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Identification of Gatifloxacin Derivative Asmycobacterium Tuberculosis Dnagyrase Inhibitor Using a Knowledge-Based Computational Molecular Screening Approach

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ABSTRACT

Tuberculosis (TB) is a respiratory disease caused by Mycobacterium tuberculosis (MTB) infection. The rapid emergence of MTB strains resistant to numerous antimicrobial drugs is posing a great challenge in controlling the tuberculosis related morbidity or mortality. Therefore, in this study, chemical derivatives of Gatifloxacin (GTF), which could act against the MTB-DNA gyrase enzyme that plays important role in DNA's negative supercoiling during DNA replication, gene expression, and recombination processes, were designed. In this regard, a series of computational molecular screening approaches like sequence extraction and 3D modelling of DNA gyrase and its binding analysis with Gatifloxacin derivatives with molecular dynamics and molecular docking analysis were performed. Findings from molecular docking have concluded GTF-18 (1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-1-piperazinyl) 1,4-dihydro-3-quinolinyl] carbonyl borodifluoridate) as the best anti-MTB DNA gyrase compound with significant physicochemical properties. This GTF-18 compound demonstrates better affinity towards MTB-DNA gyrase (ΔG is -18.27) compared to parental Gatifloxacin molecule. This study advises future laboratory assays to develop GTF-18 as a novel drug compound for targeting MTB-DNA gyrase.

INTRODUCTION

Tuberculosis (TB) is a respiratory disease caused by the chronic infection of the lungs by Mycobacterium*tuberculosis*(MTB)(Furin et al. 2019).It normally remains inert in the body without any symptoms, but 5-10% of individuals develop clinical manifestations that can be exacerbated by co-infection with HIV (Harries and Dye 2006). Patients may have lethargy, night

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sweats, fever, cough, and hemoptysis when the infection is active. Multidrug-resistant tuberculosis (MDR-TB) strains have afflicted more than 50 million individuals worldwide, but only a few potentialdrugs have been discovered in the last 40 years (MacNeil et al. 2020)(Alvi et al. 2015). With the emergence of strains resistant to numerous antimicrobial drugs, the situation surrounding tuberculosis control has drastically worsened during the previous decade.Given the rapid emergence of drug resistance in MTB, researchers need to look for new alternative therapeutic compounds that are more effective and less harmful(Allué-Guardia et al. 2021). These chemicals ought to be capable of penetrating the mycobacterial cell wall and have beenshown to be beneficial in controlling bacterial growth both in the acute and chronic growth phases (Abrahams and Besra 2018).

DNA gyrase is a well-known therapeutic target among the 150 metabolic pathways and 197 pharmacological targets in M.*tuberculosis*(Gibson et al. 2018).DNA gyrase is a unique tetrameric enzyme involved in DNA's negative supercoiling during DNA replication, gene expression, and recombination processes. MTB-DNA gyrase is a promising target for small molecule inhibitors due to the absence of a homologue in eukaryotic cells. Fluoroquinolones are a class of broad-spectrum antibiotics, which impede the unwinding and replication of bacterial DNA (Fu et al. 2017). Because bacteria and humans use distinct enzymes to unwind DNA, most of the topoisomerases in humans are unaffected (Velayati et al. 2009). Screening bioactive chemicals in the laboratory using cellular systems and model organisms can be a difficult and time-consuming approach. Computer Aided Drug Designing (CADD) along with bioinformatics methods, on the other hand, speeds up drug target identification by enabling rapid drug candidate screening, refinement, and predictions of pharmacodynamic features (Shaker et al. 2021)(Alsulaimany et al. 2022). Therefore, in this study, we used a diverse computational approach to find a potential lead molecule against DNA gyrase in multidrug-resistantMycobacterium tuberculosis.

MATERIALS AND METHODS

Sequence Analysis

The amino acid sequence of MTB-DNA gyrase (MTB: TBMG_00040) initially obtained from NCBI was used to search for homologous sequences through the BLASTp server (Altschul et al., 1990). Then the resultant MTB-DNA gyrase sequences were aligned with each other through the

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multiple sequence alignment method using the ClustalW1.8 webserver using default parameters (Hung and Weng 2016). To discover functionally relevant conserved amino acid residues in the DNA gyrase family of enzymes, they were categorised based on their sequence sources. In this procedure, the sequence of MTB-DNa Gyrase was entered into the PDB database, along with structures of E. *coli* DNA Gyrase (PDB code 1AB4) and S. *aureus* topoisomerasae IV (PDB code 2INR). Clustal-W (Sievers and Higgins 2018) was used to align the homologous sequences of DNA gyrase protein by calculating the best sequence matches and lining them up to enable the easier identification of similarities, and divergences in the protein models built.

Homology modeling and optimization of MTB-DNA gyrase

The MTB-DNA gyraseprotein model was constructed by homology modelling approach in correspondence to the crystal structure coordinates of topoisomerase IV enzymes of *S.aureus*(2INR) and *E. coli* (1AB4). We used MODELLER 9v1 webserver for this process.The most reliable protein model of MTB-DNA gyrase was obtained after many iterations of models created by homology approach. On the graphic screen, a primary structural analysis was done to check the consistency of the alignment models of variable loops at different sub sites in the built protein model.

