Research in Pharmaceutical Sciences, May 2016; 11(3): 250-258
Received: Aug 2015
Accepted: Oct 2015

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Identification of novel bacterial DNA gyrase inhibitors: An *in silico* study

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Abstract

Owing to essential role in bacterial survival, DNA gyrase has been exploited as a validated drug target. However, rapidly emerging resistance to gyrase-targeted drugs such as widely utilized fluoroquinolones reveals the necessity to develop novel compounds with new mechanism of actions against this enzyme. Here, an attempt has been made to identify new drug-like molecules for *Shigella flexneri* DNA gyrase inhibition through *in silico* approaches. The structural similarity search was carried out using the natural product simocyclinone D8, a unique gyrase inhibitor, to virtually screen ZINC database. A total of 11830 retrieved hits were further screened for selection of high-affinity compounds by implementing molecular docking followed by investigation of druggability according to Lipinski's rule, biological activity and physiochemical properties. Among the hits initially identified, three molecules were then confirmed to have reasonable gyrase-binding affinity and to follow Lipinski's rule. Based on these *in silico* findings, three compounds with different chemical structures from previously identified gyrase inhibitors were proposed as potential candidates for the treatment of fluoroquinolone-resistant strains and deserve further investigations.

Keywords: DNA gyrase inhibitor; Structural similarity; Molecular docking; *Shigella flexneri*; Simocyclinone D8

INTRODUCTION

DNA gyrase, also known as DNA topoisomerase II, plays a critical role in bacterial cell survival, and hence has been used as a successful drug target for antibacterial chemotherapy (1). This enzyme is composed of two subunits, namely A and B. The A subunit is engaged in interactions with DNA and contains the active site tyrosine responsible for DNA cleavage, while the B subunit encompasses the active site of ATPase.

The gyrase inhibitor drugs, classified based on their chemical structures, includes aminocoumarins and fluoroquinolones that accomplish their antibacterial activity through two main mechanisms (2). The first mechanism involves inhibition of gyrase enzymatic activity via competition with ATP or substrate while the latter one comprises stabilization of the covalent gyrase–DNA complex or gyrase poisoning (1). These drugs have been very successful in the clinical settings, however, the number of bacterial infections resistant to gyrase inhibitors such as fluoroquinolones, are increasing. Therefore, seeking new antibacterial agents with novel mechanisms for DNA gyrase inhibition is of great practical significance (1). In this regard, fungal secondary metabolites, historically been a rich source of antibiotics, and the natural metabolite simocyclinone D8 (SD8), a compound related to aminocoumarin family, have been reported as DNA gyrase inhibitors.

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SD8 is generated by *Streptomyces antibioticus* Tü 6040 inhibits both gyrase DNA binding and the supercoiling reaction. From structural viewpoint, SD8 contains both aminocoumarin and polyketide elements (3) whose binding pocket is located in the N-terminal domain of GyrA, the same site that also attaches to DNA (4). The occupancy and interaction of the aminocoumarin and polyketide moieties of SD8 with binding pocket would prevent DNA binding at the very first step in the gyrase reaction (4). Recently, the second binding site of SD8 in the C-terminal domain of GyrB has been reported (3), which indicates that the mode of SD8 action is different from that of the fluoroquinolones, and implies a wholly distinctive mechanism as well. Moreover, the two binding sites of SD8 are adjacent to but do not overlap with the one that binds to fluoroquinolones. Accordingly, the discovery of SD8 raises the prospect of developing inhibitors, particularly bi-functional inhibitors that bind simultaneously to the two different sites on their target, i.e., the gyrase molecule (4,5).

Considering these unique properties of SD8, in the present study, structural similarity search and virtual screening docking were performed to identify new compounds for inhibition of *S. flexneri* DNA gyrase based on SD8 structure. To achieve this, the retrieved hits from the similarity search in ZINC database against SD8 were used as input materials for library construction and the compounds library was then subjected to virtual screening by means of AutoDock4.2 package. Subsequently, the chemical ligands with higher binding affinity to gyrase compared to SD8 were selected and further evaluated according to Lipinski's 'rule-of-five', biological activity and physiochemical properties.

