International Journal of Biomedical Investigation

journal homepage: https://ijbi.edwiserinternational.com

Original Article

Structure-based virtual screening approach for identifying DNA gyrase inhibitors of mycobacterium tuberculosis

Surega A and Annapoorna Vadivelu*

Department of Pharmaceutical Chemistry, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, Gorimedu, Puducherry-605006, India

ARTICLE INFO

Received 17 April 2024
Revised 23 June 2024
Available Online 30 June 2024

Keywords:
DNA gyrase
Molecular docking
Compounds
Various modes
Prime MM-GBSA

ABSTRACT

To combat drug resistance tuberculosis, new drugs and methodologies are emerging. The present study focuses on identifying new chemical entity with potent DNA gyrase inhibitory activity. DNA gyrase is a type II topoisomerase enzyme, which encodes two subunits namely gyrA and gyrB, former contains tyrosine active site, which is essential for cleavage and relegation of DNA, while the latter is required for ATP hydrolysis. A small library of 485 compounds were designed and docked into DNA gyrase core in order to identify the potential inhibitor against target enzyme. Molecular docking was performed by using Glide. The crystal structures of the target were retrieved from RCSB PDB. The docking was performed by 3 modes namely HTVS, SP and XP. Prime MM-GBSA was used to calculate binding affinity and energy interactions are studied and hydrogen bond distance was calculated. All the designed compounds showed significant activity with A34 and A232 displaying maximum binding score.

Abbreviations

MM/GBSA: Molecular mechanics with generalised Born and surface area solvation; Asp: Aspartic Acid; Asn: Asparagine; Arg: Arginine; Gly, Glycine; Thr: Threonine; Val: Valine; Ile: Isoleucine; Pro: Proline; Met: Methionine; Leu: Leucine; Ser: Serine; Glu: Glutamic Acid; OPLS3e: Extending Force Field Coverage for Drug-Like Small Molecules; TB: Tuberculosis; ATP: Adenosine triphosphate.

*Corresponding author: Annapoorna Vadivelu, Department of Pharmaceutical Chemistry, College of Pharmacy, Mother Theresa Postgraduate and Research Institute of Health Sciences, Gorimedu, Puducherry-605006, India.

https://doi.org/10.31531/2581-4745.1000153

Introduction

DNA gyrase is a group of topoisomerases that are categorised according to their mode of operation, specifically as type I and type II (which induce temporary single- and double-stranded breaks in DNA, respectively).

They are additionally categorised into type IA, IB, IIA, and IIB. DNA gyrase is classified as a type IIA DNA topoisomerase, making it a valuable target for antitubercular therapies. DNA gyrase causes the formation of negative supercoiling in DNA, which helps to alleviate tension during the process of DNA unwinding [1-3]. DNA gyrase is composed of two GyrA and GyrB subunits, forming a heterotetrameric (A2B2) structure [4]. The GyrA subunit possesses a tyrosine residue in the active site, which is necessary for the cleavage and relegation of DNA. On the other hand, the GyrB subunit assists in the hydrolysis of ATP,

supplying the energy needed for the process of DNA replication [5-7].

During replication, DNA winding is the main catalytic function of DNA gyrase. The active site that is involved for second-line TB drugs (Fluoroquinolones) is DNA gyrase subunit A(GyrA)[5] (Figure 1).

Therefore, the suppression of M. tuberculosis DNA gyrase led to potent mycobactericidal action, establishing it as a validated target for the creation of antitubercular medications. Compounds that hinder the activity of this particular enzyme are also successful in combating mycobacteria that are not actively reproducing, a characteristic that is essential in reducing the duration of tuberculosis treatment. A novel inhibitor of M. tuberculosis DNA gyrase would not only be effective against fluoroquinolone-resistant M. tuberculosis, but also highly efficient against multi-drug resistant (MDR)-TB [8-10].