Refinement and molecular dynamics (MD) stimulation analysis of MTB-DNA Gyrase

The Gromacs Molecular Dyanamics tool, specifically the force field "ffG43a1" (GROMOS96) was used to refine the 3-D model of MTB-DNAgyrase(Rahimi et al. 2016). A Linear Constraint Solver for molecular simulations (LINCS) algorithm was used to optimize all types of hydrogen and ionic bonds in the protein structure (Hess., et al., 1997). First, 100ps of molecular dynamics at 300 K was carried out using the water molecules inside box (10 Å buffer distance), allowingsolventsettlement around the amino acids of the target protein. All protein atoms were found to be restricted in their orbits in this molecular dynamics' procedure. Then, for long-range interactions, a full molecular dynamics simulation was run for 5000 ps at 300 K with no restrictions using 1 fs of integration time and a cutoff of 14. For these simulations, a dual-range threshold was used for long-range interactions: 1.5 nm for electrostatic interactions and 1.2 nm for van der Waals interactions, since these proteins possess only a residual net charge that was balanced by counter-ions. The lengths of hydrogen bonds were constrained using the Shake method. At 14Å, it was observed that effects of

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electrical potential truncations were minimal. Considering that protein and water in the simulation box can reach a total of 130,000 atoms during the simulation, the average fluctuations of several energies for the 5000ps trajectory were determined to investigate the stability of MTB-DNA gyrase.

Evaluation of MTB-DNA gyrasemodel

Different tools such as PROCHECK (Laskowski et al. 1993), WHAT-IF (Vriend 1990), and ProSA-web were used to examine the accuracy of the refined MTB-DNA gyrase model (Sippl 1993).

Secondary structure determination of MTB-DNA gyrase

The PDBSUM online server was used for simulating the secondary structure of MTB-DNA gyrase. This website provides detailed information on the structural organization of helices, beta sheets, and turns (Laskowski et al. 2018). The Motif Scan server was utilized to identify motifs or domains contained in the MTB-DNA Gyrase model. First Glance Jmol (http://firstglance.jmol.org/) was used to check the ionic interactions of amino acid residues in the MTB-DNA gyrase model. The PDBSUM server was used to look at the active sites of MTB-DNA gyrase. These sites were then confirmed by looking at the binding sites of other proteins that are likeMTB-DNA gyrase.

Docking of Flouroquinoeinhibitors withMTB-DNA gyrase

Gatifloxacin is used as a major frontline therapy against MTB worldwide. Thus, we selected Gatifloxacin as parental lead molecule (scaffold for lead design), and appropriate chemical changes were performed on it to generate series of ligands. In designing ligand molecules, all the chemical changes were done by considering a database of substituents and spacers (linkers) obtained by substructure analysis of drugs, and other biological molecules using Molinspiration server (http://www.molinspiration.com). Gatifloxacinand its chemical derivatives were docked againstMTB-DNA gyrase to find out the best possible inhibitory pose. HyperChem Professional software was used to design and perform complex chemical calculations of Gatifloxacin derivatives.During the ligand designing, rotatable bonds were manually assigned, side chains rotated,while the backbone was kept rigid. Bond rotations were kept minimal at one side of the molecule while designing the ligand molecules. Besides, assigning rotations, all the non-polar hydrogens were eliminated, and then partial charges were added to the central carbon molecule holding the hydrogen. These 100 molecules were then subjected to Chem Office Ultra 7.0 to find their log P values and these molecules were given chemical formulae names by using the same

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software. All these pharmacophore molecules were then analyzed against Lipinski's rule-of-five by Molinspiration server (http://www.molinspiration.com). This server gives topological surface area (TPSA), Log P (partition coefficient) values, molecular weight (MW), number of H-bonding donors, number H-bonding acceptors and number of rotatable bonds for the ligands(Lipinski et al. 1997). The rule explains how molecular characteristics of a drug affecting*in vivo*pharmacokinetics such as absorption, distribution, metabolism, and excretion (ADME).According to Lipinski's rule of five, an orally active drug should possess less than 5 H_2-bond donors (OH and NH groups), less than 10 H_2 - bond acceptors (particularly N and O), a MW of less than 500g/mol, and a partition coefficient log P of < 5. The high-ranking ligand molecules following Lipinski's were chosen for docking on the MTB-DNA gyrase model using the AutoDock Tool.

Mtb-DNA gyrasemodel docked with designed Gatifloxacin lead molecules

The docking interactions of ligand molecules on the MTB-DNA gyrase model were analyzed using AutoDock 4.0 (ADT) (Morris et al. 2009). To run ADT, histidine hydrogens and polar hydrogens were introduced to the PDB file of the Mtb-DNA gyrase model, the C- and N-terminal ends of the molecule were charged, and Kollman atom partial charges were assigned. PRODRG2 Server was received from the Ligand pdbq file (Schüttelkopf and van Aalten 2004). In the ligand, all atom types were examined. The Gasteiger charges for the atoms were calculated after the charged ligands were chosen and hydrogens were added to cover all empty valencies. Grid maps were calculated to perform AutoDock procedure. This was accomplished by using the AutoGrid module with set up of X, Y, and Z grid points to 60x60x60 with 0.375 Å as grid space.