MATERIALS AND METHODS

Virtual screening and docking

Firstly, the 3D structure of SD8 was taken from PDB accession code 4CKL and used as a primary backbone for structural similarity search in ZINC database (http://zinc.docking.org/). This database contains more than 35 million compounds with high propensity of being pharmacologically active in a target organism. The environment was set up for searching the compounds with at least 50% structural similarity to SD8, and consequently 11,830 small molecules were found. These molecules were used as ligand library in virtual screening which was performed by AutoDock Tools 4.2. Briefly, for each compound the non-polar hydrogen was merged and Gasteiger-Marsili charges were added, and then, the atoms were adjusted to AutoDock atom types. In addition, rotatable bonds were assigned, and ultimately, prepared ligands were saved in the PDBQT format for docking with the receptor.

Since the structure of N-terminal region of *Escherichia coli* GyrA (PDB accession code: 4CKK) has a sequence identical to the *S. flexneri* DNA gyrase (6) and is also conserved in different strains of enterobacteriaceae (Table 1) as evidenced by multiple alignment studies, we used it as the receptor. The receptor structure file was energy minimized using the Amber99SB-ILDN force field (7) in Gromacs 4.5 package (8) and tripos force field with Kollman united atom charges was added for all atoms of the receptor. Finally, the edited structure of receptor was saved in the PDBQT format for molecular docking studies.

It should be noted that the binding pocket residues were selected in the receptor structure file prior to ligand docking (Fig. 1). This was performed by automatic sequence alignment mode in ClustalW2 software as well as structural superposition based on the specifications of the SD8 binding site in the templates (PDB accession codes: 4CKL and 2Y3P). Docking calculations were peformed by AutoDock Vina (9) and the size of the grid box was set at 33Å × 44Å × 44Å (x, y, and z) with 0.375 Å spacing between the grid points. AutoGrid was used for the preparation of the grid map using a grid box. The results of docking for each ligand were clustered on the basis of root-mean square deviation between the Cartesian coordinates of the ligand atoms and were ranked according to the binding free energy. The most suitable docking mode for ligands with a high score from consensus scoring functions was finally selected. The two-dimensional view of the binding pattern was then visualized by LigPlot+v.1.5 (10).

Table 1. List of sequences used in multiple sequence alignment.

Protein name	Organism	Uniprot ID
DNA gyrase subunit A	*Escherichia coli* str. K-12	U6NB78
DNA gyrase subunit A	*Escherichia coli* C321.deltaA	U5M3T1
DNA gyrase subunit A	*Shigella boydii* serotype 4	Q31Z64
DNA gyrase subunit A	*Shigella flexneri* 5a str	I0VEV9
DNA gyrase subunit A	*Shigella boydii* serotype 18	B2TW19
DNA gyrase subunit A	*Escherichia coli* E2265	S4AJT6
DNA gyrase subunit A	*Shigella boydii* 4444-74	I6DPH2
DNA gyrase subunit A	*Shigella flexneri* K-404	I6DEX2
DNA gyrase subunit A	*Shigella flexneri* K-1770	I6BSD7
DNA gyrase subunit A	*Shigella flexneri* CCH060	I6BPC7
DNA gyrase subunit A	*Shigella flexneri* 2850-71	I6BI02
DNA gyrase subunit A	*Shigella flexneri* 6603-63	J2NHC5

