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In silico Molecular Docking Studies of 1,2,4 Triazole-norfloxacin Hybrids with Topoisomerase II as Potent Antitubercular Agents

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ABSTRACT

Tuberculosis remains a prevalent issue in global public health, necessitating the development of new anti-tubercular medications. In this study, molecular docking techniques were utilized to explore the potential of 1,2,4 triazole-norfloxacin hybrids as inhibitors of MtTopo II. Utilizing BIOVIA Software, simulations were performed to predict the binding modes and affinities of these hybrids within the active site of MtTopo II. The compounds that designed were assessed for their binding ability to the DNA gyrase target. The molecular docking performed using LibDockScore function indicated compounds N11, N10, N6, N52, N48, N9, N38 and N14 to exhibit promising binding affinity (LibDock score 128.77, 127.73, 124.47, 122.10, 119.84, 119.17, 118.49 and 117.07 respectively) when compared to the reference norfloxacin (LibDock score 106.20) with the target 5BTC (topoisomerase II). The results emphasize the efficacy of docking studies in rational drug design against tuberculosis, with the objective of advancing novel therapeutic approaches to combat TB.

INTRODUCTION

Tuberculosis (TB) remains a major public health challenge on a global scale. TB ranks as the ninth foremost cause of mortality on a worldwide scale and is the predominant cause of death attributable to a singular infectious agent, surpassing HIV/AIDS. In the 2017 publication by the WHO concerning Global Tuberculosis, it suggests that approximately 10.4 million persons endured TB in 2016, which resulted in around 1.3 million deaths among individuals free from HIV, plus an additional 374,000 fatalities in HIV-infected individuals. The disease TB is a leading factor in worldwide fatalities, particularly in nations with lower and middle incomes. The World Health Organization (WHO) takes on a vital function in leading global programs that target tuberculosis, prioritizing prevention, diagnosis, and treatment approaches. The challenges associated with the treatment of tuberculosis (TB) encompass protracted and costly therapeutic regimens involving multiple antibiotic agents, which consequently result in hepatotoxicity and adverse effects that may precipitate premature cessation of treatment. Patients tend to discontinue their treatment upon the onset of symptom relief, consequently aiding in the emergence of drug-resistant TB variants, including those that are MDR and XDR. The hurdles faced in addressing tuberculosis (TB) relate to the strength of Mycobacterium tuberculosis to endure in host lesions, avoid immune challenges, and secure resistance to a range of drugs. It is crucial to identify drug-resistant TB, especially in scenarios of unsuccessful treatments or when closely interacting with those infected with multidrug-resistant TB, which calls for the use of advanced molecular diagnostic techniques for exact diagnosis.[1,2]

The necessity for improved treatments against tuberculosis is underscored by the formidable hurdles created by multidrug-resistant strains (MDR-TB). The existing second-line pharmacological treatments exhibit diminished efficacy and heightened toxicity, culminating in suboptimal therapeutic outcomes. Investigations into both synthetic chemical compounds and phytochemical derivatives provide promising avenues for the discovery of potent antitubercular agents. The exploration of novel agents is of paramount importance to mitigate tuberculosis, a significant global health menace characterized by millions of newly reported cases each year, as well as to fulfill the pressing demand for more effective treatment modalities against this infectious pathology.[3,4,5]

Bacterial DNA gyrase serves as a key focus for antimicrobial medications, especially fluoroquinolones, which operate by hindering both DNA gyrase and topoisomerase IV, ultimately leading to bacterial cell demise. Presently, moxifloxacin and gatifloxacin, identified as fluoroquinolone antibiotics, reveal significant power against Mycobacterium tuberculosis (Mtb). Advancing fluoroquinolones illustrate significant potential for decreasing tuberculosis treatment length, accompanied by minimal resistance found in clinical isolates of M. tuberculosis. Inhibitors targeting DNA gyrase prove to be effective against non-replicating mycobacteria, thereby contributing to the eradication of persistent organisms. The development of new inhibitors that focus on M. tuberculosis DNA gyrase unveils a compelling route for addressing multidrug-resistant tuberculosis (MDR-TB) and fluoroquinolone-resistant strains successfully.[6,7]

