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RESEARCH ARTICLE

In silico Screening of Approved Drugs to Describe Novel *E. coli* DNA Gyrase A Antagonists

Rakhi Chandran[1], Archana Ayyagari[2], Prerna Diwan[3], Sanjay Gupta[4] and Vandana Gupta[3]*

[1]Department of Biotechnology and Microbiology, School of Sciences, Noida International University, Yamuna Expressway, Gautam Budh Nagar, UP-203201, India
[2]Department of Microbiology, Swami Shraddhanand College, University of Delhi, New Delhi-110036, India
[3]Department of Microbiology, Ram Lal Anand College, University of Delhi, New Delhi-110021, India
[4]Independent Scholar (former Head and Professor, Department of Biotechnology, Jaypee Institute of Information Technology, Sector 62, Noida, UP-201309, India)

ABSTRACT

The alarming multiple drug resistance developed by *Escherichia coli* towards the routine conventional antibiotics owing to their non-judicious usage is fast becoming a tough menace. This necessitates the urgent unleashing of novel and diverse strategies and antibacterial compounds. Since finding a new antibiotic from the scratch, followed by endless clinical trials is exceedingly time-consuming, a powerful alternate strategy of CADD coupled with repurposing the available drugs could save precious time and money. DNA gyrases (topoisomerase II) of *E. coli* are among the promising new drug targets. The interface between the N-terminal domain of gyrA and C-terminal domain of gyrB which is targeted by most of the available inhibitory drugs, is of particular interest. Crucial active site residues within the N-terminal domain of gyrA were delineated through a literature search. FDA approved drugs were docked using FlexX on the receptors created around the co-crystallized reference ligand. Based on the docking scores and interactions with crucial residues, 12 leads were shortlisted, namely ceforanide, tetrahydrofolic acid, azlocillin, cefazolin, adenosine triphosphate, cefixime, dihydronicotinamide adenine dinucleotide, moxalactam, leucal, cromoglicic acid, cefotetan, and cedax. Surprisingly quinolones, which are approved inhibitors of gyrases were not picked up in the top leads, rather, the most dominant class of molecules that docked successfully was cephalosporin. Our results indicated that these cephalosporins, as well as the other shortlisted leads, could be further optimized and validated through *in-vitro* experiments for their potential as gyrase A antagonists. Hence the present study holds immense promise in combating MDR of human bacterial pathogens.

ABBREVIATIONS

ADME: Absorption, Distribution, Metabolism, and Excretion; CADD: Computer-Aided Drug Discovery; ESBL: Extended Spectrum β-Lactamase; ESBL-Ec: ESBL Producing *E. coli*; MDR: Multiple Drug Resistance; NDM: New Delhi Metallo-β-Lactamase; SD8: Simocyclinone D8; UTI: Urinary Tract Infections

INTRODUCTION

Escherichia coli, a common intestinal pathogen, is known to cause gastroenteritis and a variety of extra-intestinal diseases, such as Urinary Tract Infections (UTIs), meningitis among newborns, colisepticemia, and skin and soft tissue infections [1,2]. *E. coli* infection is also reported to be responsible for several post-operative abscesses and other complications such as neonatal sepsis [3,4]. It has been developing more and more resistance towards the available antibiotics. Extended-

***Corresponding author**

Vandana Gupta, Department of Microbiology, Ram Lal Anand College, University of Delhi, New Delhi-110021, India

Tel: +91-783-800-4880
E-mail: vandanagupta@rla.du.ac.in; vandanagupta72@rediff.com