Vast research has been progressed to identify DNA gyrase inhibitors most of them derived either naturally or synthetically. Few examples of natural inhibitors include Aminocoumarin, simocyclinone, cyclothialidine, catechin-based polyphenols, haloemodin, chebulinic acid and synthetically derived compounds include quinolones, pyridine-3-carboxamide-6-yl-ureas, N-benzyl-3-sulfonamido pyrrolidines, spiropyrimidinetriones, Benzothiazinone-piperazine (Figure 1) [11].

Among the various heterocyclic compounds available, the current designed set of compounds contains scaffolds of chalcones, aryl thiosemicarbazone, aryl semicarbazone, aryl phenyl hydrazone, aryl hydrazone chalcone, aminopyrimidine and natural moiety such as caffeic acid, palmitic acid, vanillic acid and plumbagic acid (Figure 1).

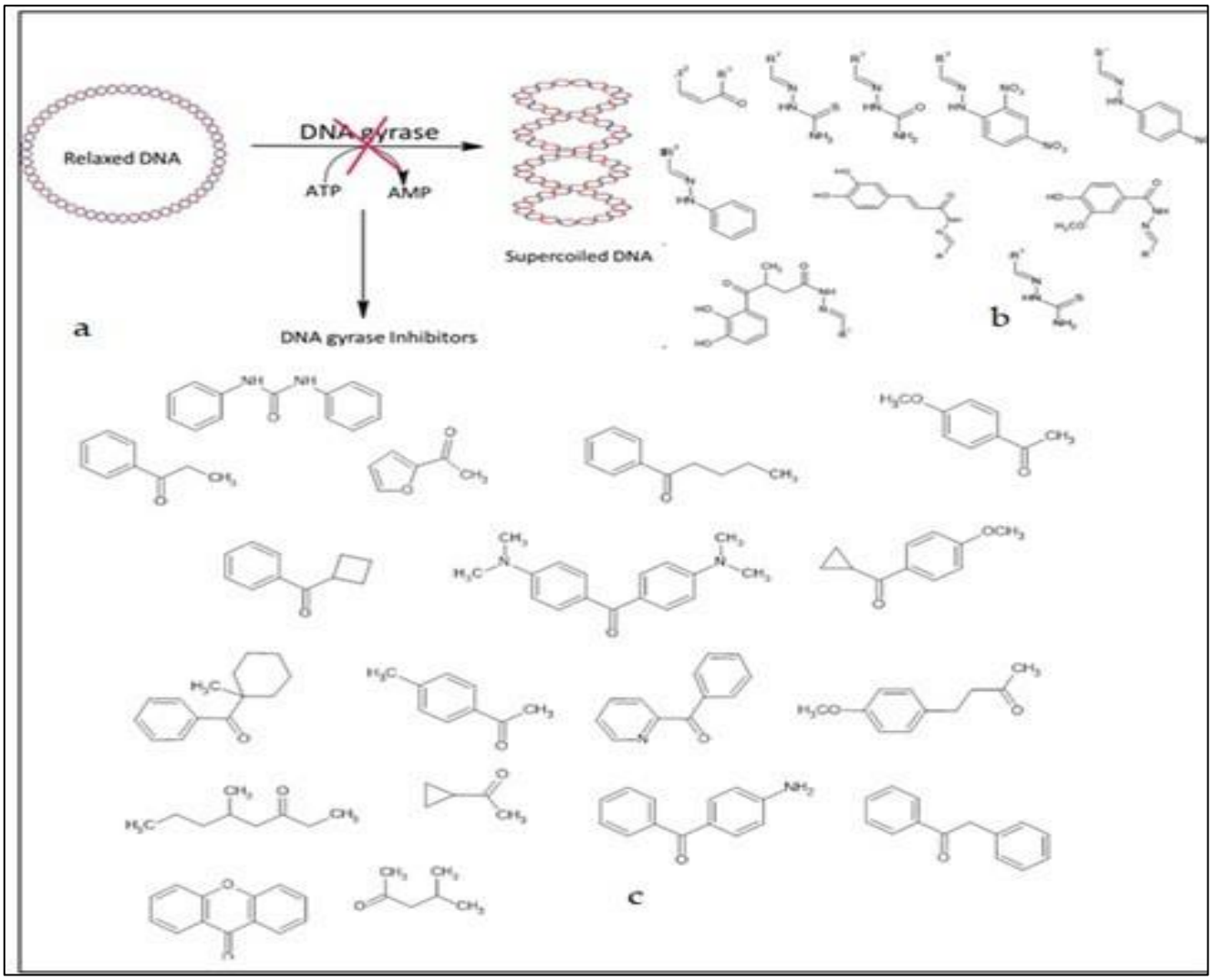

Figure 1: a. Mechanism of action of DNA gyrase and DNA gyrase inhibitors b. Core structural fragments of designed ligands. c. R1 and R2 substituent of core fragment

Materials and Methods

Docking Study

Molecular docking was performed by using Maestro (13.3) on molecular modeling interface (Schrodinger. Inc., New York, USA) against the selected mycolic acid macromolecule [12,13].

Anti-Tubercular Library Preparation

A set of 485 molecules were designed from a detailed literature survey to find effective inhibitors against DNA gyrase as to inhibit negative supercoiling of DNA in Mtb. All these compounds were prepared using the Ligprep module for geometry optimization and energy minimization using Schrodinger software for docking study with DNA gyrase.

Protein Preparation and Grid Generation

The target DNA gyrase is selected from the RCSB PDB databank (www.rcsb.org) based on the X-ray diffraction crystallography and good resolution factor having PDB ID: 6M1J. Using the protein preparation wizard of the Maestro molecular modelling interface, these protein structures were further refined for the docking study (Schrodinger). Hydrogen atoms were added after bond orders were determined. Utilizing the OPLS3e force field, the produced protein structure energy was minimized. The "Glide's Receptor Grid Generation" tool was used to create receptor grid boxes at the active site residues which are responsible for the anti-tubercular activity.