Molecular docking, a technique widely recognized in computational biology, plays a crucial role in the realm of

computer-aided pharmaceutical design, primarily aimed at anticipating how a ligand interacts and connects with its target receptor. This methodology has undergone substantial evolution from its original objective of elucidating molecular recognition mechanisms to becoming a multifaceted instrument in pharmaceutical discovery, facilitating operations such as lead optimization, biochemical pathway examination, as well as forecasting adverse effects, drug repurposing, alongside target identification and profiling. In the context of molecular docking investigations, a variety of software applications and databases are routinely employed to execute simulations, interpret outcomes, and substantiate predictions.[8,9,10]

In medicinal chemistry, 1,2,4-Triazole derivatives have emerged as a prominent subject of scholarly research, largely because of their various biological functions, particularly their prospective roles as antibacterial substances. These elements illustrate a comprehensive selection of pharmacological properties, involving antibacterial, antiviral, anticancer, anticonvulsant, and antifungal functions, thereby marking them as a multifaceted basis for pharmaceutical advancement. Through the implementation of targeted modifications to the chemical architecture, it may be feasible to enhance their potency against a wider spectrum of bacterial strains.[11,12,13] Fluoroquinolones are the type of synthetic antibacterial substances that find extensive use in the fields of human and veterinary medicine, owing to their comprehensive effectiveness and strong action against various bacterial threats. Fluoroquinolones are widely utilized for addressing urinary tract infections, stomach infections, and lung infections. The development of hybrids featuring 1,2,4-triazole and fluoroquinolone as antitubercular medications suggests a potentially meaningful tactic in addressing tuberculosis (TB). These pioneering hybrids unite the pharmacophoric qualities of 1,2,4-triazoles and fluoroquinolones, tapping into their microbial combat abilities to elevate potency against Mycobacterium tuberculosis. The hybridization of these two pharmacophoric structures is intended to formulate compounds that exhibit enhanced potency, diminished resistance, and superior pharmacokinetic characteristics.[14,15,16]

MATERIALS AND METHODS

Molecular docking studies

Molecular docking of the designed compounds into the active site of the proposed potential targets was conducted utilizing Discovery Studio (provided by BIOVIA Software Inc.). The crystal structure of Mycobacterium topoisomerase II (PDB ID: 5BTC), which was taken from the RSCB Protein Data Bank, was subjected to docking simulations. The validation of the protein structure was accomplished through a Ramachandran plot, which evaluates the quality of protein models by correlating the torsional angles (ϕ and ψ) of amino acid residues against one another, with the majority of residues residing within the permissible regions. The protein structure was prepared utilizing the clean protein tool and the prepare protein protocol in Discovery Studio to standardize atom nomenclature, rectify connectivity and bond order, protonate ionisable residues at a physiological pH of 7.4, incorporate missing residues and correct incomplete ones, and eliminate water molecules. To validate the docking protocol, the co-crystallized ligand was isolated and re-docked into the enzyme's active site to ensure proper definition of the binding site and to assess the accuracy of the docking algorithm in reproducing the pose of the co-crystallised ligand. Docking studies 1,2,4-triazole-norfloxacin hybrids were conducted against their prospective targets. The ligands were positioned within a rigid receptor framework, while a series of ligand conformations was generated. Diverse poses for each ligand were produced upon the completion of every docking computation. A LibDockScore function was employed to identify the most favorable docked structure. Additional scoring functions were applied to ascertain the optimal orientation of the ligand within the active binding site of the target. The highest score, indicative of the most robust receptor-ligand binding affinities, was taken into consideration for the refinement of binding poses. The calculation of binding energy was carried out.

Pharmacokinetic analysis

Physicochemical properties are essential characteristics for an optimal drug candidate and significantly influence the ability to cross cellular barriers to attain its intended targets. Consequently, the drug likeness of these compounds was subsequently evaluated in accordance with Lipinski's rule of five. The data regarding physicochemical properties presented in the table has been obtained through the utilization of www.swissadme.ch.