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Spectrum B-Lactamase (ESBL) producing *E. coli* (ESBL-Ec) has recently gained much importance as a common cause of contagious nosocomial and community acquired infections in India and abroad, because it resists treatment with almost all the β-lactam antibiotics including three generations of cephalosporins [5]. Multiple surveys recorded the highest rate of ESBL-Ec in India (80%), followed by China (60%), and less than 30% in East and Southeast Asia. In Europe, Australia and North America it ranges between 5-10%. Further resistance to the advanced antibiotics like carbapenems due to the production of carbapenemases/ New Delhi Metallo β-lactamases (NDM) among these pathogens has rendered treatment of such infections extremely challenging. During the last decade, the preventive measures followed to curb such infections have not proved to be adequate to prevent the rapid spread of resistant Gram-Negative Bacteria (GNB), particularly extended-spectrum β-lactamase producing *Escherichia coli* [6,7]. Approximately 67% of *E. coli* isolates from extra-intestinal infections are reported to be multidrug-resistant, of which up to 85% producing ESBL and 6% producing NDMs. These enzymes are responsible for inactivating β-lactam and carbapenem antibiotics commonly used in treating *E. coli* infections [1]. A single Extra-Intestinal Pathogenic strain *Escherichia coli* (ExPEC) clone, named Sequence Type (ST) 131, is the cause of millions of drug-resistant infections annually [1,8]. NDM was first described by Yong, et al. [9] in a Swedish national who fell ill with *Klebsiella* infection, acquired in New-Delhi. Similar trends were subsequently reported for other gram-negative pathogens including *Proteus vulgaris*, *Serratia marcescens*, *Enterococcus*, etc. Coupled with the increment in antibiotic-resistant bacteria, the extremely slow speed of newer approved antibiotics for treatment, several infectious diseases are not being addressed successfully [10].

These astonishing global health threats call for urgent accelerated research into finding novel and more effective therapeutic options for such infections. Available options for the same encompass either newer antibacterials or newer strategies to target such drug-resistant microorganisms. Given this challenging scenario, there is an urgent need to look for either newer target components of the bacterial cells or find novel inhibitors of the older ones. Conventionally, all anti-infection agents are used to target certain pathways within the pathogenic bacteria, such as cell wall production, nucleic acid synthesis, protein synthesis, and folate synthesis [11]. However, over a span of time, indiscriminate, excessive, and improper usage of these has led to astonishing unresponsiveness of these bacteria towards the same antibiotics, translating into the emergence of drug resistant mutant bacteria [12,13]. This very fact necessitates a paradigm shift in our focus towards unconventional targets presents within bacterial machinery. DNA gyrase is one such important enzyme that introduces negative supercoils in DNA, classified as topoisomerase type 2 that controls DNA topology in proper form during its replication and transcription as well as during cell division. The native DNA gyrase (370kD protein) comprises two types of subunits gyrase A (gyrA) and gyrase B (gyrB) with 875 and 804 residues respectively [14]. Its active form is made up of a hetero-tetramer complex A_2B_2. It possesses various molecular interfaces named N-gate, DNA gate, and C-gate which assist in strand passage and DNA binding in a specific manner. GyrA function is to break and re-join DNA and GyrB function is to hydrolyze ATP to provide energy for the DNA unwinding. DNA gyrase is known to be targeted by catalytic inhibitors such as aminocoumarins, or 'poison' such as quinolones. Several citations suggest that the known inhibitors of DNA gyrase mostly dock onto amino acids located near the amino-terminal of the gyrA. Gepotidasin, a novel inhibitory antibiotic is demonstrated to interact with the complete *E. coli* DNA gyrase nucleoprotein complex [15]. Similarly inhibition of gyrA by 7-oxy-4methyl coumarinyl amino alcohol derivatives 17 and 18 [16], 4,5,6,7-tetrahydrobenzeno (1,2-d) thiazole-2,6 diamine, 2-(2-aminothiazol-4-yl) acetic acid and benzol (1,2,-d) thiazole-2,6-diamine) [17], fluoroquinolones with alkylamine, alkylpnthalimide, and alkylphenyl groups introduced at N-1 position [18] and 4,5,6,7 tetrahydrobenzo(d)thiazole [19] has been reported.

Residues crucial to the activity of gyrA as reported in the literature include Lys42, Val44, His45, His78, Pro79, His80, Gly81, Asp82, Ser83, Ala84, Asp87, Arg91, Lys103, Lys110, Tyr122 Asn169, Gly170, Ser171, Ser172, and Asp424. Alanine substitutions at Asp87 and Ser83 lead to DNA cleavage, DNA supercoiling, and resistance to different quinolones [14,20,21], Lys42 stabilize interaction with ciprofloxacin [14], His45 and Arg91 are involved in binding to nalidixic acid [22], deletion of Tyr122 leads to the change in the conformation of the active site of enzyme affecting its interaction with neighboring amino acids and binding of ciprofloxacin to gyrase [23] while Asp424 is crucial to the DNA cleavage at the active site of gyrA and is involved in a conformational change, while others are either active site residues or are involved in binding to simocyclinone D8 [14].

