RJC

RASĀYAN *J. Chem.*
Vol. 17 | No. 2 |356-362| April - June | 2024
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

In-silico ANALYSIS OF *N*-PHENYL PYRAZOLINE DERIVATES AS POTENTIAL OF HUMAN EPIDERMAL GROWTH RECEPTOR-2 (HER-2) INHIBITOR USING MOLECULAR DOCKING AND MD SIMULATIONS

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ABSTRACT

One of the most typical cancers to affect women worldwide is breast cancer. Multiple routes involving different proteins that control the development of cancer. HER-2 is a protein that contributes to the progression of breast cancer cells**.** This study uses n-phenyl pyrazoline derivate compounds. The predictive binding of several forms of pyrazoline compounds to HER-2 was analyzed using docking analysis in an in silico model. Pyrazoline A, B, C, D, and M were used as ligands, and neratinib as a commercial drug. Pyrazoline C was the ligand with the highest affinity (-109.218 Kcal/mol) if compared with native ligand 03Q (-170.697 Kcal/mol) and neratinib (-83.416 Kcal/mol). Pyrazoline C has the potential to develop as a breast cancer drug with COX-2 inhibitory activity. The molecular dynamics simulation for 50 ns showed that RMSD, RMSF, and SASA are rigid and stable. Pyrazoline C has the potential to develop as a breast cancer drug with HER-2 inhibitory activity.

Keywords: Pyrazolines, HER-2, Anticancer Drugs, Molecular Docking, MD Simulation

RASĀYAN *J. Chem.*, Vol. 17, No. 2, 2024

INTRODUCTION

Breast cancer has a notably high prevalence among malignancies affecting females, ranking second in incidence alone to cervical cancer. The number of breast cancer patients is most significantly influenced by changes in lifestyle and diet. The prevalence of cancer cases and the exorbitant expenses associated with its treatment have created a compelling need for alternative medicinal options, with a special emphasis on traditional medicine.[1,2] HER-2 signaling has emerged as a promising target for cancer-targeted molecular treatments. Several chemotherapeutic drugs targeting the HER-2 receptor are under development. Trastuzumab was the first antibody to target HER-2. Nonetheless, 70% of patients who have used trastuzumab for a long time develop resistance, which leads to the advancement of metastatic cancer owing to an increase in EGFR expression.[3,4,5] Pyrazoline derivatives are nitrogenous heterocyclic compounds that exhibit diverse biological activities. Pyrazoline derivatives inhibited breast cancer, hepatocellular carcinoma, lung cancer, and breast cancer cells.[6,7,8,9] Several cancer cell lines have been examined using pyrazoline derivatives, which have been shown to inhibit cell growth and cause apoptosis. Prior study indicates that our synthesized N-phenyl pyrazolines exhibit anti-cancer activity against breast and colorectal cancer cells.[10-13] The goal of this work is to use docking and molecular dynamic analysis to investigate the

http://doi.org/10.31788/RJC.2024.1728795

activity of N-pyrazoline derivate compounds in inhibiting HER-2 expression, which plays a key role in cancer cell progression.

EXPERIMENTAL

Ligand 3D Preparation

The molecular docking process was carried out using an Aspire Vivobook running on Windows 7 Home Basic. The laptop was equipped with an Intel® CoreTM i5 processor clocked at 3.4 GHz, 64-bit architecture, a 320 GB hard disc drive, and 4 GB DDR3 L RAM. The chemicals Pyrazoline C and Pyrazoline M were shown in their 2D structures using the ChemDraw 18.1 software. Then, the 2D structure is converted into a 3D structure using the chem3D 18.1 application by performing energy minimization (perform MMFF94 minimization) and saving in SDF format.[14]

Docking Analysis

The 3D conformation of the chosen target proteins was obtained from the RSCB PDB database (https://www.rcsb.org/) for COX-2 (PDB ID: 5IKT), whilst the 3D arrangement of the reference ligand employed was taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). In addition, the re-docking process was performed via the MolDock algorithm, a particular docking approach that is incorporated into Molegro Virtual Docker 5.0. The grid box size corresponds to the control ligand (native ligand) that has been attached to the PDB protein.[15,16]

Molecular Dynamics Simulation

The protein and ligand were generated using GROMACS 2019, specifically through topological protein preparations utilizing the pdb2gmx tool. The protein force field used is AMBER99SB. Acpype is utilized to ascertain the ligand's topology. Furthermore, the procedure encompassed the incorporation of protein and ligand structure, solvent inclusion, ion incorporation, stabilization, optimization, and the implementation of molecular dynamics simulations. The MD manufacturing process lasted for a duration of 50,000 picoseconds, which is comparable to 50 nanoseconds. The graph illustrates the MD interpretation by displaying the RMSD on the backbone, RMSF on C-alpha, and SASA on the protein. The utilization of the qtGrace software facilitates this process.[17,18]