Table 1: Docking Results

S. No.	LigandCode	AbsoluteEnergy	Relative Energy	LibDockScore
1	N1	91.2596	1.29747	106.408
2	N2	86.9183	1.6076	113.717
3	N3	86.9747	1.92091	112.228
4	N4	95.4995	15.1932	112.674
5	N5	86.6812	6.77197	114.438
6	N6	105.798	4.5348	124.473
7	N7	95.649	9.83655	109.567
8	N8	100.882	11.7866	107.871
9	N9	115.114	15.6062	119.178
10	N10	116.257	16.1976	127.732
11	N11	115.072	18.7668	128.779
12	N12	112.818	11.722	116.066
13	N13	113.767	13.6511	111.928
14	N14	118.134	15.7946	117.075
15	N15	97.3089	7.38491	101.123
16	N16	101.094	15.7409	105.344
17	N17	87.0098	7.72271	110.8
18	N18	95.644	17.1198	103.377
19	N19	95.303	17.4728	114.803

20	N20	112.017	12.9786	108.901
21	N21	91.7805	7.78471	106.398
22	N22	97.9902	10.3773	113.134
23	N23	102.652	10.4212	109.241
24	N24	107.796	11.2326	112.149
25	N25	115.92	16.8043	100.414
26	N26	107.603	7.50143	108.016
27	N27	108.762	10.0067	106.445
28	N28	110.059	9.30057	105.034
29	N29	91.3017	12.2124	110.867
30	N30	96.3141	15.3747	108.5
31	N31	84.9893	12.4203	103.684
32	N32	91.1193	7.44334	94.3278
33	N33	87.9217	13.4877	115.782
34	N34	104.956	11.6798	113.887
35	N35	93.4195	15.8111	109.37
36	N36	101.458	19.8758	115.434
37	N37	101.873	11.2928	111.975
38	N38	105.038	11.2801	118.498
39	N39	107.62	14.7428	113.185
40	N40	105.38	14.5616	114.951
41	N41	104.8	12.3633	101.902
42	N42	103.325	12.565	115.546
43	N43	89.8128	6.9638	114.188
44	N44	95.8234	15.0525	111.994
45	N45	114.764	11.8199	99.0799
46	N46	90.8723	15.4754	115.402
47	N47	107.43	11.6479	92.9089
48	N48	95.2943	2.51592	119.849
49	N49	96.5096	16.1071	108.377
50	N50	91.0182	12.2825	111.605
51	N51	102.267	15.5126	112.701
52	N52	102.979	9.49008	122.108
53	N53	96.1877	5.91724	115.431
54	N54	111.769	9.6677	93.4376
55	N55	109.651	14.331	115.956
56	N56	110.329	16.6968	115.333
57	N57	84.2339	0.183245	111.803
58	N58	85.1229	5.87866	106.861
59	N59	89.0924	16.0069	112.157
60	N60	106.638	10.856	93.9595
NRF	NRF	42.1504	11.4829	106.206

Table 2: Pharmacokinetic analysis

S.No.	Ligand Code	Mol. Wt	No. of Rotatable Bonds	H acceptor	H donor	CLogP
1	N1	568.62	8	8	1	2.95