The *in silico* research is very promising in this direction and also is much quicker. Further, to make it more rewarding and fruitful for successfully dealing with the emergence of AMR in the bacterial pathogens, these studies could be combined with the stimulating possibility of repurposing the already available drugs, indicated for other ailments [24]. Various computational tools may be used for *in silico* screening of libraries of these approved drugs. Scaffold hopping of the top leads from each protein without altering the ADME properties significantly helps in further optimizing these leads. Molecular docking paves the way quite effectively towards establishing a quantitative structure-activity relationship between the antibacterial and the target enzyme of infecting pathogens [25].

This particular study was planned by carrying out an extensive literature search on the bacterial gyrases

concerning their active sites and inhibitor binding residues, and an attempt was made to explore the potential of existing FDA Approved drugs to be repurposed as DNA gyrase inhibitors. Such a piece of work certainly holds a paramount potential for competent healthcare and well-being of the society at large, and therefore, is worth attempting.

MATERIALS AND METHODS

To accomplish the stated goals, an extensive literature search was carried out and many target proteins were delineated and explored. The suitable 3D structures of the target enzymes DNA GyrA were retrieved from the PDB database. From the available PDB structures of *E. coli* DNA GyrA, 4CKL [14] was selected. It is a 55kD N-terminal domain of gyrA in complex with the antibiotic Simocyclinone D8 (SD8) obtained from *Streptomyces antibioticus*. The library of 2924 molecules FDA approved drugs was downloaded from www.zincdocking.org in mol2 file format. FlexX docking and scoring module from BiosolveIT was used for docking. It is an effective flexible docking module as it flexibly docks every molecule on the target site in up to 2000 iterations while using the graphic user interface. It ranks each pose and gives the output as the best-docked pose score for all the compounds in the library and rank them based on docking score and lower the score better is the binding. Receptor Intelligence™ is included in FlexX includes, which is a fundamentally simple and different way to design and perform a docking. It helps in accurately predicting the geometry of the protein-ligand complex within a few seconds.

The active site residues of *E. coli* DNA gyrA were delineated after an extensive literature search. Gyrase A crystal structure 3CKL has a co-crystallized ligand (SM8) which was used as a reference ligand to create a binding pocket. The active site pocket was further modified to include some of the remaining crucial conserved residues delineated by the literature search while some of the non-conserved residues from the pocket were removed without disturbing the integrity of the core binding pocket so that less specific or non-specific interactions can be avoided. We used PyMOL for visualizing the pocket created automatically by the lead IT software and all the changes in residues were made after confirming the position of each residue in the surface view using PyMOL. DNA Gyrase A pocket was created with the residues Gly40, Leu41, Lys42, Val44, His45, Arg47, Ile74, His78, Pro79, His80, Gly81, Asp82, Ser83, Ala84, Asp87, Thr88, Arg91, Met92, Phe96, Ser97, Leu98, Arg99, Leu102, Asn165, Asn169, Gly170, Ser171, Ser172, Gly173, Ile182, Tyr266, and Gln267. The lead selection was done based on the docking score obtained from Flex X. The binding of the leads in comparison to the reference ligand and the details of the interaction of leads with the residues within the docking pocket was analyzed using the PyMOL and the Discovery Studio Visualizer platforms.

RESULTS AND DISCUSSION

The crystal structure of the DNA gyrA N-terminal domain 4CKL is a 55kD dimer with the co-crystallized ligand SD8. The structure is resolved at 2.05 Å. As the interface between the N-terminal of gyrase A and C-terminal of gyrase B in a functional tetrameric DNA gyrase is the site involved in DNA cleavage and strand passage, 4CLK was considered to be a suitable structure for the docking studies. Moreover, it also has a co-crystallized ligand to be used as a reference point in docking studies. DNA Gyrase A (4CKL) docking pocket created (Figure 1) with the residues Gly40, Leu41, **Lys42**, **Val44**, **His45**, Arg47, Ile74, **His78, Pro79**, **His80, Gly81**, **Asp82**, **Ser83**, **Ala84, Asp87**, Thr88, **Arg91**, Met92, Phe96, Ser97, Leu98, Arg99, Leu102, Asn165, **Asn169, Gly170, Ser171, Ser172**, Gly173, Ile182, Tyr266, Gln267 include 16 of the crucial functional residues reported in the literature (in bold), their significance is summarized in table 1. Lys42 forms polar contacts and helps in protein stability in association with ciprofloxacin a fluoroquinolone gyrase inhibitor and mutation lys42Ala also results in resistance to SD8. His45 and Arg91 are involved in the interaction with nalidixic acid and SD8 [14,26]. Whereas Ser83, Ala84, and Asp87 are involved in drug binding and are considered as hot spots for quinolone resistance mutations [14,20,21]. Alanine substitution at these positions leads to DNA cleavage, DNA supercoiling, and resistance to different quinolones, hence implying the importance of these residues in the functioning of gyrA [20,21,26].