Structure-Based Virtual Screening

The compounds were removed through the application of a structure-based virtual screening technique that relied on docking scores. Molecular docking data are utilised to ascertain the connections between crucial amino acid residues in the protein and molecules exhibiting low-energy conformations. GLIDE (Grid-based Ligand Docking with Energies) was utilised to conduct molecular docking studies. GLIDE was used to keep the active sites of the target enzymes rigid during docking, while allowing the ligands to be flexible. The results are compiled and organised in a tabular format, as shown in Tables 1-3.

Prime MM-GBSA

The prime MMGBSA method (Prime Version 4.8) exhibited the relative binding-free energy (ΔG bind) of each ligand molecule, and results are given in Table 4.

Formula expanded is given below:

$$\Delta G(\text{bind}) = \Delta G(\text{solv}) + \Delta E(\text{MM}) + \Delta G(\text{SA})$$

where:

- Δ*G*solv is the difference in GBSA solvation energy of the DNA gyrase-inhibitor complex and the sum of the solvation energies for unliganded DNA gyrase and inhibitor.
- Δ*E*MM is a difference in the minimized energies between DNA gyrase-inhibitor complex and the sum of the energies of the unliganded DNA gyrase and inhibitor.
- Δ*G*SA is a difference in surface area energies of the complex and the sum of the surface area energies for the unliganded DNA gyrase and inhibitor [14].

The compounds designed in the dataset (Figure 1) underwent structure-based virtual screening using the HTVS (High-throughput virtual screening), SP (standard precision), and XP (Extra precision) docking technique. The XP docking analysis provided a strong correlation between the position and rating of the ligand. However, the therapeutic potential of the ligand-receptor complex was mostly determined by the free binding energy.

The free binding energy studies of designed molecules with DNA gyrase depicts binding affinity in the range of -6.1 to 8 for 49 compounds, -4.1 to -6 for 289 and -1 to -4 for 155 compounds. The glide score and different interactions of the top molecules are shown in the Table 1.