To avoid any asymmetrical issues, the grid center was set to be somewhat off the active site's center axis. A Python script that uses AutoDock methods created the docking parameter files (dpf) in the docking matrix. The script reads one pdbqs file, cycles through the pdbq files, and sets the map and ligand names in the parameter file. It also specifies a population size of 150 people for the Lamarckian genetic algorithm (LGA). Thus, 150 individuals were estimated throughout 100 separate runs (i.e., 100 dockings), with two stop criteria: a maximum of 2,500,000 energy assessments or a maximum of 27,000 generations for each run. The ligands were configured to start in a random position and conformation, with a maximum translation speed of 2 Å/step and a maximal quarterion and tortion speed of 50^o/step. The number of elitists was fixed at one. The

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crossover rates were 0.02 and 0.80, respectively. The individual population search parameter was set to 0.06, and 150 iterations were used for each pseudo-solis local search. Local search space was set to 1.0, and the least step that could be taken before the local search ended was set to 0.01. These standardized docking settings generate a file for each ligand, which was then run using the AutoDock application. The substrate, flavin adenine dinucleotide (FAD), was docked to the MTB-DNA gyrase model using the parameters same as the lead molecules as mentioned above. Docking reaction took between 7 and 30 minutes depending on the ligand complexity, number of rotatable, and atom number. PMV (Python Molecular Viewer) 1.4.5 was used to assess the docking results in a graphical format.

RESULTS AND DISCUSSION

Sequence Analysis

The amino acid sequence of MTB-DNA Gyrase retrieved from NCBI is found to be composed of 838 amino acids. The multiple sequence alignment of MTB-DNA Gyrase sequences from *S.aureus*topoisomerase IV (2INR) and *E.* coli (1AB4) revealed the mutagenic status of DNA Gyrase. In the MTB-DNA gyrase, there were 48 conserved amino acid residues. In the C-terminal region, amino acids are deleted, and more than 120 amino acid insertions are found in the N-terminal regions of the MTB-DNA Gyrase protein sequence. The distances between the different forms of DNA gyrase have been calculated using cladogram trees (MTB-DNA: 027.898, S. aureus-DNA Gyrase: 0.28912, E. coli-DNA Gyrase: 0.24841).

Homology Model of MTB-DNA Gyrase

To generate MTB-DNA gyrase model at atomic scale resolution, an alignment file and script file were necessary. So, first MTB- DNA gyrase model was built by Modeller 9v1 program using the crystal co-ordinates from organisms such as E. coli (1AB4) and S.Aureus (2INR). The Modeller program uses the spatial constraints in the template structures and builds a tertiary structural model of the target molecule. 100 runs were carried out to obtain the most reasonable model, which was later optimized using Modeler. Various secondary structural elements **(Figure 1A)** were visualized in the built model using the Pymol software.

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Validation of the models

PROCHECK, WHATIF and PROSA analysis software tools have assessed the stereochemical and structural evaluation of the homology model of MTB-DNA gyrase. The quality of the energy minimized model of MTB-DNA gyrase was determined with help of PROCHECK (Laskowski, 1993). The Ramachandran plot constructed for MTB-DNA Gyrase model showed a good distribution for the Φ/ψ angles of all amino acid residues where, 99.09% of the residues are in the most favored regions, while only 0.1% of amino acids fell in disallowed regions (Figure 1B). Val 58 is situated in the first part of 17^{th} beta turn and Leucine 78 is situated in the 3^{rd} alpha-alpha-beta motif. According to the main chain properties of the MTB-DNA Gyrase model, there are no significant bad contacts. In addition, the average G-factor, which measures the degree of normality of the protein's properties, is within the permitted range(Laskowski, MacArthur, Moss and Thornton 1993). The side chain parameters of MTB-DNA gyrase shows that the χ1- gouche minus standard deviation, trans standard deviation, gouche plus standard deviation and pooled standard deviation and χ2- trans deviation are in good agreement with the expected values (Dunbrack and Karplus 1994). Bond and hydrogen bond lengths did not significantly deviate from standard mean values. On the basis of the PROCHECK criteria, the MTB-DNA gyrase model was determined to be a good structure suitable for molecular docking and dynamics. From WHATIF analysis, it is seen that Ramachandran- z score values for bonds and angle parameters for the model were within the typical of highly refined structures. Furthermore, the MTB-DNA gyrase was analyzed after refinement with GROMACS, which explains the larger deviations in bond length and angles. This result shows that Z-score average packing quality of MTB-DNA gyrase model is -1.149 and back bone conformation is -2.536 which is within range of good quality (Figure 1C).The ProSA-web analysis of MTB-DNA gyrase revealed that the z-score and energy graphs were nearly identical and correlated with the NMR energy pattern. The analysis revealed that the Z scores for MTB-DNA gyrasewas -4.5. The analysis of a 3D structure reveals that energy graphs with negative values correspond to stable parts of the structure, and that the z-score indicates overall model quality and measures the deviation of the structure's total energy from a distribution of energy deduced from random conformations(Sippl 1995).

