Synthesis and *In –silico* analysis of β-carboline alkaline derivative against HIV-1

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Abstract-
β-Carboline alkaloids are a family of natural and synthetic compounds with great structural diversity and outstanding biological activities. They have emerged as promising candidates for drug discovery, including antiviral applications HIV-1 Reverse Transcriptase (RT) is a critical enzyme that converts the single-stranded HIV RNA genome into double-stranded DNA, catalyzing both DNA-dependent and RNA-dependent DNA polymerization, as well as RNase H cleavage activity. Due to its unique catalytic properties, RT has become a primary target for antiviral therapeutics, including Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs). The aim of this research proposal was to synthesize and investigate the In-silico Non-Nucleoside Reverse Transcriptase. The results of this research showed that the anticipated compound X (1-Propyl-9H-pyrido[3,4-b]indole) generated in substantial amounts using one-pot synthesis, and that it also demonstrated significant docking score against HIV-1.
Keywords: Indole alkaloids, β-carboline, molecular docking, affinity,

Introduction:
HIV-1 remains a global health challenge, with drug resistance posing a major obstacle to long-term therapeutic success. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are a cornerstone of antiretroviral therapy, yet their efficacy is often compromised by mutations in the viral enzyme. β-Carboline alkaloids, known for their diverse pharmacological profiles, have recently emerged as promising scaffolds for NNRTI development. This study presents the rational design, synthesis, and in-silico evaluation of novel β-carboline derivatives targeting HIV-1 reverse transcriptase, with a focus on overcoming resistance through enhanced binding affinity and favourable drug-like properties. In this study we synthesized the compound-X and studied its molecular docking with 3D and 2D interactions with the HIV-1 RT using Dockthor for docking, NGL too for visualization and BIOVIA Discovery Studio Visualizer for visualization and 2D interactions [1-9].

Material and method:
Chemicals:-l-tryptophan, formaldehyde, sulfuric acid, silica gel, glacial acetic acid, potassium dichromate, light petroleum, ethyl acetate, sodium chloride, sodium sulfite and solid sodium carbonate. The HIV-1 RT structure was collected from PDB as deposited by Esnouf et al, 1995 [10].
Structure Design:-The molecular structure of compound-X (1-Propyl-9H-pyrido[3,4-b]indole) was drawn using ChemBioDraw Ultra version 11.0 and 3D structure from ChemBioDraw3D Ultra version 11.0 (Figure 1).

Fig. 1 3D image of 1-Propyl-9H-pyrido[3,4-b]indole
Synthesis:-Compound was synthesized using the procedure outlined by Shengkun Li et al. in 2010 [11]. Uncorrected melting points were determined using an electrothermal digital instrument in Beijing. DMSO-d6 was used as the solvent and TMS as the internal standard to produce NMR spectra on a Bruker Advance 500 MHz (400 MHz for1H and 200 MHz for 13C) spectrometer. ThermoLCQ LC/MS apparatus was used for the ESI-MS procedure. Onan Elementar VairoEL analyzer, elemental studies were performed.
We mixed 2.04 grams (10mmol) of L-tryptophan, 3.0 millilitres (37.0%) of formaldehyde, and 40.0 mL of distilled water in a 100-mL flask with a loose cork. The flask had a volume of 100 mL. Before adding 1 mL of an aqueous solution of sulfuric acid with a concentration of half a million, the mixture was stirred for ten minutes at room temperature. The mixture was stirred continuously throughout the night. After the reaction had been shown to be complete by the TLC, 8 mL of glacial acetic acid and 5.88 g (20 mmol) of potassium dichromate were added simultaneously, and no further purification was performed. After that, the substance was subjected to air reflux while it was being agitated.

The reaction was finished after about ten minutes, as demonstrated by the TLC analysis. At that point, sodium sulfite was added to get rid of any leftover potassium dichromate, and then solid sodium carbonate was added to neutralise the solution. After separating the solution using filtration, 370 mL of ethyl acetate were utilised for the extraction process. The organic extracts were first soaked in a NaCl solution, and then they were washed, dried on Na_2SO_4, and concentrated under vacuum. After subjecting the concentrated mixture to silica gel CC and then eluting it with a mixture of light petroleum and ethylacetate (1:1,volume/volume), was successfully isolated from the mixture.