Table 1: Best G score (kcal/mol) of compounds and their H-bond interactions, interacting amino acid residues and distance calculated against DNA gyrase.

Compound	G Score	H Bond Score	Lipophilic Score	Interacting residue	No. of H bonds	Distance
A34	-7.057	-1.18	-4.91	H_2O molecule	1	2.73
A58	-6.830	-1.02	-4.10	Thr167, Asp75	2	3.98; 4.43
A73	-6.704	-1.28	-4.59	Arg78, H_2O molecule	3	3.23; 4.86; 4.53
A98	-6.690	-1.09	-4.01	H_2O molecule, Asp75	3	2.83; 4.20; 3.44
A36	-6.743	-1.15	-4.55	H_2O molecule	1	2.72
A223	-6.610	-1.48	-4.22	H_2O molecule	1	3.07
A148	-6.847	-1.36	-4.40	Val73, Asp75, H_2O mol	3	4.28; 3.44; 2.86
A46	-6.572	-1.20	-4.11	Asp75, H_2O mol, Gly79	3	5.94; 3.88; 3.63
C1	-6.545	-1.47	-3.57	H_2O molecule	2	2.73; 3.14
A230	-6.406	-1.01	-4.44	Asp75, H_2O molecule	3	4.37; 3.07; 3.53

Table 2: Hydrophobic, polar, positive and negative interactions of the amino acid for the selected ligand.

Compounds	Hydrophobic	Polar	Negative	Positive
A34	Val45, Val73, Ile80, Pro81, Ile 96, Val122, Val169	Asn48, Ser49, Thr167	Glu52, Asp75, Glu168	Arg74, Arg78, Arg138
A58	Val45, Val73, Ile80, Pro81, Ile96, Met97, Val122, Leu134, Val169	Asn48, Ser49, Thr167	Glu52, Asp75, Glu168	Arg74, Arg78
A73	Val45, Val73, Ile80, Pro81, Ile 96, Met97, Val122, Val169	Asn48, Ser49, Thr167	Glu52, Asp75	Arg78, Arg138
A98	Val45, Val73, Ile80, Pro81, Ile 96, Met97, Val122, Leu134, Val169	Asn48, Ser49, Thr167	Glu52, Asp75, Glu168	Arg74, Arg78
A36	Val45, Val73, Ile80, Pro81, Ile 96, Val122, Val169	Asn48, Ser49, Thr167	Glu52, Asp75, Glu168	Arg74, Arg78, Arg138
A223	Val45, Val73, Ile80, Pro81, Ile 96, Val122, Val169	Asn48, Ser49, Thr167	Glu52, Asp75, Glu168	Arg74, Arg78, Arg138
A148	Val45, Val73, Ile80, Pro81, Ile 96, Met97, Val122, Leu134, Val169	Asn48, Ser49, Thr167	Glu52, Asp75, Glu168	Arg74, Arg78
A46	Val45, Ile80, Pro81, Ala92, Val95, Ile 96, Met97, Val122, Val169	Asn48, Ser49, Thr167	Glu52, Asp75	Arg78, Arg138
C1	Val45, Val73, Ile80, Pro81, Ile 96, Val122, Val169	Asn48, Ser49, Thr167	Glu52, Asp75	Arg74, Arg78, Arg138
A230	Val45, Cys58, Ile80, Pro81, Ile 96, Met97, Val122, Val169	Asn48, Ser49, Thr167	Glu52, Asp75	Arg78, Arg138

Table 3: Glide energy, Vander Waal's energy, coulomb energy and lipophilic Vander Waal's for the top-scored ligands.