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Refinement and MD Simulation

The energy of the MTB-DNA gyrase model was lowered with the Gromacs force field. Then, this energy-minimized crystal structure of MTB-DNA gyrase was selected to complete molecular dynamics simulations utilizing the GROMOS 96 force field. The purpose of this procedure was to place both enzymes in the same physiologic conditions for further superposition, as well as to provide a method for additional validation and refinement of the structure of MTB-DNA gyrase. It showed an energy of -1.4571 K.cal. after the refinement step. A model can only be deemed acceptable if its average structure is conserved during the entire molecular dynamics simulation period. To examine the stability of the MTB-DNA gyrase, fluctuations of several energies were calculated for the 5000 ps trajectory after the dynamics simulations. The total energy fluctuation, mean square fluctuation (RMSF), was found to be as low as 0.2% **(Figure 2A).** The kinetic and potential energies fluctuated by 3.5% and 0.49%, respectively. The time evolution of the root mean square deviation (RMSD) of the MD trajectory from the X-ray structure was utilized to determine the stability of the MTB-DNA gyrase structure after the 2000ps MD simulation procedure.

Within the first 10 psec of the simulation, the RMSD increased rapidly to 0.6 Å for the main chain atoms and 0.4 Å for the side chain atoms, respectively. Thereafter, the RMSD values decreased gradually for the main chain atoms while increasing for the side chain atoms. The total RMSD values were < 0.6 Å, indicating that MTB-DNA gyrase possesses a high degree of structural stability **(Figure 2B).** Our findings confirmthe MD results of Murc in MTB(Alahari et al. 2009), serine hydroxymethyltransferase in MTB. The RMSF graphs of Cα-chain, backbone, main chain and side chains indicated that N-terminal residues have greater RMSF values than active site residues. Throughout the duration of the dynamic simulations, the total protein's largest fluctuations exceeded 0.1 nm. Dynamic plots showed fluctuations close to 0.5 to 0.6 nm for the N-terminal amino acid residues (1-15), as previously reported in case of MurC of MTB (Alahari, Alibaud, Trivelli, Gupta, Lamichhane, Reynolds, Bishai, Guerardel and Kremer 2009). Based on MD simulations, the side chains of Leu105, Ser1478, Gly177, and Asn176 in MTB-DNA gyrase are found to be oriented at approximately 3Å to 4Å. This orientation enables efficient binding of inhibitors to MTB-DNA gyrase, as compared to substrate. These significant binding differences lead to the development of new anti-MTB drugs.

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Secondary Structure Analysis

The secondary structure characterization of MTB-DNA gyrasemodel carried out through the PDBSUM online server showed various secondary structure elements like helices, sheets, strands, turns and loops etc., The secondary structure analysis of 487 residues of MTB-DNA Gyrasemodel shown 6 beta sheets, 7 beta hairpins, 17 strands, 18 helices, 31 beta turns and 4 gamma turns and over all the MTB-DNA Gyraseconsist of 68 amino acids strands (68%), 240 amino acids Alpha helix (49.3%), 4 amino acids helix (0.8%) and remaining 175 amino acids belongs to other secondary structural elements (35.5%).

Active site Analysis

Active site analysis carried out through the PDBSUM server and with multiple sequence alignment, the result revels that all the DNA gyrase and MTB-DNA gyrase active site residues are highly conserved (Leu105, Ser1478, Gly177 & Asn176). PDBSUM results revels the similar active site residues (Leu105, Ser1478, Gly177 & Asn176) are interacting with the crystallized complex of DNA Gyrase (Figure 2C).

Electrostatic potential Analysis

The protein electrostatic potential, which is influenced by charged side chains as well as bound ions determines different parameters of the protein like its polypeptide folding and stability, enzyme activity, and protein-protein recognition. The electrostatic surface of MTB-DNA Gyrase was calculated using APBS (Baker et al. 2001) by assigning the Amber force field (default 99 charges and 0.46 raddi). The electrostatic images generated were viewed and analyzed using Pymol. The majority of the MTB-DNA Gyrase active site residues are seen to be belonging to the negative potential surface, where the inhibitor interactions occur (Figure 2D).

Analysis of docking results for best lead molecules

MTB-DNA gyraseinhibitors and new designed lead molecules were analyzed with AUTODOCK 4.0 output file docking log file information. The lead molecules, the torsional energy of ligand was constant with 2.18 kcal/mol. Binding energy of ligand is the sum of intermolecular energy and torsional free energy (ΔG). Docked energy is the sum of intermolecular energy and internal energy

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of ligand. Results were obtained under 298.15 K temperatures with solvation of water molecule as the solvent parameter. Based on docking energy and binding energy parameters all eight molecules were subjected to screening to obtain the best molecules for further pharmacological studies. Based on the inhibitors interaction with Leu105, Ser1478, Gly177 & Asn176 it is confirmed that crystal structure and docking procedures are correct and results are valid for further analysis (Figure 3A).