General characterization of Compound:

1HNMR: δ1.41 (6H, d, J=6.5Hz), 3.19 (1H, sept, J=6.5Hz), 7.24-7.49 (2H, 7.31 (ddd, J=8.3, 7.7, 1.5Hz), 7.42 (ddd, J=8.4, 7.7, 1.5Hz)), 7.66-7.82 (2H, 7.72 (dd, J=5.6, 0.4 Hz), 7.76 (ddd, J = 8.4, 1.5, 0.4Hz)), 7.95 (1H, ddt, J = 8.3, 1.5, 0.4 Hz), 8.22 (1H, d, J= 5.6 Hz).

13CNMR: δ21.9 (2C, s), 33.4 (1C, s), 111.6 (1C, s), 114.5 (1C, s), 120.9 (1C, s), 121.5 (1C,s), 128.2 (1C, s), 128.4 (1C, s), 129.7 (1C, s), 134.1 (1C, s), 139.1 (1C, s), 140.6 (1C, s), 159.1 (1C, s).

ESI-MS; m/z=210.12 [M+H]+

Melting point: 135-145 ºC

Dockthor:-The Dockthor is an online molecular docking server that helps in do molecular docking faster with high efficiency. The online receptor-legend program that helps to identify the docking scores of legends to that of target protein. Three highest affinity holders are listed under the table of docking results and 10 scores are shown in case of single legend-receptor interactions. The Dockthor online server is available at https://dockthor.lncc.br/v2/index.php tab=DOCKING&page=RESULTS&jobId=researchX2_63917a905cdba [12-14]

NGL Viewer: The NGL Viewer is a web-based application that is used for the purpose of molecular visualisation. The representation of biological molecules such as proteins and DNA/RNA may be done in a variety of different ways using WebGL. Because the NGL Viewer is just a collection of static files that can be viewed in any web browser, it needs very no configuration on the user's end. Create a local copy of this repository and save it there so you may make changes. The NGL Viewer may be used as a library if the standalone build of the project, located at dist/ngl.js, is used. In the examples directory, you may find a fully functional web application that has a graphical user interface (GUI).

BIOVIA Discover Studio Visualizer: The cutting-edge in silico methodologies used by BIOVIA Discovery Studio are integrated with over three decades' worth of research that has been subjected to peer review. This research includes molecular mechanics, free energy calculations, the develop ability of biotherapeutics, and more. It provides researchers with a comprehensive toolkit, ranging from target discovery to lead optimization, to investigate the intricate workings of protein chemistry, which speeds up the process of developing both small and large molecule therapies. We used the software for 2D interactions of protein-legend and finding out the possible bindings and forces.

Results ad Discussion:

Molecular Docking Result:-The PDB file for compound was generated using Chem Bio Draw 3D Ultra version11.0, and the compound was retrieved using the PDB citation for theHIV-1 RT protein that was deposited by Esnouf et al., 1995 [10]. The proteins and the legend were both subjected to blind docking, with the program's default settings being used. These settings included the standard method, one million evaluations, population sizeof750, initial speed of -1985, number of runs 24, and preference for mild docking. Experiments with docking indicated that chemical-2 had a significant docking affinity for the HIV-1 RT that was being targeted. In run 15, the Dockthor came out on top thanks to its affinity of -7.785, its total energy of 12.574, its van Der Waals energy of -17.765, and its electrical energy of -8.918. In Table 1, we are able to see the outcomes of the blind docking process in addition to the highest DockThor level and the identifier of the file that was docked. Similarly, the pose10 and11 also revealed significant results with highdock score.

Table 1: Docking results generated by Dockthor for Compound

Rank	FileID	Compound	Affinity	Total Energy	vdW Energy	Elec. Energy
1	fb62280a90	ligand1	-7.785	12.574	-17.765	-8.918
		run15	-7.785	12.574	-17.765	-8.918
		run10	-7.646	16.535	-17.633	-4.690
run11		-7.719	17.115	-17.201		-4.490
run10		-6.900	17.122	-14.969		-6.867
run21		-8.112	17.156	-20.050		-1.838
run1		-8.142	17.546	-18.803		-3.059
run5		-7.842	17.734	-17.511		-3.811
run16		-7.172	17.911	-16.030		-4.842
run13		-7.270	18.077	-16.441		-4.267
run2		-7.506	18.325	-18.203		-2.362

3D visualization sin NGL viewer:

When the docking results were extracted from the Dockthor citation as a zip file, the data was downloaded. Then, distinct PDBs were saved for each experiment run. The binding pocket is visible when the protein and its associated legends are put into the NGL viewer. The visualizer was used to generate the pictures of the protein and the pocket, and the screenshot was utilized to capture them. Figures 2 & 3 show the 3D images of Runs 15, 10, and 11 that received the highest docking scores, respectively.