In silico studies revealed that the docking site created exhibited reasonable binding to ligands with the formation of polar as well as nonpolar contacts between the atoms of the ligand and the docking site residues that contribute significantly to the stability of docked conformations. Scores as low as -39.9848 were obtained. Analysis of the 20 top scoring zinc Ids for the compound name, binding score, and current therapeutic indications is summarized in table 2.

A list of top 20 leads was further sorted after removing the duplicated molecules as the compounds appearing again with lesser scores were eliminated. The shortlisted 12 leads

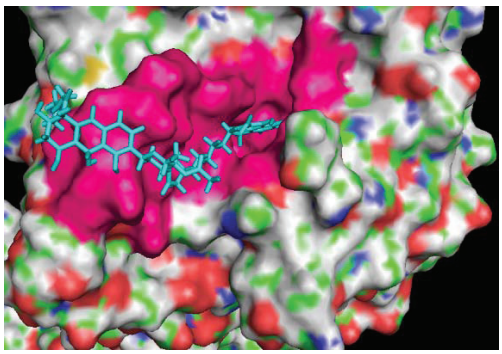

Figure 1 The docking pocket created within the N-terminal domain of the *E. coli* DNA gyrase A (pink) with the bound reference ligand SD8 (cyan).

Table 1: Residues of the docking pocket and the significance of crucial residues in the pocket [14].

Residues involved in binding to SD8 in the co-crystal structure	Residues involved in quinolone/ fluoroquinolone resistance	Residues involved in SD8 resistance	Active site residues
Lys42, Val44, His45, His78, Pro79, His80, Gly81, Asp87, Arg91, Ser171, Ser172,	Lys42, His45, Ser83, Ala84, Asp87, Arg91,	Lys42, His45, His80, Gly81, Ser83, Ala84, Asp87, Arg91,	Lys42, Val44, His45, His80, Gly81, Asp82, Ser83, Arg91, Asn169, Gly170, Ser172

Table 2: List of top-scoring molecules with their Zinc Ids, common names, current indications, and Flex-X scores.

S.no.	ZincID/ Compounds	Current Indications	Flex-X SCORE
1	zinc03830434 Ceforanide	Second generation cephalosporin indicated for bacterial infections	-39.9848
2	zinc13513942 Tetrahydrofolic acid	Treat hematologic complications eg. macrocytic anaemia, topical sprue, and megaloblastic	-38.3155
3	zinc03830262 Azlocillin	Antibacterial, broad spectrum semisynthetic penicillin	-37.6348
4	zinc03830407 Cefazolin	First generation cephalosporin indicated for bacterial infections	-37.4504
5	zinc18456332 Adenosine triphosphate	Seems to improve appetite and quality of life in people with weight loss due to tumors or other etiologies	-37.1850
6	zinc03830435 Ceforanide	Second generation cephalosporin indicated for bacterial infections	-36.7278
7	zinc03830410 Cefixime	Third generation cephalosporin indicated for bacterial infections	-36.3434
8	zinc53682927 1,4-Dihydronicotinamide adenine dinucleotide	To treat simple fatigue, energy sapping disorders, chronic fatigue, and fibromyalgia	-35.5141
9	zinc03871613 Adenosine triphosphate	Seems to improve appetite, food intake, and quality of life in people with weight loss due to tumors or other etiologies	-35.3634
10	zinc18456284 Leucal	Chemically reduced derivative of folic acid to treat overdose of anti-neoplastic folic acid antagonist methotrexate	-35.1048
11	zinc03831157 moxalactam (latamoxef)	Oxacephem antibiotic grouped with cephalosporins indicated in bacterial infections	-34.8578
12	zinc03871615 Adenosine triphosphate	Neurotransmitter	-34.5817
13	zinc03831159 moxalactam (latamoxef)	Treat bacterial infections	-34.2649
14	zinc04468778 Cefixime	Third generation cephalosporin indicated for bacterial infections	-34.0983
15	zinc03871612 **Adenosine triphosphate**	Neurotransmitter	-33.8784
16	zinc01530604 Cromoglicic acid	Prevents inflammatory release from eosinophil and mast cell and inhibits calcium influx	-33.7909
17	zinc09212428 Leucal	Chemically reduced derivatives of folic acid to treat overdose of anti-neoplastic folic acid antagonist methotrexate	-33.5305
18	zinc03830433 Ceforanide	Second generation cephalosporin indicated for bacterial infections	-33.3712
19	zinc03830441 Cefotetan	Second generation cephalosporin indicated for bacterial infections	-33.3351
20	zinc03871967 Ceftibuten	Third generation cephalosporin indicated in UTI and other bacterial infections	-33.1164