Docking with Available Inhibitors with MTB-DNA gyrase

Studying the MTB proteins and inhibitor interactions by their crystal structures continue to serve as a starting point for the evaluation of various docking methods(Blanco et al. 2015). The first docking study of MTB-DNA gyrase was conducted by the Kuntz laboratory (Shoichet and Kuntz 1991), which revealed that haloperidol may inhibit this enzyme. However, subsequent determination of the crystal structure of haloperidol revealed a different orientation than predicted (Drlica and Zhao 1997). Docking methods and algorithms were evaluated using the known structural data and experimental characteristics by Monte Carlo docking (Cavalli et al. 2003)or by comparing with de novo constructed antagonists by a fragment-based approach(Ganapathy et al. 2021)(Rotstein and Murcko 1993), where the inhibitors were constructed entirely from individual functional groups selected from a predefined library. SCULP's method of continuous energy minimization was a new paradigm for modelling proteins in interactive computer graphics systems (Rustin et al. 1994). This physically realistic effort made it possible to model very large conformational changes and enhanced our understanding of how different energy terms relate to stabilize a given conformation. Other recent studies have explored evidence based free energy target function in docking and design, demonstrating the advantages of this approach over studies using the calculation of interaction energy (King et al. 1996)(GL et al. 2020).

The inhibitor Gatifloxacin tightly packed with 4 important residues are Leu105, Ser1478, Gly177 & Asn176 of MTB-DNA Gyrase (Figure 3A). The analysis of the number of occurrences below zero and its distribution it indicates that Gatifloxacin have the best response inhibiting the DNA Gyrase.Of the 100 Gatifloxacin analogs designed 24 molecules passed the Lipinski rule of 5 (Table 1). These Gatifloxacin analogs were found to be interact with the enzyme molecule same as parental inhibitors (Table 2).

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Docking of selective high ranked ligands on to MTB-DNA gyrase model

From these 24 ligand molecules the first 5 ligands, were chosen for docking simulations on to MTB-DNA gyrasecrystal structure using Auto Dock 4.0. The dock program searches for best conformation and best place of binding of the ligand within a fixed structure of MTB-DNA Gyrase model. Each of the molecules was set with docking simulations of 100 for docking on to the MTB-DNA gyrase model and simulations allow us to efficiently study the ligand binding processes to design those ligands with the best binding kinetics.To orientthe ligand molecules to active sites, all the spheres are matched to ligand atoms in order to pair the center of sphere with the ligand atom in case if the distance between different spheres is equivalent to the corresponding atomic ligands in the active sites of interest (Ewing and Kuntz 1997).

a) GTF-18:The docking results for MTB-DNA Gyrase and Gatifloxacin 18 (GTF-18) ([1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-1-piperazinyl)1,4-dihydro-3-quinolinyl]carbonyl borodifluoridate) analoghave achieved good convergence, lowest binding energy, high number of conformations per cluster over the series of other GTF inhibitor analogues (Table 2). For the most Stable conformer, with the GTF-18 conformation for both arms of L-CA, 100 docking runs converged on a top-ranked cluster with a lowest energy binding mode as shown in Fig.6.16 (Table. 6.4). The complex of MTB-DNA Gyrase and Gatifloxacin 18 forms strong hydrogen bonds with Thr26, Asn176, Gly177 and Ser178 residues with the lowest binding energy of -18.27 Kcal/mol, RMSD of 0.02Å and inhibitory constant (Ki) value of $+3.48^{-07}$(Figure 3B).

b) GTF-21:The molecule GTF-21 (1-Cyclopropyl-6-fluoro-8-methoxy-7-[3-methyl(2,2,5,5-2H4)-1-piperazinyl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid) has 4 hydrogen bond interactions with Thr26, Ans176, Gly177, and Ser178 with the -15.45 K.cal/mol of binding energy at RMSD of 0.45 Å and its inhibition constant (Ki) is $+3.68^{-07}$. Amino group of Asn176-OD1atom is interacting with amino group of GTF-21 tail region. The best of GTF-21 molecular conformation in the cavity is exactly matching with molecular surface (Figure 3C).

In conclusion, we constructed the tertiary structure of MTB-DNA gyrase by the homology modeling and performedthe molecular docking reactions with currently market available drug,Gatifloxacinand its chemical analogues. Findings from molecular docking have concluded GTF-18 (1-Cyclopropyl-6-

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fluoro-8-methoxy-7-(3-methyl-1-piperazinyl) 1,4-dihydro-3-quinolinyl]carbonyl borodifluoridate) as the best anti-MTB DNA gyrase compound with significant physicochemical properties.This GTF-18 compound demonstrates better affinity towards MTB-DNA gyrase (ΔG is -18.27) compared to parental Gatifloxacin. This study advises future laboratory assays to develop GTF-18 as a novel drug compound for targeting DNA gyrase in tuberculosis samples.