Compounds	Glide energy	Vander Waal's energy	Coulomb energy	Electrostatic	Lipophilic Vander Waal's
A34	-40.65	-36.003	-4.646	-0.348	-4.917
A58	-38.839	-33.989	-4.850	-0.363	-4.102
A73	-42.415	-31.812	-10.603	-0.795	-4.591
A98	-42.394	-36.017	-6.376	-0.478	-4.013
A36	-41.133	-35.473	-5.659	-0.424	-4.556
A223	-43.193	-34.788	-8.404	-0.630	-4.229
A148	-40.564	-37.086	-3.477	-0.260	-4.409
A46	-40.637	-32.125	-8.512	-0.638	-4.111
C1	-42.958	-34.971	-7.987	-0.599	-3.579
A230	-37.001	-31.454	-5.546	-0.416	-4.447

Table 4: The relative binding-free energies (kcal/mol) obtained by Prime MM–GBSA.

Compounds	MMGBSA-dG-binding energy	MMGBSA-dG-bind in Coulomb	MMGBSA-dG-Bind covalent	MMGBSA-dG-Bind Hydrogen bond	MMGBSA-dG-bind Lipophilic
A58	-48.577	-1.429	4.621	-3.403	-34.050
A73	-59.782	-25.289	4.356	-1.747	-43.689
A98	-58.432	-11.753	4.329	-3.705	-33.287
A223	-64.519	-20.438	-0.842	-1.066	-31.915
A148	-66.796	-8.899	2.465	-3.467	-41.393
A46	-45.919	-16.215	11.820	-1.442	-33.839
A230	-57.202	-17.826	3.919	-3.731	-28.859

Result and Discussion

The present study studied the DNA gyrase inhibitory activity of the designed molecule and their evaluation through molecular docking study using glide. The structures of the compounds were drawn by using Marvin sketch and the energy minimization was done. All the designed compounds show different glide score, coulomb energy and Vander Waal's energy due to difference in structural features.

The docking study showed that all the compounds displayed binding affinity by forming hydrogen bond and hydrophobic interaction with DNA gyrase. All the compounds docked by HTVS mode showed glide score -10 to -4 (Table 1). The top 75 compounds with binding score in the range of -8 to -10 were selected for docking by XP mode. The interaction of top 10 compounds is tabulated in Table 2.

A34 interacted with H_2O molecule by forming 1 hydrogen bond. A58 interacted with Thr167 and Asp75 by forming 2 hydrogen bonds. A73 interacted with Arg78 and H_2O molecule by forming 3 hydrogen bonds. A98 interacted with H_2O molecule Asp75 by forming 3 hydrogen bonds. A36 interacted with H_2O molecule by forming 1 hydrogen bond. A223 interacted with H_2O molecule by forming 1 hydrogen bond. A148 interacted with Val73, Asp75 and H_2O molecule by forming 3 hydrogen bonds. A46 interacted with Asp75, H_2O molecule and Gly79 by forming 3 hydrogen bonds. C1 interacted with H_2O molecule by forming 1 hydrogen bond.

A230 interacted with Asp75 and water molecule by forming 3 hydrogen bonds. Binding interactions are shown in the Table 3. The top binding score was obtained for the compound A34, A223 and A73 (Figures 2-3).

Most of the top scored compounds formed hydrogen bond with Asp75, Val73, H2O molecule and Arg73. The compounds displayed hydrophobic bonds with Val 45, Val 73,122, 169, Ile 80, 96 and Pro 81.

The compounds displayed polar interactions with Thr167, Asn38 and Ser49. The compounds showed

negative interaction with Glu52, Asp75 whereas positive interaction with Arg74, 78 and 148.

Prime MM-GBSA analysis improves the binding energy calculations than the molecular docking energies.

There upon, MM-GBSA analysis reveals the stronger binding of the ligands to the receptors. The free energy of binding of the designed compounds with the DNA gyrase was calculated by employing prime MM-GBSA approach.