2	N2	582.65	9	8	1	3.14
3	N3	573.04	7	7	1	3.35
4	N4	556.58	7	8	1	3.16
5	N5	554.59	7	8	2	2.55
6	N6	616.67	8	9	1	2.68
7	N7	604.65	8	8	1	3.38
8	N8	620.72	8	7	1	4.03
9	N9	630.69	8	8	2	3.64
10	N10	630.69	8	8	2	3.67
11	N11	630.69	8	8	2	3.66
12	N12	615.68	8	8	1	3.4
13	N13	615.68	8	8	1	3.32
14	N14	615.68	8	8	1	3.34
15	N15	582.65	9	8	1	3.15
16	N16	596.67	10	8	1	3.51
17	N17	587.07	8	7	1	3.53
18	N18	570.61	8	8	1	3.32
19	N19	568.62	8	8	2	2.65
20	N20	630.69	9	9	1	3.08
21	N21	618.68	9	8	1	3.6
22	N22	634.74	9	7	1	3.97
23	N23	644.72	9	8	2	3.8
24	N24	644.72	9	8	2	3.85
25	N25	644.72	9	8	2	3.84
26	N26	629.7	9	8	1	3.6
27	N27	629.7	9	8	1	3.5
28	N28	629.7	9	8	1	3.54
29	N29	573.04	7	7	1	3.48
30	N30	587.07	8	7	1	3.71
31	N31	577.46	6	6	1	3.69
32	N32	561	6	7	1	3.53
33	N33	559.01	6	7	2	2.96
34	N34	621.08	7	8	1	3.27
35	N35	609.07	7	7	1	3.73

36	N36	625.14	7	6	1	4.38
37	N37	635.11	7	7	2	3.93
38	N38	635.11	7	7	2	3.94
39	N39	635.11	7	7	2	4.03
40	N40	620.1	7	7	1	3.8
41	N41	620.1	7	7	1	3.7
42	N42	620.1	7	7	1	3.71
43	N43	556.58	7	8	1	3.26
44	N44	570.61	8	8	1	3.49
45	N45	561	6	7	1	3.49
46	N46	544.55	6	8	1	3.28
47	N47	542.56	6	8	2	2.71
48	N48	604.63	7	9	1	3.09
49	N49	592.62	7	8	1	3.52
50	N50	608.68	7	7	1	4.12
51	N51	618.65	7	8	2	3.85
52	N52	618.65	7	8	2	3.89
53	N53	618.65	7	8	2	3.9
54	N54	603.64	7	8	1	3.64
55	N55	603.64	7	8	1	3.51
56	N56	603.64	7	8	1	3.52
57	N57	554.59	7	8	2	2.57
58	N58	568.62	8	8	2	2.78
59	N59	559.01	6	7	2	2.76
60	N60	542.56	6	8	2	2.62

