding energy value of -9.21kcal/mol as shown in Table 1. Within the tyrosine kinase active cavity, the molecule R4 was predicted to create a crucial contact with ALA-52 and LYS-75 of tyrosine kinase active residues of enzyme. These interactions include the formation of three hydrogen bonds between the ligand and the enzyme. It make one H-bond with ALA-52(CO....NH, 2.4Å) at hinge region, and two H-bonds with LYS-75 (CO....NH, 2.3Å) and (CO...NH, 2.3Å) represented in Table 2. The said compound R4 also form Conventional Hydrogen Bonding, Halogen (Fluorine), Pi-Anion, Pi-Sigma, Pi-Alkyl with active residues of tyrosine kinase. As shown in the Figure 6.

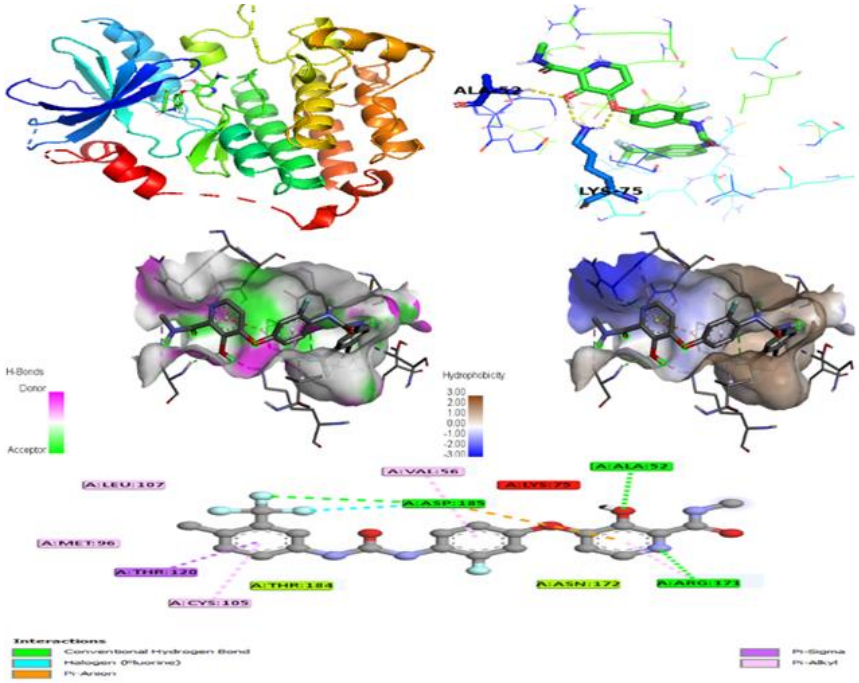


Figure 6. Molecular pose of R4 with Tyrosine kinase (A) Complex, (B) 3D Interactions, (C) Hydrogen bonding, (D) Hydrophobic Interactions (E) 2D interactions

3.6. R5 interaction with Tyrosine Kinase:

According to Table 1, the binding positions of candidate ligand R5 demonstrated good inhibition against tyrosine kinase with binding energy value of -11.03kcal/mol and an inhibition constant of 8.01nM(Nano molar). According to the docking analysis, hit compound R5 has a high probability of bonding with the target protein and becoming highly stable inside the active pocket of the enzyme. The said ligand form three hydrogen bonds with enzyme. The compound R5 make different type of interactions with amino acids such as LYS-75,ASP-185 and ASN-172.It make two H-bond with different bond lengths with LYS-75(CO....HN, 2.2Å) and (O...NH, 2.6Å) at hing region, and one H-bonds with ASP-185(NH....O, 2.2 Å) and two H-Bond with different bond lengths with ASN-172(CO....NH, 2.2Å) and (CO....NH, 2.2Å) demonstrated in Table 2. In addition, the residue LYS-75, ASP-185 and ASN-172 make π sigma bonds with R5. Additionally the compound R5 also show different type of interactions such as Conventional Hydrogen Bond, Halogen (Fluorine), Pi-Sigma and Pi-Alkyl with amino acids LYS-75, ASP-185 and ASN-172 further stabilizes the complex shown in Figure 7.