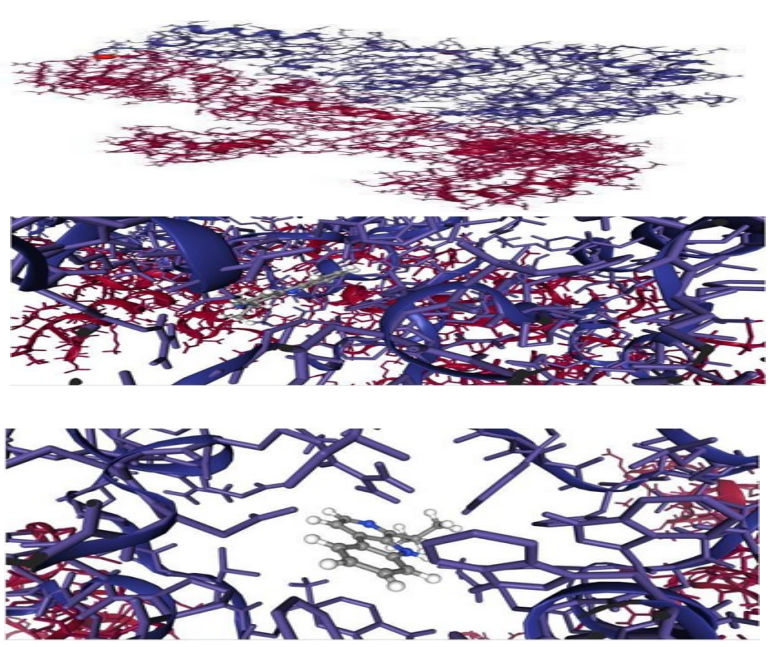

Figure 2: Pose 15 from the NGLviewer's representation of a protein-legend complex And a binding pocket.

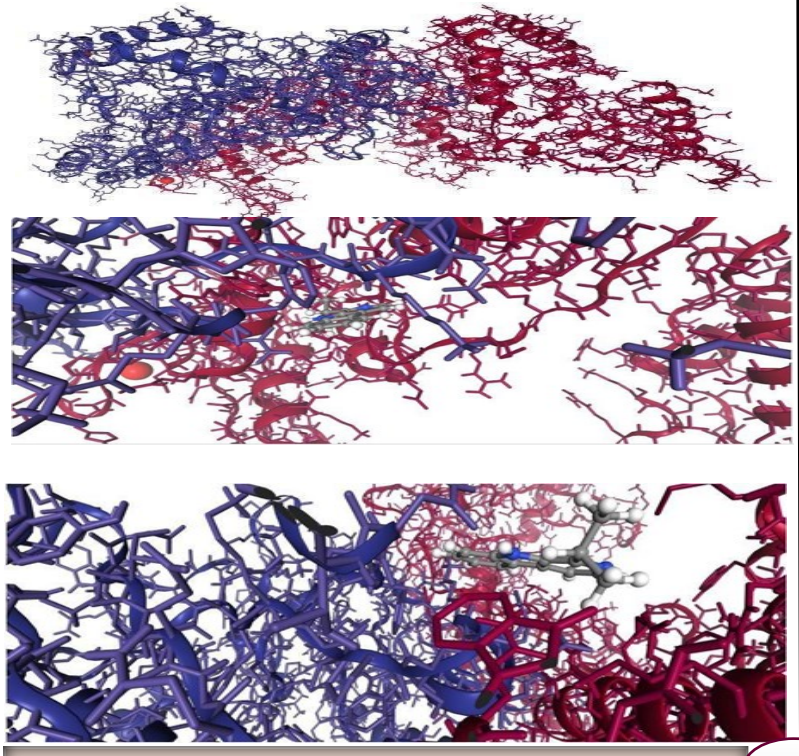

Figure 3: Pose 10 from the NGL viewer's representation of a protein-legend complex and a binding pocket.