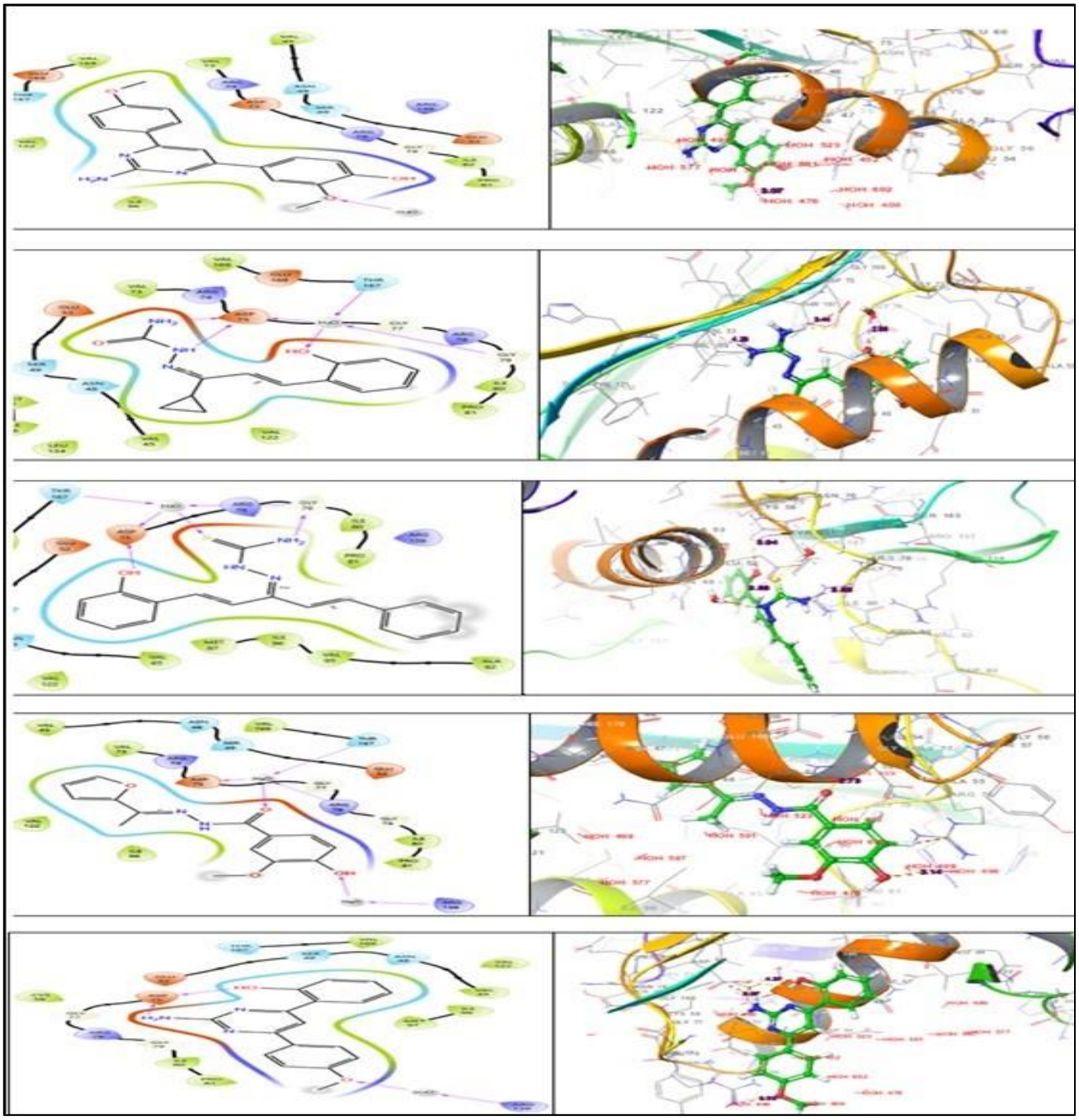

Figure 2: Interaction and Hydrogen bond details of A36, A223, A148, A46, C1 and A230 with DNA gyrase.

Conclusion

The study initially involves the identification of active pharmacophore so that we can identify potent molecules capable of giving good promising candidates for DNA gyrase inhibitory action. The MMGB/SA methodology has been much more reliable than docking for rank ordering the poses. The most stable conformer (ΔG_{bind}= -66.796) from the screened library was found to be A148. The next stable conformer (ΔG_{bind}= -64.519) was found to be A223 and those interactions are analysed.