along with the residues interacted with in the docking pocket are summarized in table 3. The interacting key residues are highlighted in bold font. Each lead has interacted with at least 3 to at most 5 crucial residues with multiple hydrogen bonds, salt bridges, and hydrophobic interactions (Figure 2 and 3).

Results obtained from these studies were very surprising and unexpected. Even though quinolones and fluoroquinolones are the known DNA gyrase inhibitors, they were not picked up among the top scoring leads. Rather, the cephalosporins appeared to be a dominant class of molecules binding to the DNA gyrase A, as out of the 12 top selected leads 6 are cephalosporins. The analysis of docked poses (with reference to the binding of reference ligand, as per the co-crystal structure) of cephalosporins (Figure 2), as well as the other lead molecules including tetrahydrofolic acid, azlocillin, ATP, 1,4-dihydronicotinamide adenine dinucleotide, leucal and cromoglicic acid (Figure 3), revealed that all of them bind within the deep seated pocket, where

Table 3: Top leads and their interacting residues as determined using Discovery Studio Visualizer.

DNA GYRASE A [4CKL]		
S.no.	**Compounds**	**Interacting residues**
1	zinc03830434, Ceforanide	**Lys42, His45, Arg91,** Phe96**,** Ser97**,** Tyr266, Gln267
2	zinc13513942, Tetrahydrofolic acid	**Lys42, Arg91**, Leu98, **Asn169, Gly170**, **Ser172**
3	zinc03830262, Azlocillin	**Lys42, His45, Arg91, Asn169, Gly170**
4	zinc03830407, Cefazolin	Leu41**, Lys42, Arg91**, **Gly170**,
5	zinc18456332, Adenosine triphosphate (ATP)	**Lys42**, Thr88, **Arg91**, Leu98, Asn165, **Asn169**, **Ser172**, Tyr266
6	zinc03830410, Cefixime	**Lys42**, **His45,** Thr88, **Arg91,** Ser97, Leu98, Asn169, **Ser172,** Tyr266
7	zinc53682927, 1,4-Dihydronicotinamide adenine dinucleotide	**Lys42, His45**, **Ala84**, Thr88, **Arg91**, Ser97, Tyr266, Gln267
8	zinc18456284, Leucal	**Lys42, His45**, **Ala84**, Thr88, **Arg91**, Ser97, Gln267
9	zinc03831157, moxalactam	**Lys42**, **Asp87**, Thr88, **Arg91**, **Asn169**, Ser171, Tyr266
10	zinc01530604, Cromoglicic acid	**Lys42, His45**, Thr88, **Arg91**, Ser97, **Ser172,** Tyr266, Gln267
11	zinc03830441, cefotetan	**His45**, Thr88, **Arg91**, **Asn169**
12	zinc03871967, Ceftibuten	**Lys42**, **His45**, Thr88, **Arg91**, Ser97, **Asn169**, **Ser172**, Tyr266