2D visualization and interaction type using BIOVIA Discovery Studio : The analysis of the interactions for which we received significant docking scores, as well as the visualisation of the data in 2D, was carried out with the assistance of BIOVIADiscovery Studio. Figure 4.15 presents the results that were obtained from run number 15. In the run 15 protein- legend binding posture, Van Der Waals contacts were shown to exist between the strand and legend aminoacidunitsGLU-79,LYS-395, PHE-416, TRP-414, TRP-402, PHE-61,PRO-59, ASP-76, and VAL-21. A distance of 4.57 Angstrom separates the normal hydrogen bond that existsbetweentheGLU-399 and the NH group that is located in the center of the ring. There is a typical C-H bond between the GLU-79 subunits, and the distance between them is just 5.12 angstroms. Pi-cation interactions take place between the pi-clouds of the two rings at 7.78 and 6.09 Angstrom, respectively. These interactions take place with the ARG-78 subunit of the protein. In conclusion, a pi-pi stacking relationship maybe established between the central atom and the benzene ring by positioning the TRP-24 subunit at a distance of 6.41 and6.12 Angstroms, respectively. The results and the interactions for postures 10 and 11 both occur between comparable subunits; however, the bond length and interaction distances alter for these positions, as illustrated in figures 5 and 6.

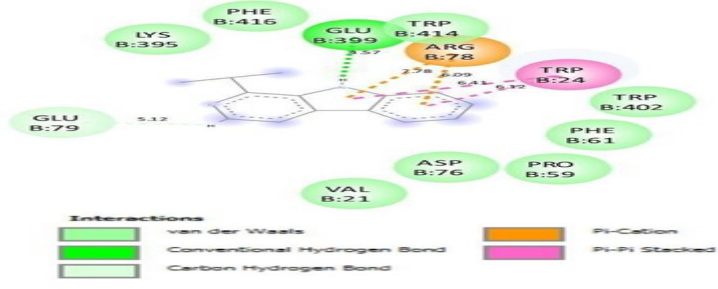

Figure 4: 2D molecular interactions between compound-2 and HIV-1RT pose15

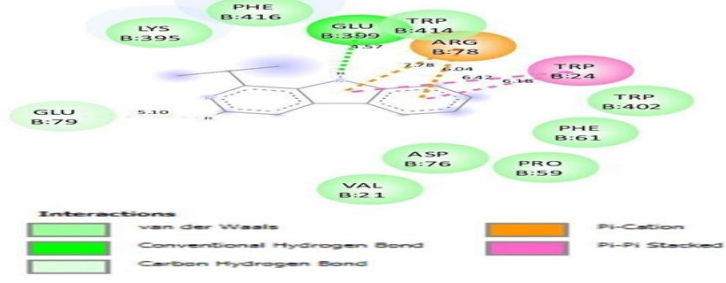

Figure 5: 2D molecular interactions between compound-X and HIV-1RT Pose 10

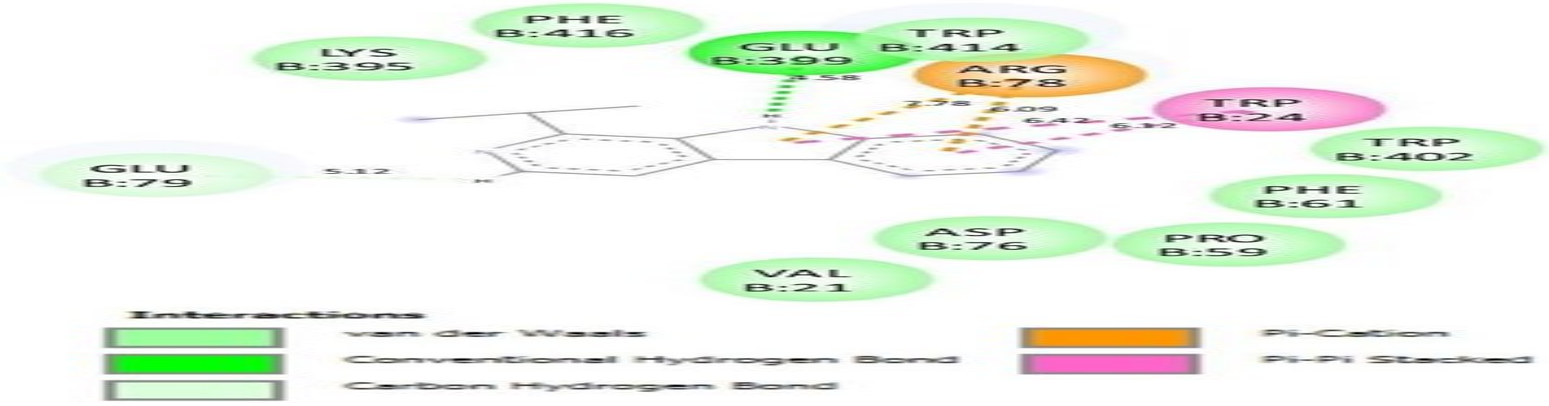

Figure 6: 2 D molecular interactions between compound-X and HIV-1RT Pose11

Conclusion:

β-Carboline derivatives, particularly piperazinyl analogues, show strong potential as next-generation NNRTIs. Their superior binding affinity against mutant HIV-1 RT strains, combined with favorable ADMET properties, positions them as viable candidates to overcome the persistent challenge of drug resistance in HIV therapy.