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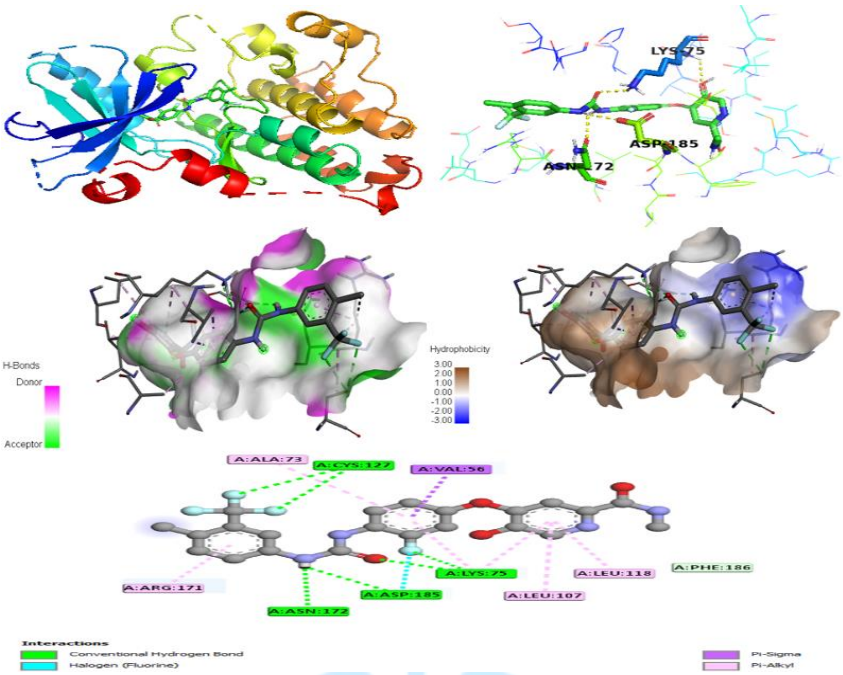


Figure 7. Molecular pose of R5 with Tyrosine kinase (A) Complex, (B) 3D Interactions, (C) Hydrogen bonding, (D) Hydrophobic Interactions (E) 2D interactions

3.7. R6 interaction with Tyrosine Kinase:

The title ligand R6 was shown to bind with tyrosine kinase based on the docking parameter, with a binding energy value of -8.01kcal/mol and an inhibition constant of 1.33 µM (Micro molar), as shown in Table 1. Within the tyrosine kinase active cavity, the molecule R6 was predicted to create a crucial contact with ASP-185 active residue of enzyme. Among these interactions, the selected ligand form three hydrogen bonds with residue ASP-185(NH...OC, 2.2A°) represented in Table 2. Further, R6 compound also make weak Vander Waal, π –sigma, π-alkyl and alkyl bonds with ASP-185 active residue of tyrosine kinase expressed in figure. Additionally, the compound R6 also show interactions like Conventional Hydrogen Bond, Halogen (Fluorine) and Pi-Alkyl with the receptor, expressed in figure. As shown in the Figure 8.

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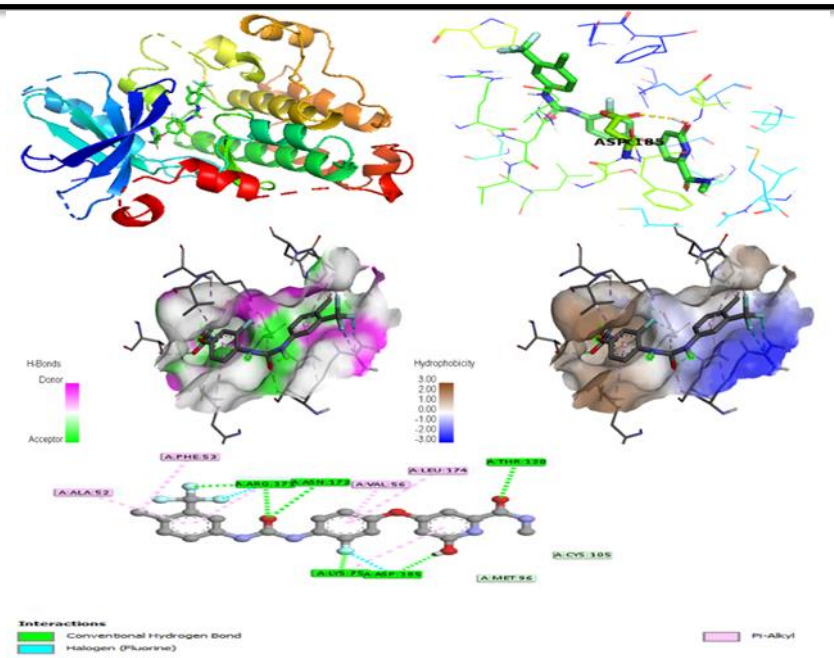


Figure 8. Molecular pose of R6 with Tyrosine kinase (A) Complex, (B) 3D Interactions, (C) Hydrogen bonding, (D) Hydrophobic Interactions (E) 2D interactions