S.No.	Compound Name	miLogP	TPSA	Natoms	MW	nON	nOHNH	Nviolations	Nrotb	Volume
	Gatifloxacin	-0.036	83.803	27	375.4	7	2	0	4	327.592
1	GTF-1	-1.024	104.031	27	377.372	8	3	0	4	319.049
2	GTF-2	-0.108	95.242	28	391.399	8	2	0	4	335.992
3	GTF-3	-0.694	104.031	28	391.399	8	3	0	4	335.636
4	GTF-4	-0.353	104.031	27	377.372	8	3	0	4	319.049
5	GTF-5	-0.131	104.031	28	391.399	8	3	0	4	335.61
6	GTF-6	-0.093	93.037	28	391.399	8	2	0	5	336.577
7	GTF-7	-3.876	98.13	28	392.407	8	3	0	4	339.32
8	GTF-8	-2.011	98.13	28	392.407	8	3	0	4	339.32
9	GTF-9	1.489	104.031	28	391.399	8	3	0	4	335.61
10	GTF-10	-1.15	104.031	28	392.407	8	3	0	5	338.745
11	GTF-11	-0.694	104.031	28	391.399	8	3	0	4	335.636
12	GTF-12	-0.981	109.826	27	376.388	8	4	0	4	322.32
13	GTF-13	-0.441	95.83	27	374.372	8	3	0	2	312.618
14	GTF-14	-0.651	109.826	27	376.388	8	4	0	4	322.32
15	GTF-15	-0.187	83.803	27	373.384	7	2	0	2	317.017
16	GTF-16	-1.096	115.47	28	393.371	9	3	0	4	327.448
17	GTF-17	-1.754	135.698	29	409.37	10	4	0	4	335.492
18	GTF-18	-1.959	144.932	29	409.326	11	4	1	2	317.1
19	GTF-19	-2.399	155.926	30	425.369	11	5	1	5	343.751
20	GTF-20	-1.849	155.926	30	425.369	11	5	1	4	343.51
21	GTF-21	-1.811	144.932	30	425.369	11	4	1	5	344.477
22	GTF-22	-0.41	113.265	28	393.371	9	3	0	5	328.034
23	GTF-23	-1.398	133.493	28	395.343	10	4	0	5	319.491
24	GTF-24	-1.341	124.259	27	379.344	9	4	0	4	310.506

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Table 1: Physicochemical properties of the GTF analogs

miLogP=Moriguchi logP, nOHNH=number of hydrogen bonds donors, TPSA=topological polar surface area, nAtoms=number of atoms, MW=molecular weight, **Nviolations=** number of Rule of 5 violations, Number of Rotatable Bonds – nrotb.

S.No.	Protein	Inhibitors	Cluster[a]	RMSD[b]	Lowest Binding Energy[c] (Kcal/mol)	Inhibition Constant[d] (*Ki*)
1.	MTB-DNA Gyrase	**GTF-18**	**5**	**0.02**	**-18.27**	**-3.52**
2.		GTF-21	45	0.45	-15.45	-3.32
3.		GTF-10	87	0.02	-14.51	-3.75
4.		GTF-16	32	0.04	-13.36	-3.98
5.		GTF-20	41	0.88	-11.25	-4.14
6.		GTF-24	2	1.16	-10.04	2.0207
7.		GTF-03	78	1.18	-7.44	1.8107
8.		GTF-11	85	1.18	-5.4	1.0507
9.		GTF-05	9	0.55	-4.28	-6
10.		GTF-15	50	2.02	-4.14	-6.02

Table 2. Docking energies of GTF analogs with MTB-DNA Gyrase

[Ta]Indicative of the total number of binding modes produced

[b]Heavy atoms root-mean-square deviation with respect to the experimental structure.

[c]The change in binding free energy is related to the inhibition constant using the equation: $\Delta G = RT$ in *Ki* where R is the gas constant 1.987 cal K-1 mol-1 and T is the absolute temperature assumed to be 298.15 K.

[d]Estimated inhibition constant at 298.15 K.

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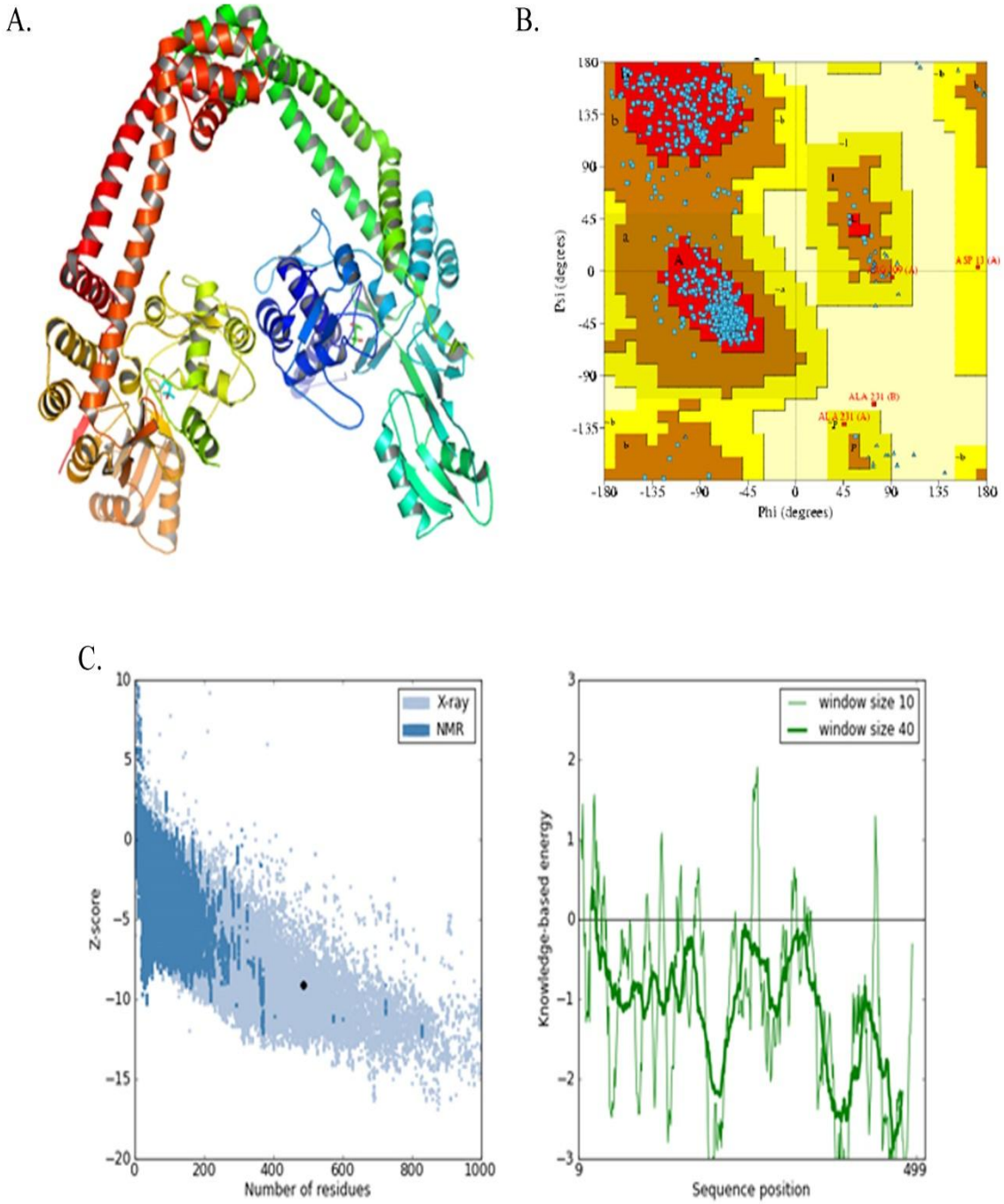