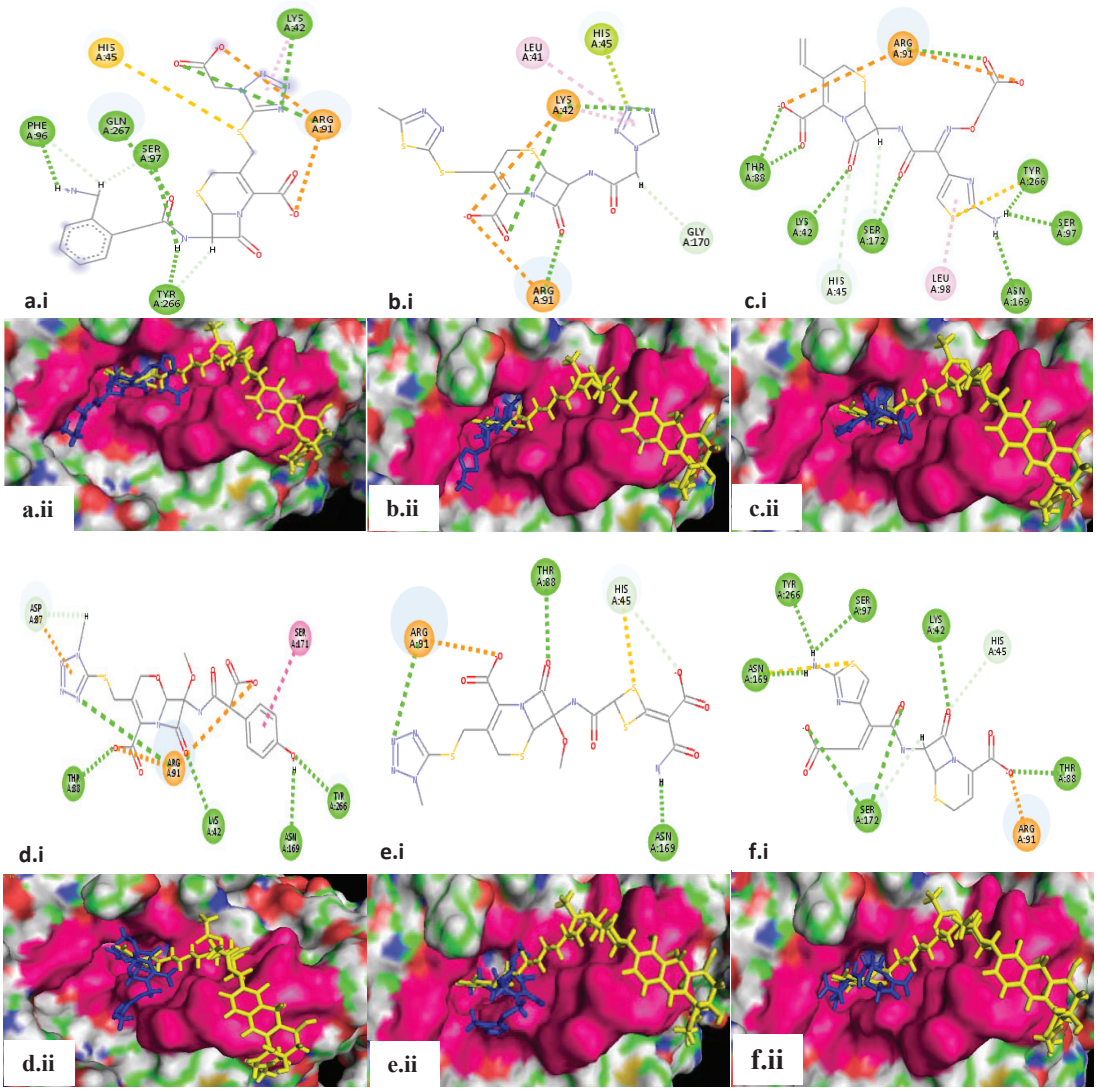

Figure 2 Interaction and docking analysis of cephalosporins i. Interaction with docking site residues in a 2-D view created using DSV; ii. Showing comparative positioning of the reference ligand and leads in a surface view created using PyMOL respectively. a.i and a.ii. Ceforamide; b.i and b.ii. Cefazolin; c.i and c.ii. Cefixime; d.i and d.ii. Moxalactam; e.i and e.ii. Cefotenan; f.i and f.ii. Ceftibuten.