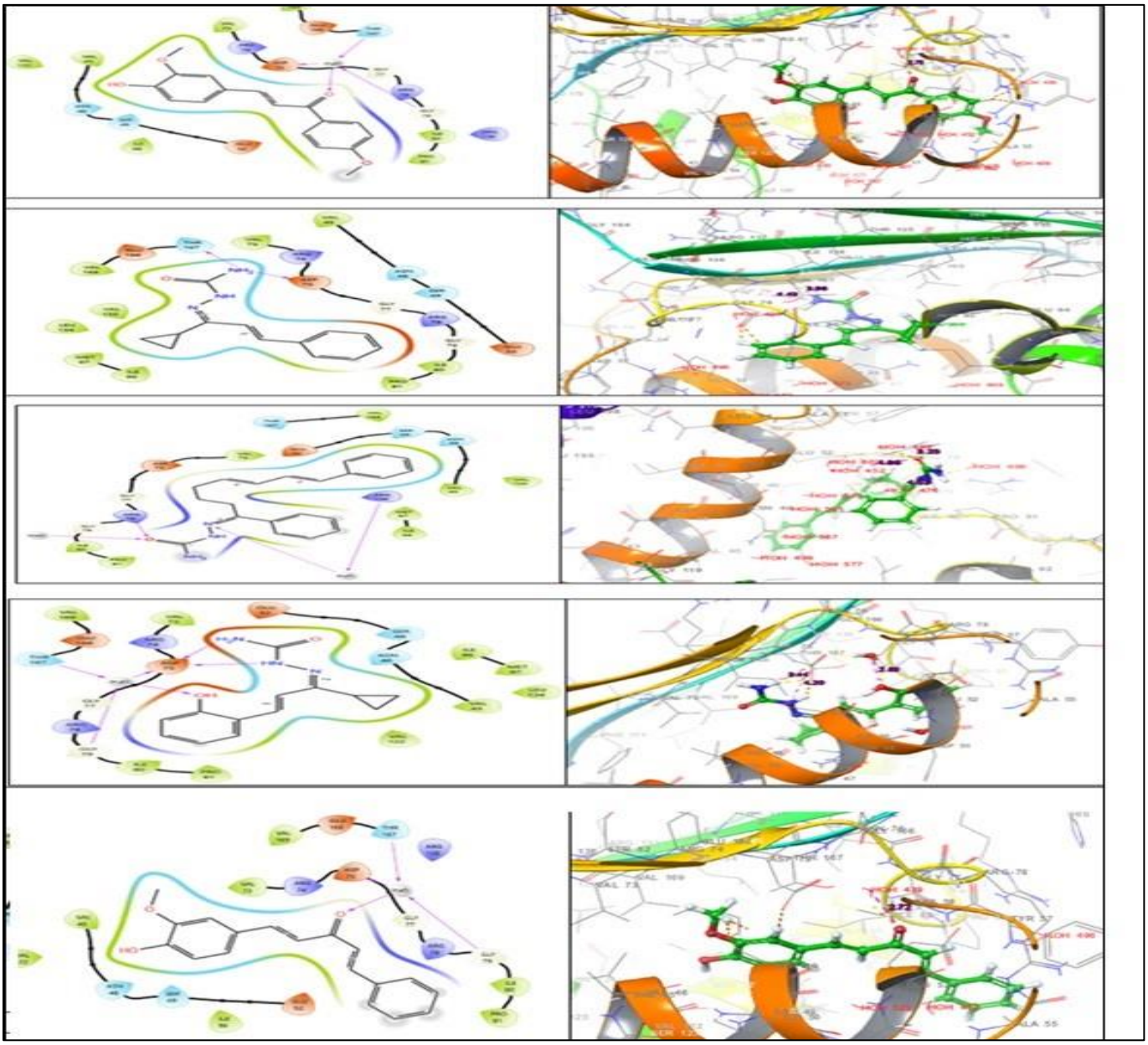

Figure 3: Interaction and Hydrogen bond details of compound A34, A58, A73, A98, & A73 with DNA gyrase.