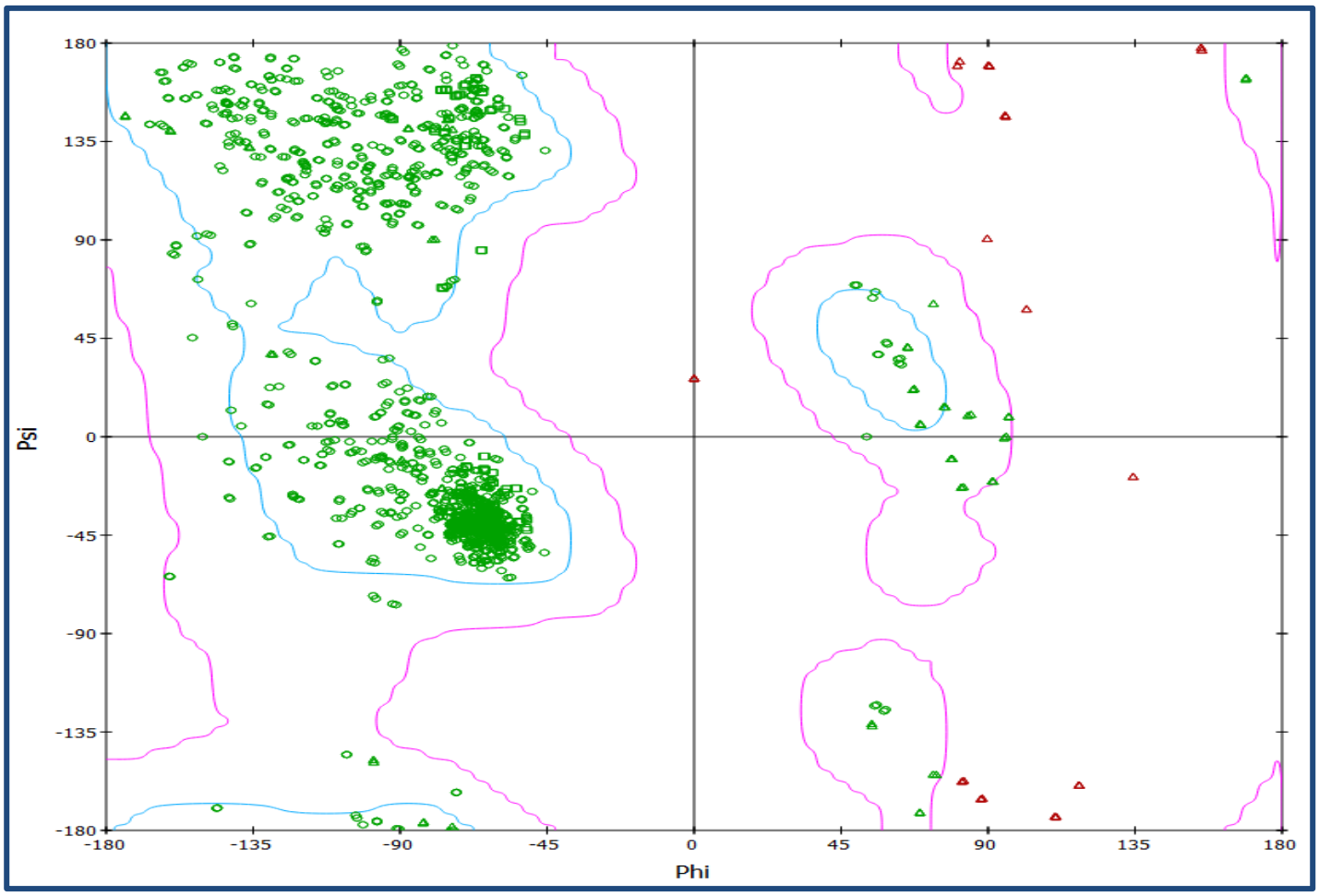

Fig.1. Ramachandran Plot of Protein (5BTC) utilizing Discovery Studio (provided by BIOVIA Software Inc)