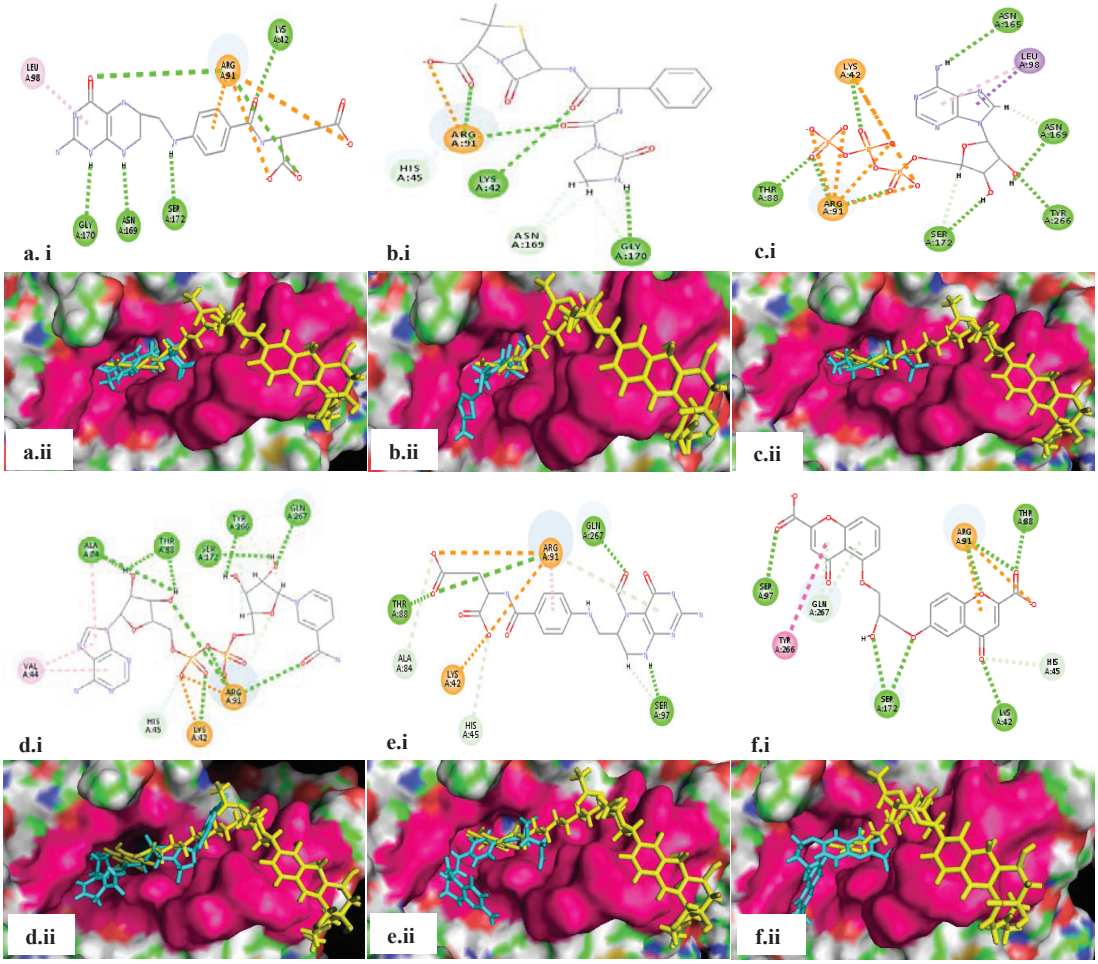

Figure 3 Interaction and docking analysis of tetrahydrofolic acid a.i and a.ii: Azlocillin; b.i and b.ii: ATP; c.i and c.ii: 1,4-DihydroNAD; d.i and d.ii: Leucal; e.i and e.ii: Cromoglicic acid; f.i and f.ii:Interaction with docking site residues in a 2-D view created using DSV. f.iii: Showing comparative positioning of the reference ligand and leads in a surface view created using PyMOL respectively.

the aminocoumarin moiety of the reference ligand SD8 also binds. None of the selected leads docked at the pocket at the interface of the two monomers in this 55kD N-terminal domain homodimer of gyrA where the polyketide moiety of SD8 was reported to be binding [14]. The binding site and affinity of polyketide moiety change if the 55kD partial gyrA protein is replaced with 59kD partial gyrA protein, whereas the binding of aminocaumarin moiety remains essentially the same [14]. This signifies our results as in native tetrameric gyrA there is a high probability of the retention of the integrity of the aminocaumarin binding pocket, where all our lead molecules are apparently binding. Moreover, as the aminocaumarin moiety of SD8 interacted with Lys42, His45, Arg91, and Ser172, the leads molecules identified in our study also interacted with at least two to all four of these residues along with other crucial residues, signifying the distinction of our study.