3.8. R7 interaction with Tyrosine Kinase: The title ligand R7 was shown to bind with tyrosine kinase with an inhibition constant of 2.57nM (Nano molar) based on the docking parameter, and binding energy value of -11.72 kcal/mol and given in Table 1. The chemical R7 was predicted to be present in the tyrosine kinase active cavity and established a crucial contact with THR-184 and PHE-186 of tyrosine kinase active residues of enzyme. Among these interactions, the said ligand forms three hydrogen bonds with enzyme. It makes one H-bond with THR-184(O....HN, 3.0 Å) at hinge region with oxygen atom, and two H-bonds with PHE-186(NH....O, 1.7 Å) at the Domain region (urea containing group), represented in Table 2 It also form π-π interactions with THR-184 and PHE-186 amino acids. The GLU-917 make bond with fluorine atom of flat aromatic region (hinge region). Moreover, the said compound R7 also make Conventional Hydrogen Bond, Halogen (Fluorine), Pi-Sigma and Pi-Alkyl with THR-184 and PHE-186 active amino acids of tyrosine kinase expressed in Figure 9.

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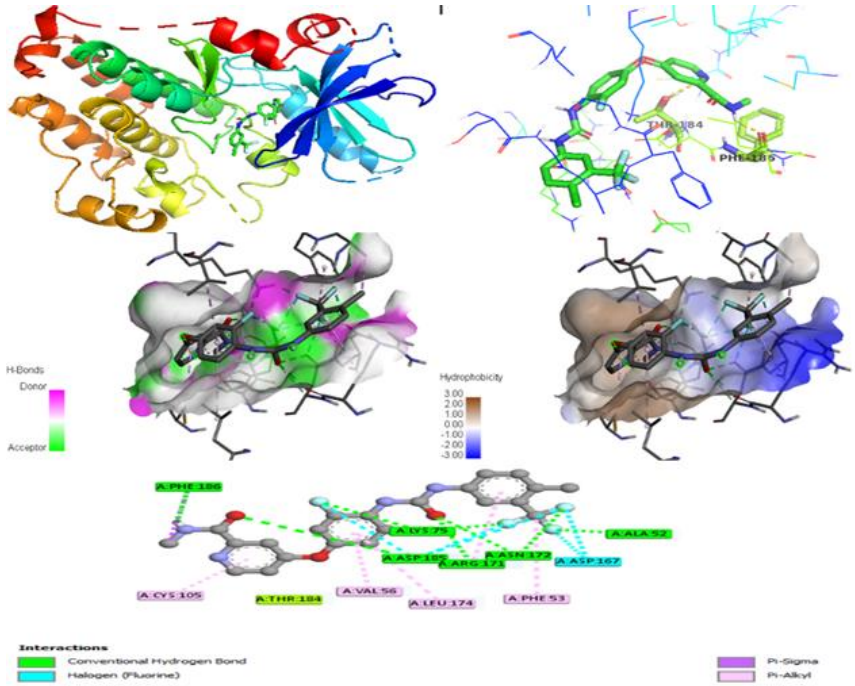


Figure 9. Molecular pose of R7 with Tyrosine kinase (A) Complex, (B) 3D Interactions, (C) Hydrogen bonding, (D) Hydrophobic Interactions (E) 2D interactions

Table 1. Binding energies and inhibition constant values of Regorafenib and its derivatives

Compounds	Binding Energy	Inhibition Constant
R1	-14.03	52.31 pM
R2	-3.83	1.56mM
R3	-6.24	26.72µM
R4	-9.21	177.70nM
R5	-11.03	8.26nM
R6	-8.01	1.33µM
R7	-11.72	2.57nM
Regorafenib	-12.87	369.24 pM

Table 2. Hydrogen bond distance and interacting residue of Regorafenib and their derivative

Compounds	Interacting amino acids	Distances(Å)
R1	ASP-185, ARG-171, LYS-75	1.7, 2.4, 2.1, 2.1, 2.2, 2.4
R2	ALA-52	2.1
R3	-	-
R4	ALA-52, LYS-75	2.4, 2.3, 2.3
R5	LYS-75, ASP-185, ASN-172	2.2, 2.6, 2.2, 2.2, 2.5
R6	ASP-185	2.2
R7	CYS-105, ARG-171, ASP-185, PHE-186 THR-184, PHE-186	3.0, 2.1
Regorafenib	PHE-53	2.4

Conclusion

The molecular docking investigation demonstrated that hydroxyl-substituted Regorafenib derivatives can exhibit promising binding interactions with the leukemia-associated tyrosine kinase target. Among the

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studied compounds, R1 showed the most favorable binding energy (-14.03 kcal/mol) and lowest inhibition constant (52.31 pM), outperforming the parent Regorafenib, while R7 also displayed strong binding affinity. These findings suggest that hydroxyl functionalization can enhance ligand-kinase interactions and may provide a useful strategy for developing improved Regorafenib-based tyrosine kinase inhibitors. However, experimental validation and further pharmacological studies are required to confirm their anti-leukemic potential.

Acknowledgments.
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Conflict of Interest
The authors declare no conflict of interest

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