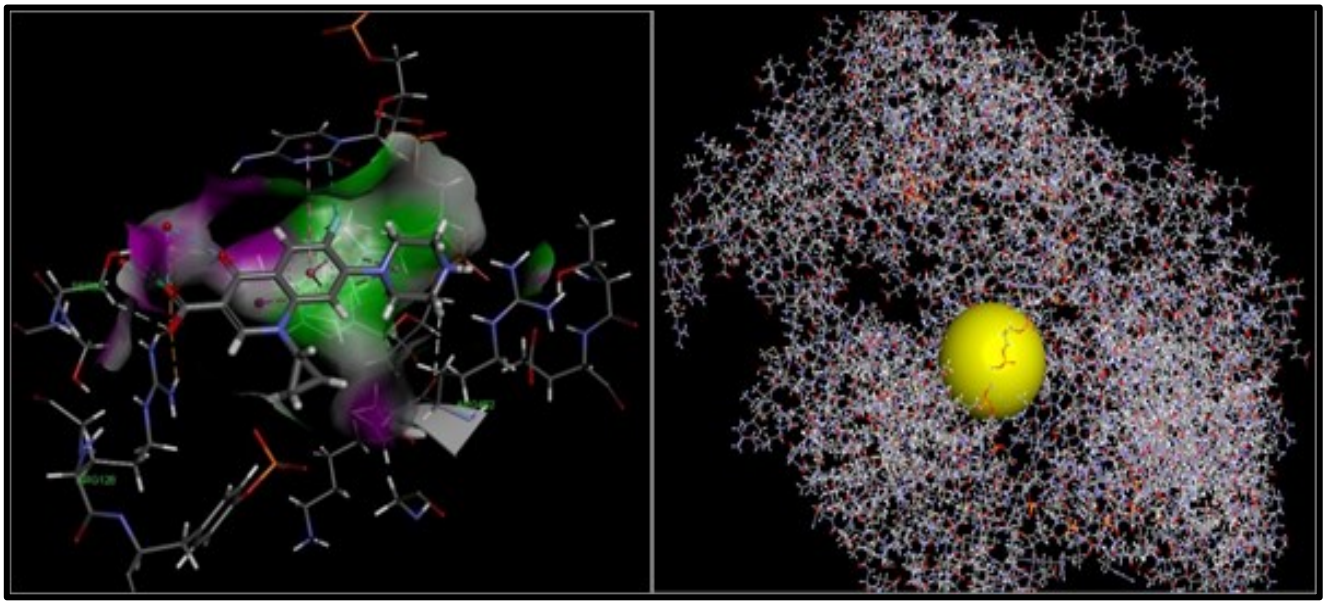

Fig.2. Surface Interaction & Active Site Sphere utilizing Discovery Studio (provided by BIOVIA Software Inc)

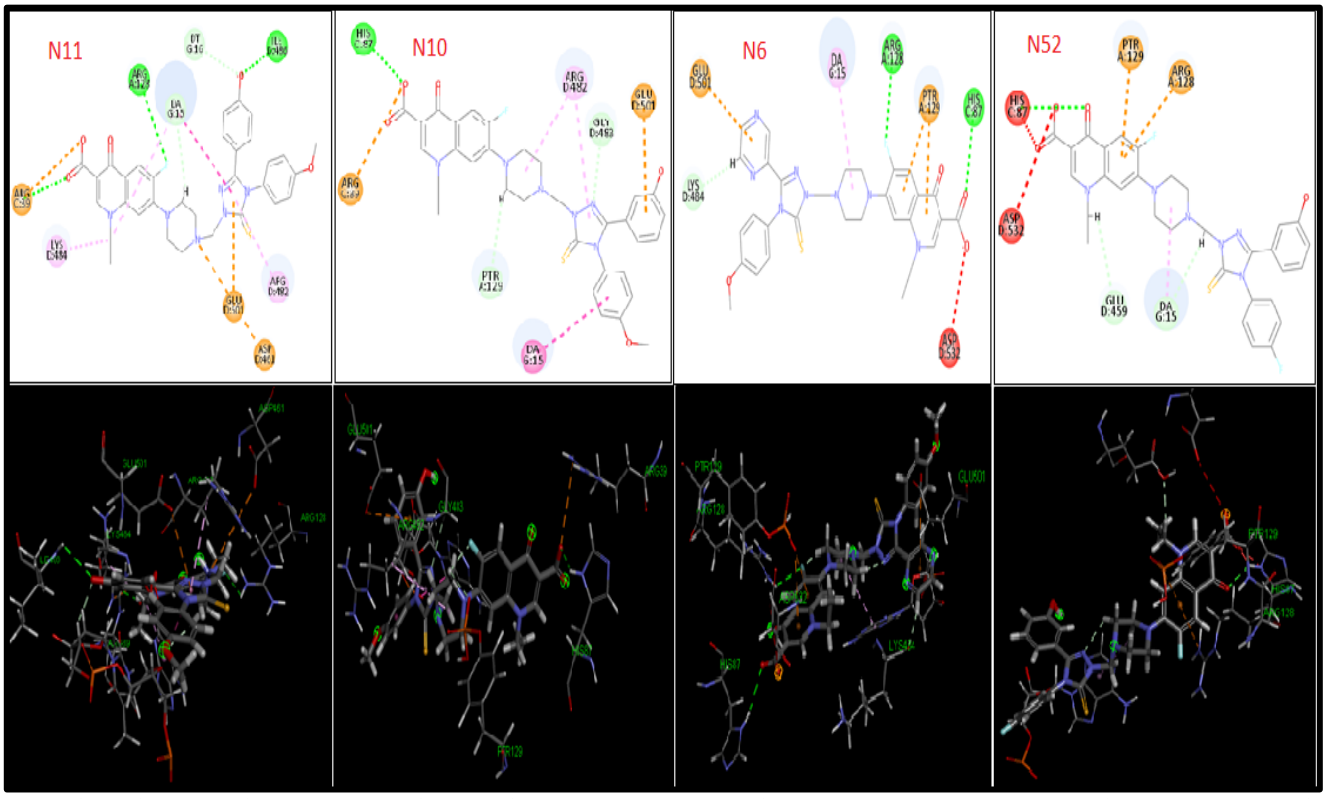

Fig.3. Three dimensional interactions of the Ligand N11, N10, N6 & N52 with active site of protein (5BTC), utilizing Discovery Studio (provided by BIOVIA Software Inc)