Figure 1A. 3D model of MTB-DNA Gyrase; B. Ramachandra plot of refined crystallographic MTB-DNA Gyrase; C.MTB-DNA Gyrase Overall model quality (Left) and Local model quality (Right)

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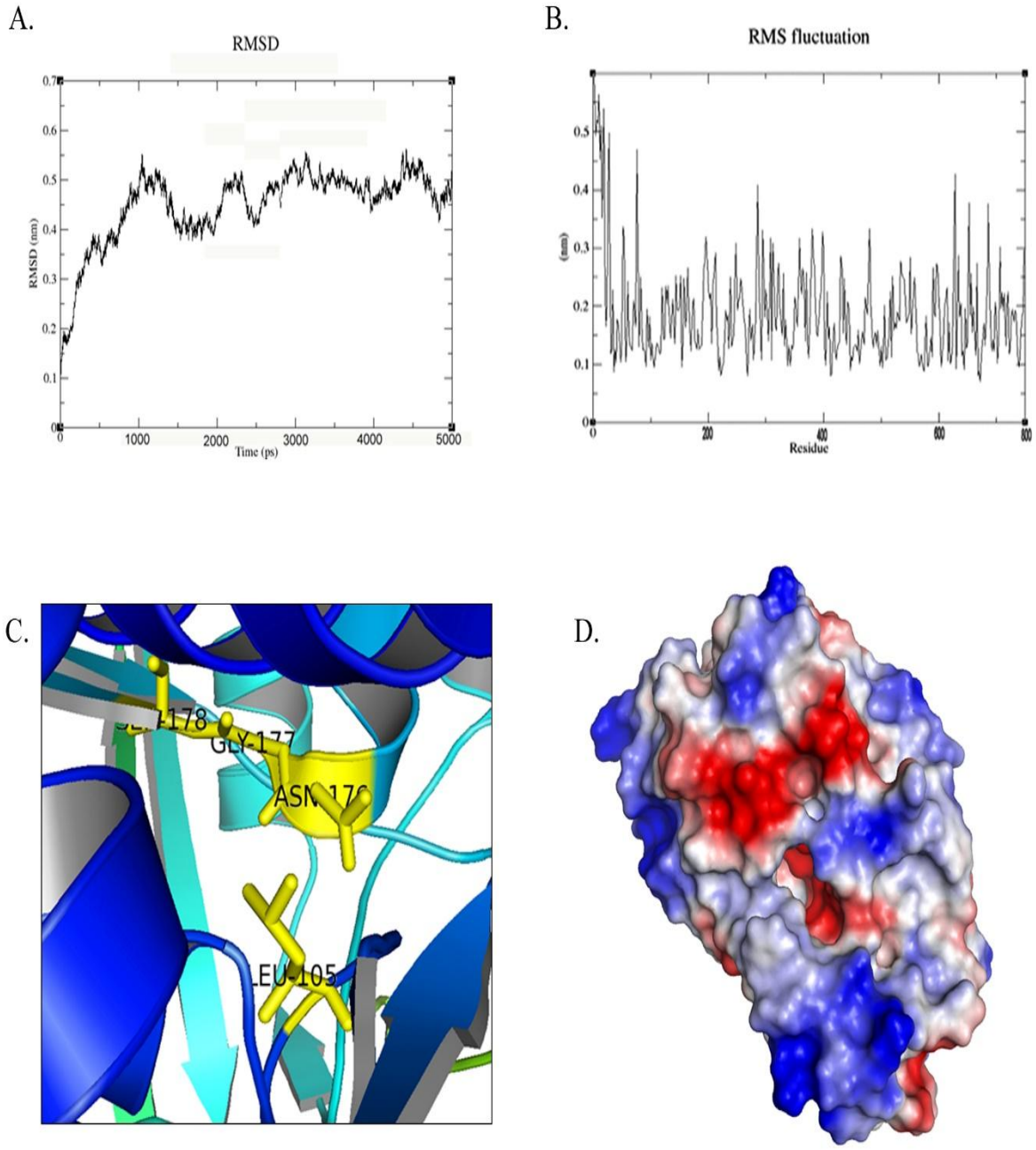