Acknowledgements

The authors would like to thank to the glide (Schrödinger) for providing us license and the department of pharmaceutical chemistry for their support.

Conflict of Interest

The authors declare no conflict of interest.

Funding

The authors did not receive any fundings from any private or government sources.

References

1. Medapi B, Renuka J, Saxena S, Sridevi JP, Medishetti R, Kulkarni P, Yogeeswari P, Sriram D. Design and synthesis of novel quinoline–aminopiperidine hybrid analogues as Mycobacterium tuberculosis DNA gyraseB inhibitors. Bioorgan Medicinal Chem. 2015 May 1;23(9):2062-78.
2. Dighe SN, Collet TA. Recent advances in DNA gyrase-targeted antimicrobial agents. Eur J Med Chem 2020 Aug 1;199:112326.
3. Campanico A, Moreira R, Lopes F. Drug discovery in tuberculosis. New drug targets and antimycobacterial agents. Eur J Med Chem. 2018 Apr 25;150:525-545.

4. Kashyap A, Singh PK, Silakari O. In silico designing of domain B selective gyrase inhibitors for effective treatment of resistant tuberculosis. Tuberculosis (Edinb). 2018 Sep;112:83-88.
5. Shetye GS, Franzblau SG, Cho S. New tuberculosis drug targets, their inhibitors, and potential therapeutic impact, Translational Research, vol. 220. Mosby Inc., pp. 68–97, Jun. 01, 2020.
6. Salve PS. Design and synthesis of new 3-((7-chloroquinolin-4-yl) amino) thiazolidin-4-one analogs as Mycobacterium tuberculosis DNA gyrase inhibitors, Futur J Pharm Sci, vol. 7, no. 1, Dec. 2021.
7. Rajendram M, Hurley KA, Foss MH, Thornton KM, Moore JT, Shaw JT, Weibel DB. Gyramides prevent bacterial growth by inhibiting DNA gyrase and altering chromosome topology. ACS Chem Biol. 2014 Jun 20;9(6):1312-9.
8. Mdluli K, Ma Z. Mycobacterium tuberculosis DNA gyrase as a target for drug discovery. Infect Disord Drug Targets. 2007 Jun;7(2):159-68.
9. Aubry A, Pan XS, Fisher LM, Jarlier V, Cambau E. Mycobacterium tuberculosis DNA gyrase: interaction with quinolones and correlation with antimycobacterial drug activity. Antimicrob Agents Chemother. 2004 Apr;48(4):1281-8.
10. Barberis I, Bragazzi NL, Galluzzo L, Martini M. The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. J Prev Med Hyg. 2017 Mar;58(1):E9-E12.
11. Khan T, Sankhe K, Suvarna V, Sherje A, Patel K, Dravyakar B. DNA gyrase inhibitors: Progress and synthesis of potent compounds as antibacterial agents. Biomed Pharmacother. 2018 Jul;103:923-938.
12. Surega, A., Annapoorna vadivelu. “Structure based virtual screening and identification of potential inhibitors of mycolic acid biosynthesis enzymes (kasA, InhA, PkS13) for treatment of tuberculosis”International journal of pharmaceutical sciences and research 2023, (Accepted)
13. Mishra GP, Panigrahi D. Molecular docking and ADMET study for searching multi targeted antiviral compounds against SARS-CoV-2: A computational approach, International Journal of Applied Science and Engineering, vol. 18, no. 2, pp. 1–10, Jun. 2021.
14. Pattar SV, Adhoni SA, Kamanavalli CM, Kumbar SS. In silico molecular docking studies and MM/GBSA analysis of coumarin-carbonodithioate hybrid derivatives divulge the anticancer potential against breast cancer, Beni Suef Univ J Basic Appl Sci, vol. 9, no. 1, Dec. 2020.