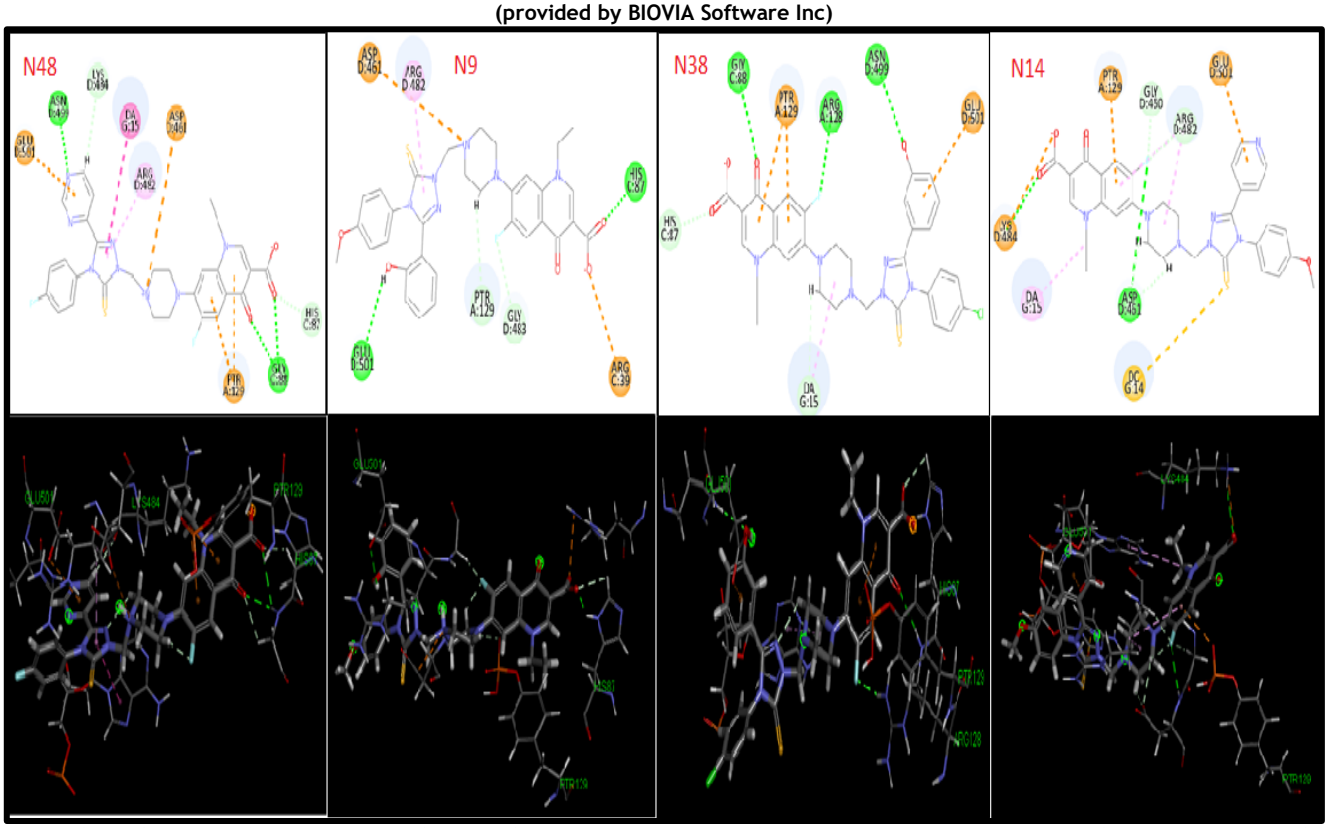

Fig.4. Three dimensional interactions of the Ligand N48, N9, N38 & N14with active site of protein (5BTC), utilizing Discovery Studio (provided by BIOVIA Software Inc)