RESULTS AND DISCUSSION

HER-2 Protein Docking Analysis

Molecular docking, a computer approach, is employed to monitor the formation of a stable protein-ligand complex at the active region of the protein.[19,20] The control RMSD results are by the standard, which is less than 2 Å.[21] The molecular docking results of HER-2 protein (ID: 3PP0) with test compounds revealed that pyrazoline C and M, among three pyrazoline compounds, exhibited a higher binding affinity than the typical commercial ligand neratinib. In comparison, their affinity was lower than that of the control ligand 03Q.[15] (Table-1). The grid box settings used are as follows:

Protein	X	Y	Z	Radius	RMSD Re-docking	Binding affinity (kcal/mol)
3PP0	16.71	16.04	27.10	10	0.55	-170.216

As indicated by the results, Pyrazoline C and M exhibit superior activity in inhibiting HER-2 compared to Pyrazoline A, B, D, and neratinib. The ligand-protein bond with the lowest energy has superior inhibitory action.[22]

Table-1: Binding Affinity Between HER-2 Protein and Test Compounds

Compounds	*Binding Affinity (kcal/mol)*	RMSD (Å)
03Q (control)	-170.697	0.35
Pyrazoline A	-101.006	0.01
Pyrazoline B	-105.533	35.33
Pyrazoline C	-109.218	0.01
Pyrazoline D	-101.759	0
Pyrazoline M	-108.729	0
Neratinib	-83.416	0.66