Figure 2: A-B RMSD and RMSFluctuation of Amino acids of MTB-DNA Gyrase at 5000ps; C. Active site amino acids (Yellow sticks) of MTB-DNA Gyrase; D.Electrostatic surface with active site residues and the extreme left with electrostatic poten- tials proposed binding site (Blue + charge and Red – charge). These figures were generated with Pymol

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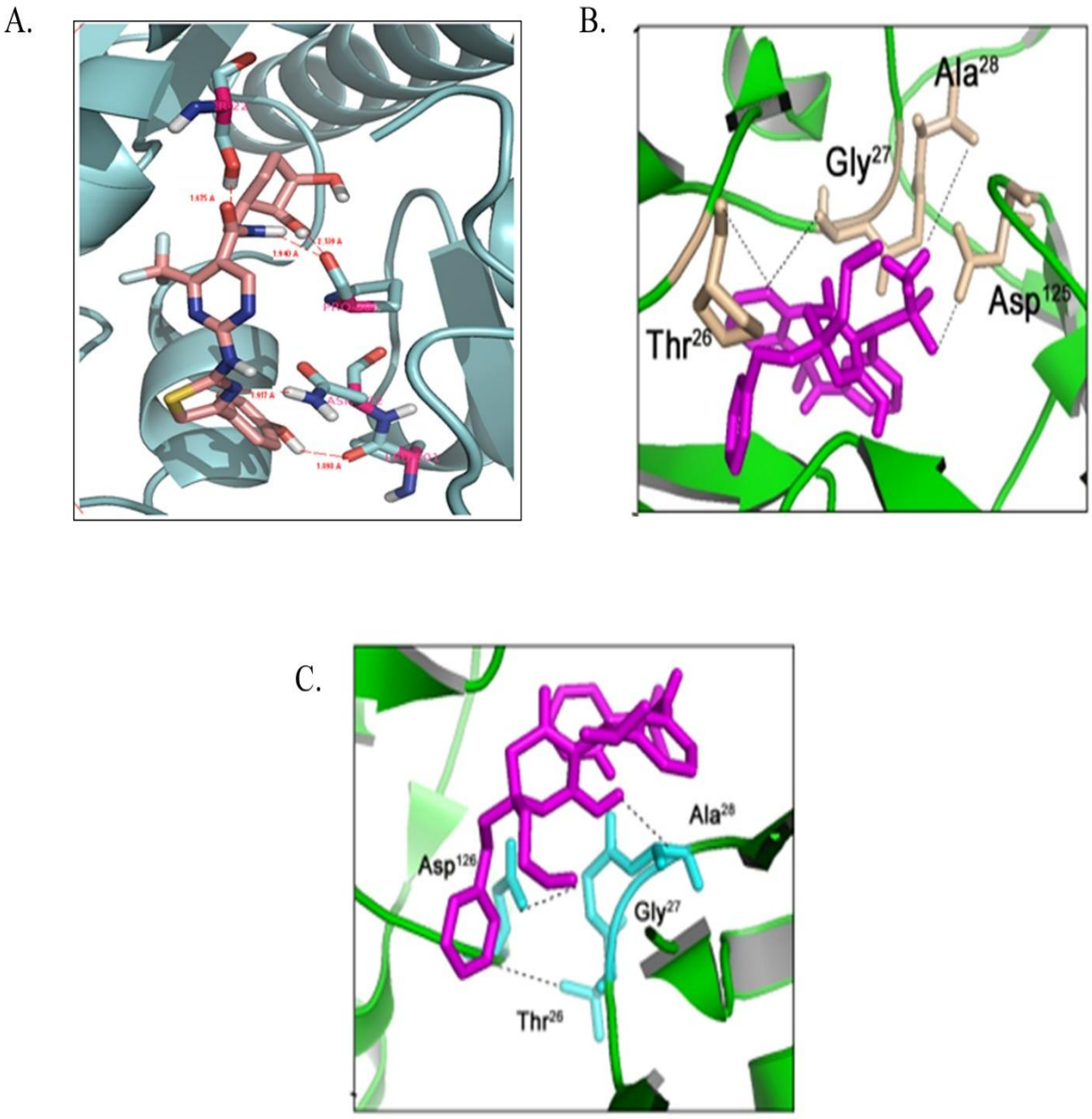

Figure 3: A. Strong hydrogen bonding of Gatifloxacin withMTB-DNA Gyrase Active site residues; B. Docking interaction of GTF best analog GTF -18 with MTB-DNA Gyrase active site residues ; C. Docking interaction of TP best analog GTF-21 with MTB-DNA Gyrase active site residues

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