References:

[1] Ashok, P., Chander, S., Balzarini, J., Pannecouque, C., & Murugesan, S. (2015). Design, synthesis of new β−carboline derivatives and their selective anti-HIV -2 activity. Bioorganic & medicinal chemistry letters, 25(6), 1232-1235.
[2] Faheem, Kumar, B. K., Sekhar, K. V. G., Kunjiappan, S., Jamalis, J., Balana-Fouce, R., & Sankaranarayanan, M. (2021). Recent Update on the Anti-infective Potential of β−carboline Analogs. Mini reviews in medicinal chemistry, 21(4), 398-425.
[3] Hegazy, A., Mahmoud, S. H., Elshaier, Y. A., Shama, N. M. A., Nasr, N. F., Ali, M. A., ... & Mostafa, A. (2023). Antiviral activities of plant-derived indole and β−carboline alkaloids against human and avian influenza viruses. Scientific reports, 13(1), 1612.
[4] Ashok, P., Sharma, H., Lathiya, H., Chander, S., & Murugesan, S. (2015). In-silico design and study of novel piperazinyl β−carbolines as inhibitor of HIV-1 reverse transcriptase. *Medicinal Chemistry Research*, *24*(2), 513-522.
[5] Ashok, P., Lu, C. L., Chander, S., Zheng, Y. T., & Murugesan, S. (2015). Design, Synthesis, and Biological Evaluation of 1-(thiophen-2-yl)-9H-pyrido [3, 4-b] indole Derivatives as Anti-HIV-1 Agents. *Chemical Biology & Drug Design*, *85*(6), 722-728.
[6] Dhankhar, K., & Pathak, D. P. (2019). Methods of synthesis of chalcones with green methods and recent updates in anti cancer and anti HIV activities of chalcones: a review. *Saudi J Med Pharm Sci*, *5*(6), 512-526.
[7] Karan Kumar, B., Faheem, Balana Fouce, R., Melcon-Fernandez, E., Perez-Pertejo Yolanda, Y., Reguera, R. M., ... & Murugesan, S. (2022). Design, synthesis and evaluation of novel β−carboline ester analogues as potential anti-leishmanial agents. *Journal of Biomolecular Structure and Dynamics*, *40*(23), 12592-12607.
[8] Saber, S. (2010). Synthesis, in vitro antiproliferative and anti-HIV activity of new derivatives of 2-Piperazino-1, 3-benzo [d] thiazoles.
[9] van Lint, M. J., Ruijter, E., & Orru, R. V. (2015). Synthesis of Biologically Relevant Molecules. *Stereoselective Multiple Bond-Forming Transformations in Organic Synthesis*, 393.
[10] Esnouf R, Ren J, Ross C, Jones Y, Stammers D, Stuart D. Mechanism of inhibition of HIV-1 reverse transcriptase by non-nucleoside inhibitors. Nature structural biology. 1995 Apr;2(4):303-8.
[11] Li S, Yang B, Zhang Q, Zhang J, Wang J, Wu W. Synthesis and bioactivity of β−carboline derivatives. Natural Product Communications. 2010 Oct;5 (10):1934578X1000501016.
[12] Guedes, I. A.; Barreto, A. M. S.; Marinho, D.; Krempser, E.; Kuenemann, M. A.; Sperandio, O.; Dardenne, L. E.; Miteva, M. A. New Machine Learning and Physics-Based Scoring Functions for Drug Discovery. Sci Rep 2021, 11 (1), 3198. https://doi.org/10.1038/s41598-021-82410-1.
[13] K. B. dos Santos, I. A. Guedes, A. L. M. Karl, and L. Dardenne. Highly Flexible Ligand Docking: Benchmarking of the DockThor Program on the LEADS-PEP Protein-peptide Dataset, J. Chem. Inf. Model., Jan. 2020, doi: 10.1021/acs.jcim.9b00905.
[14] C. S. de Magalhães, D. M. Almeida, H. J. C. Barbosa, and L. E. Dardenne, A dynamic niching genetic algorithm strategy for docking highly flexible ligands, Information Sciences, vol. 289, pp. 206–224, Dec. 2014, doi: 10.1016/j.ins.2014.08.002.

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