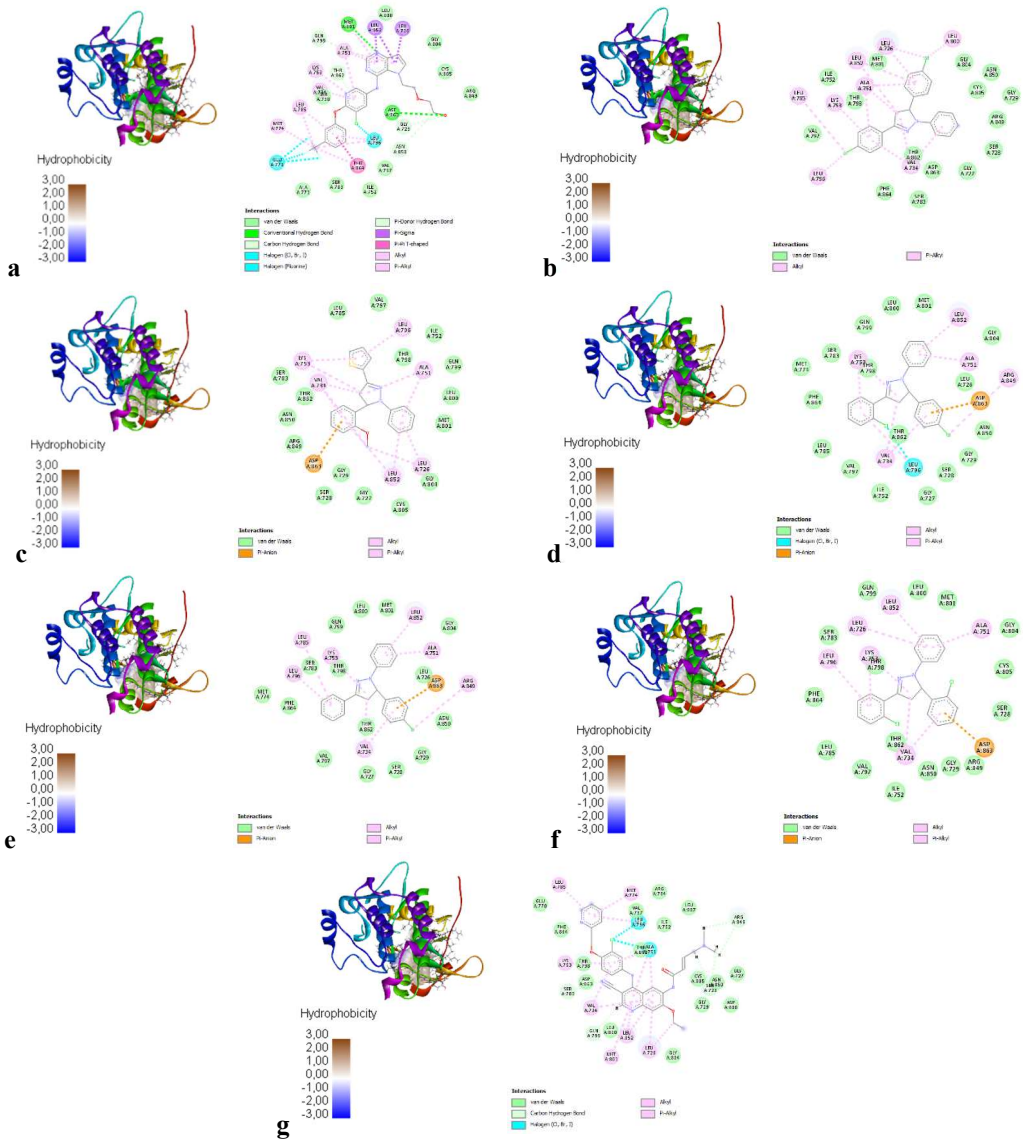


Fig.-1: Visualization of Docking Results, a) HER-2-Control, b) HER-2-pyrazoline A, c) HER-2-pyrazoline B, d) HER-2-pyrazoline C, e) HER-2-pyrazoline D,) f) HER-2-pyrazoline M, g) HER-2-neratinib. The Left Part Shows the 3D Visualization and the Right Part Shows the Type of Bond Produced Between the Ligand-Protein

Table-2: Interaction Amino Acid Residue Towards HER-2 Protein with Compounds

Ligand	Interaction			
	Van der Waals	Hydrogen Conventional	Hydrogen Carbon	Hidrofobic
03Q (Control)	-	-	-	-
Pyrazoline A	A: ILE752; A: THR798; A: GLY804; A: CYS805; A: ARG849; A: SER783; A: VAL797	-	-	A: VAL734; A: ALA751; A: LEU852; A: LEU726; A: LEU785; A: LEU796; A: LYS753
Pyrazoline B	A: SER783; A: ARG849; A: CYS805; A: GLY804; A: THR798; A: ILE752; A: VAL797; A: LEU800	-	-	A: VAL734; A: ALA751; A: LYS753; A: LEU726; A: LEU796; A: LEU852
Pyrazoline C	A: VAL797; A:I LE752; A: GLY804; A: LEU800; A: THR798; A: SER783	-	-	A: VAL734; A: LYS753; A: LEU796; A: ALA751; A: LEU852

Ligand	Interaction			
	Van der Waals	Hydrogen Conventional	Hydrogen Carbon	Hidrofobic
Pyrazoline D	A: VAL797; A: GLY804; A: LEU800; A: THR798; A: SER783	-	-	A: LEU796; A: LEU785; A: VAL734; A: LEU852; A: ALA751; A: LYS753
Pyrazoline M	A: VAL797; A: ILE752; A: SER728; A: CYS805; A: GLY804; A: LEU800; A: THR798; A: SER783	-	-	A: VAL734; A: LYS753; A: LEU796; A: LEU726; A: ALA751; A: LEU852
Neratinib	A: THR798; A: LEU800; A: GLY804; A: CYS805; A: ILE752; A: VAL797	-	A: GLN799	A: LEU726; A: VAL734; A: ALA751; A: LYS753; A: MET801; A: LEU852; A: MET774; A: LEU785; A: LEU796

Molecular Dynamics Simulation

Root Mean Square Deviation (RMSD)

RMSD quantifies the mean discrepancy between a protein's structure and its initial conformation at a specific moment, serving as a crucial metric for assessing the stability of a protein's structure. A Molecular Dynamics Simulation was conducted for a duration of 50,000 picoseconds to assess the stability of the kinase domain of human HER-2 during its interaction with various test chemicals, namely 03Q, Neratinib, Pyrazoline C, and Pyrazoline M. The molecular dynamics (MD) data reveal that the native protein, specifically the kinase domain of human HER-2, exhibits a root mean square deviation (RMSD) of around 0.2 nm. In contrast, the kinase domain of human HER-2 exhibited a marginal elevation in the RMSD value upon interaction with the test chemicals 03Q, Neratinib, Pyrazoline C, and Pyrazoline M, with respective scores of 0.25 nm, 0.24 nm, 0.29 nm, and 0.24 nm (Fig.-2). Despite the rise in the root mean square deviation (RMSD) value, it remained insignificant, measuring below 1 nm.

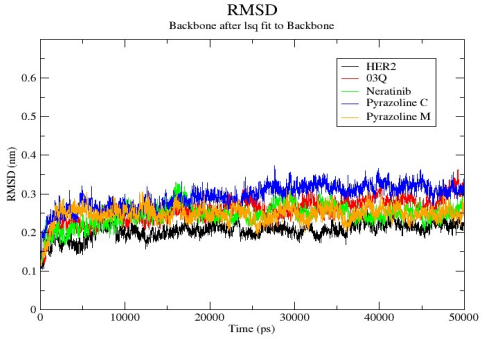
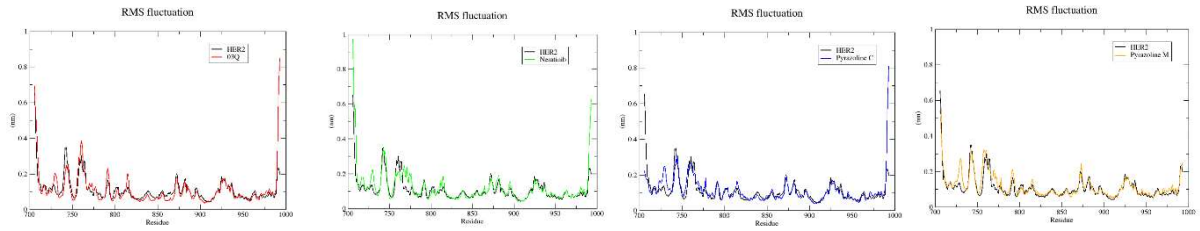