In comparison to the docking score of the reference ligand SD8 which is calculated to be -15.16 using Flex-X, lead molecules identified in our study demonstrated much lower scores and hence stable docking. The not so good docking score with the reference ligand could be attributed to the stearic bumps observed when we analyzed the co-crystallized structure 4CKL and the top scored docking pose we obtained with Flex-X (Figure 4a and b respectively). Recently in line with our results, Fois, et al. [27] demonstrated the efficacy of the hybrid molecule of DNA gyrase B (GyrB) inhibitor and ciprofloxacin against *E. coli*. They speculated that conjugation with ciprofloxacin facilitates the access of non-permeating GyrB inhibitors into the bacterial cells, facilitating their interaction with the fluoroquinolone binding site in the GyrA [27].

Further, to target ESBL-Ec, which are resistant to most of the generations of cephalosporins by virtue of production of ESBL, that can cleave the six-carbon beta-lactam ring in cephalosporins, we suggest detailed QSAR analysis of the selected leads. The leads can be optimized by fragment building and/or replacement to enhance their bioactivity and resistance to cleavage by ESBL. The addition of active groups from cephalosporins to non-cephalosporin leads, and also to the fluoroquinolones and vice versa to create a new library of

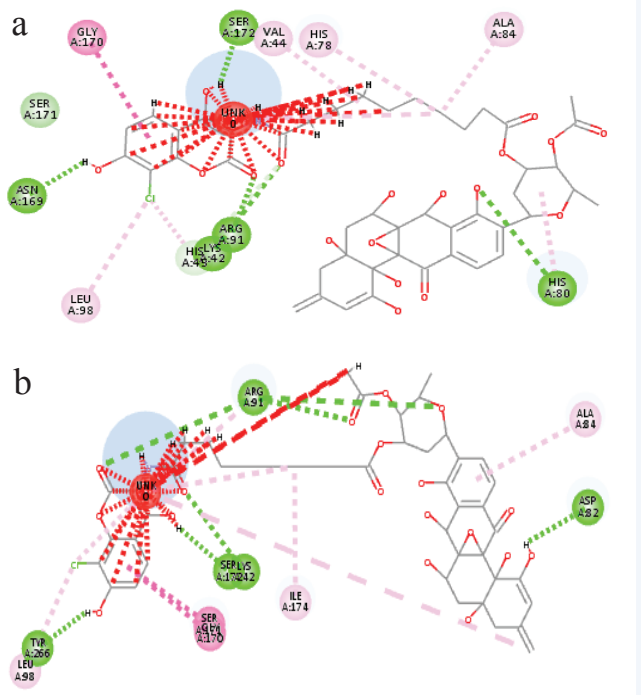

Figure 4 Stearic bumps (depicted in red dotted lines) observed in the a. co-crystallized structure of SD8 and Gyrase A and b. the top-scored docking pose we obtained with Flex-X.

chemically synthesizable molecules, and validation of these hybrid molecules will possibly lead to a newer class of gyrA inhibitors. Alternatively, simple *in vitro* validation using antibiotic sensitivity assays could be planned for different combinations of lead drugs to formulate newer concoctions in a pursuit to combat the drug-resistant *E. coli.*

CONCLUSION AND FUTURE ENHANCEMENT

CADD in conjunction with drug repurposing has already resulted in the worthwhile possibility of second medical use for many drugs, as is quite evident from the trends towards the discovery of effective therapeutics to SARS-CoV-2 to curtail the ongoing COVID-19 pandemic (reviewed by Singh and Gupta, unpublished) [28]. In this research, 12 repurposed drugs with reasonably good binding scores are reported. Out of these, six are cephalosporins and the rest are rather simple molecules like the antibiotic azlocillin, tetrahydrofolate, ATP, 1,4-dihydroNAD, and leucal. Hence, this study has widened the scope of developing promising leads in significantly less time and cost for the most part anti *E. coli* compounds. Accurate *in vitro* studies could be planned in the future based on CADD studies and their outcome may be expected to be fruitful to mankind.

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