RESULT AND DISCUSSION

The docking studies involving 1,2,4-triazole-norfloxacin hybrids with Mycobacterium topoisomerase II (5BTC) shown significant findings. The compounds N11, N10, N6, N52, N48, N9, N38, and N14, which achieved the highest LibDock scores of 128.77, 127.73, 124.47, 122.10, 119.84, 119.17, 118.49, and 117.07, respectively, exhibit promising binding affinities in comparison to the reference compound NRFX norfloxacin (LibDock score 106.20) with the target enzyme 5BTC (topoisomerase II), as illustrated in Table 1. Molecular docking analyses revealed that the compounds predicted the lowest binding energies with topoisomerase II (PDB ID: 5BTC), suggesting a strong likelihood of favourable binding interactions with the target enzyme. Moreover, the predicted lowest binding energies were found to be superior to norfloxacin. The majority of the ligands show interaction with the amino acids ARG482, GLU501, LYS484, and ARG482 in relation to the topoisomerase II target. The test compounds exhibited favorable interactions with the active site of the Topo II enzyme, displaying a binding pattern that closely resembles that of norfloxacin. Norfloxacin showed interaction with the amino acids GLU501 and ARG482. Ligands N6, N10, N14, and N48 exhibited a similar interaction pattern with the amino acid ARG482. Norfloxacin's piperazine moiety's N-terminal region exhibited a relationship with the GLU501 residue in topoisomerase II. However, in the context of the hybrid compounds, the N-1 position of the triazole was anticipated to interact with GLU501 due to its deeper penetration into the binding pocket. The analysis of docking results suggested that adding 1,2,4-triazole groups at the N4 site of piperazine in norfloxacin might greatly enhance its binding strength to topoisomerase II. The carboxyl group present in norfloxacin exhibited ionic interaction with Mg2+. In comparison to norfloxacin, the hybrid compounds also demonstrated additional binding interactions involving aromatic rings with residues DA15 and DC14, while the hydrogen atom at the N4 position of the piperazine ring interacted with DC14. The introduction of substituents that either electrons withdrawing or donating groups play a significant role in this investigation. The incorporation of electron-donating substituents within the phenyl rings of triazole enhances the binding affinity towards the target site.

The Pharmacokinetic analysis constitute fundamental attributes for an ideal drug candidate and play a crucial role in determining the capacity to traverse cellular membranes to reach designated targets. As a result, the drug likeness of these molecules was subsequently assessed in alignment with Lipinski's rule of five. All hybrids had calculated cLogP values ≤5, indicating favourable solubility and permeability. The triazole-norfloxacin hybrids exhibited cLogP values ranging from 2.55 to 4.38, reflecting their lipophilic properties (Table 2). The compounds were determined to possess 1-2 hydrogen bond donors and 6-9 hydrogen bond acceptors, thereby conforming to the established criteria. Although the molecular weights were slight higher than the stipulated guidelines, they were deemed acceptable within reasonable limits. Therefore, all these hybrids were considered to possessing favourable drug likeness.

CONCLUSION

The hybrids exhibit promising binding affinities toward the active site of Mycobacterium tuberculosis topoisomerase II, suggesting a significant potential for interaction, and alterations at the 1,2,4-triazole position improved the binding affinity. Docking simulations elucidate precise binding orientations and interactions between the hybrids and the enzyme, thereby clarifying the structural foundation for their prospective role as inhibitors. Consequently, this investigation establishes a preliminary framework for the innovation of novel analogues characterized by enhanced potency as topoisomerase II inhibitors. These finding provide a robust basis for future research and development initiatives designed to more effectively address the challenge of tuberculosis.

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CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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