Fig.-2: Root Mean Square Deviation (RMSD) Kinase Domain of Human HER-2 When Interaction with 03Q, Neratinib, Pyrazoline C, and Pyrazoline M

Root Mean Square Fluctuation (RMSF)

We analyzed the flexibility of the kinase domain of human HER-2 and its interactions with the test drugs, as shown in Fig.-3. The root mean square fluctuation (RMSF) quantifies the average movement of individual amino acid residues within a protein relative to the average shape. A direct relationship exists between the size of residual variation and the degree of flexibility the residue exhibits upon pressure treatment.[23] The RMSF kinase domain of human HER-2 value when interacting with 03Q, Neratinib, Pyrazoline C, and Pyrazoline M compounds is slightly bigger especially at residue number 754 even though the number is still in a small number (around 0.1 nm), and the RMSF score is consistent with RMSD score. The SASA analysis quantifies the extent of protein surface area accessible to solvents. Higher SASA levels indicate a greater degree of growth.[24] The surface area of the kinase domain of human HER-2 without ligand molecules is 151.27 nm^2, as determined by the SASA value. Concurrently, the SASA kinase domain of human HER-2 exhibited a marginal alteration in size, measuring

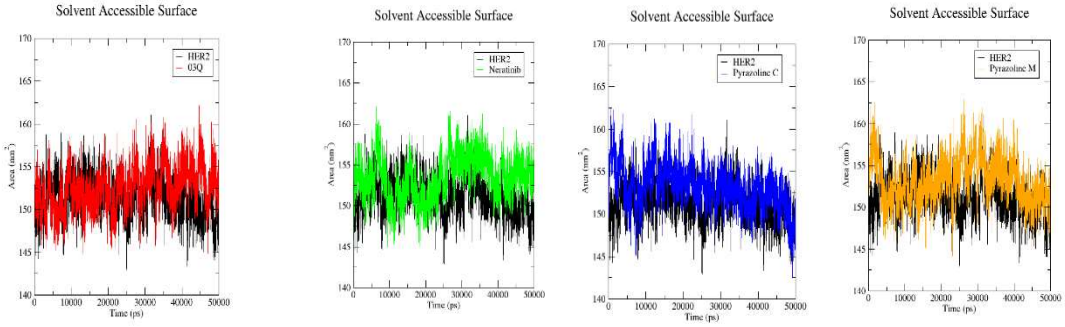
152.56 nm^2, 153.40 nm^2, 153.14 nm^2, and 153.24 n nm^2, respectively, upon interaction with the test chemicals 03Q, Neratinib, Pyrazoline C, and Pyrazoline M.



(a) (b) (c) (d)

Fig.-3: Root Mean Square Fluctuation (RMSF) Kinase Domain of Human HER-2 When Interaction with 03Q (a), Neratinib (b), Pyrazoline C (c), and Pyrazoline M (d)

Nevertheless, this increment is insignificant. A rise in the SASA value signifies an augmentation in the accessibility to the surface of the Kinase domain of Human HER-2.
(Fig.-4).



(a) (b) (c) (d)

Fig.-4: Solvent-Accessible Surface Area (SASA) HER-2 When Interaction with 03Q (a), Neratinib (b), Pyrazoline C (c), and Pyrazoline M (d)

CONCLUSION

Based on docking analysis Pyrazoline C is the best compound to inhibit HER-2 expression and the MD results for 50 ns, 03Q, Neratinib, Pyrazoline C and Pyrazoline M had a slight increase in RMSD, RMSF, and SASA values. However, these compounds may still have the ability to bind to the Kinase domain of Human HER-2. In order to verify the accuracy of these computer-simulated results, it is essential to conduct experimental studies in a controlled laboratory setting (*in vitro*) and in living organisms (*in vivo*).

ACKNOWLEDGMENTS

Universitas Sumatera Utara provided financial support for this study through the "Indonesia Research Collaboration" research grant 2023. The project was supported under Contract No: 26/UN5.2.3.1/PPM/KP-RKI/2023, dated 11 May 2023.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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