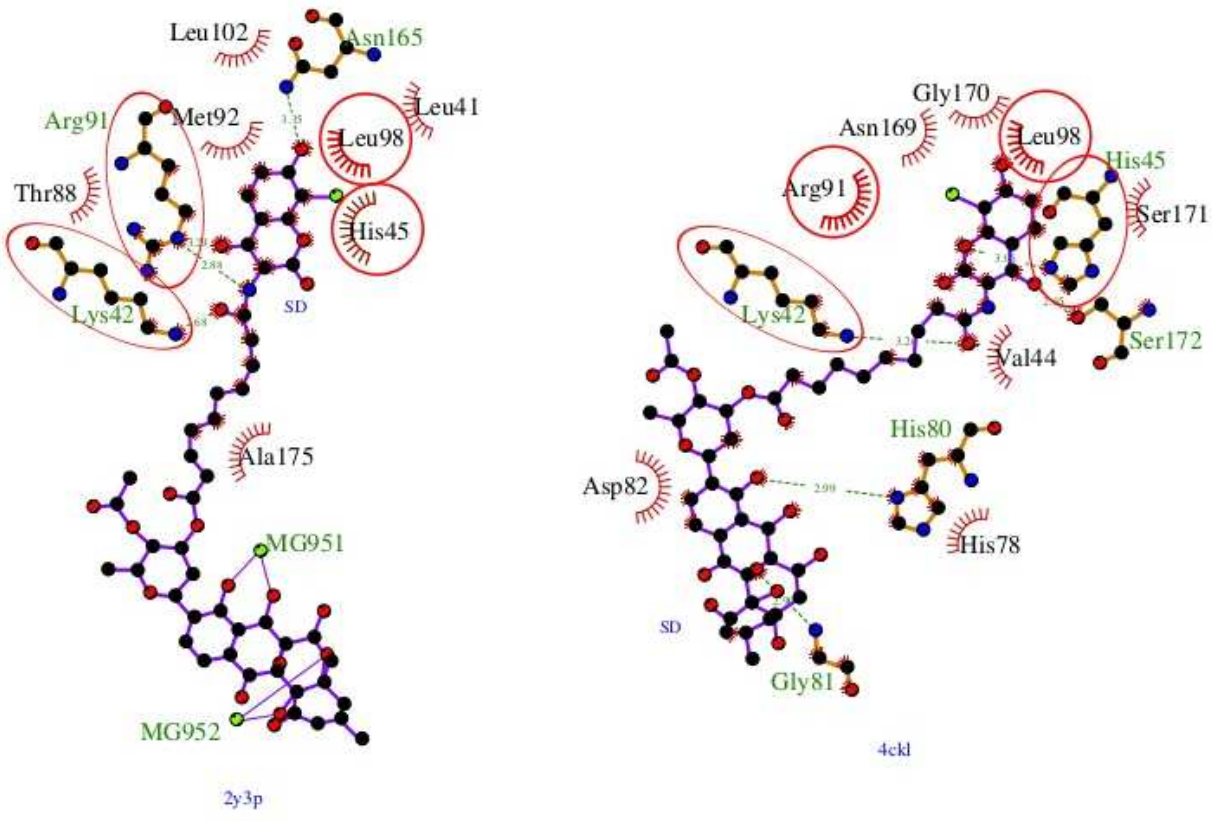

Fig. 1. Binding pocket of SD8 in DNA gyrase. This binding pocket determined via analysis of x-ray structures using LigPlot+. This pocket was used as search space in docking process.

Descriptors calculation and clustering

Structural descriptors were calculated through utilizing ChemMine tool including Atom Pairs, PubChem fingerprints and maximum common substructure descriptors for each compound (11). These structural features are beneficial for binning clustering, hierarchical clustering and multidimensional scaling (11,12).

The distance matrices were generated using all-against-all comparisons of compounds with the Tanimoto coefficient as similarity measures for clustering (13,14). Next, the calculated similarity scores were transformed into distance values. The resulting trees and scatter plots are presented as interactive plots generated with the CanvasXpress javascript library.

RESULTS

The SMILE format of already known DNA gyrase inhibitor, SD8, was used as input for similarity search in the ZINC database and finally 11830 molecules were obtained. They were used for ligands library establishment. Concerning receptor sequence, the results of multiple alignment revealed that the sequence of N-terminal region was conserved among *Escherichia* family (Table 1). In the first step of the docking, the binding pocket and search space were determined by analysis of x-ray structures of SD8 and DNA gyrase complexes (PDB accession codes: 4CKL and 2Y3P) using ligplot and autodocktools4.2 grid plugin Fig. 1), which its coordination was 33 x × 44 × 44 with 39.27 × 30.78 × -39.41 center. The 787 compounds with higher affinity to gyrase than SD8 were clustered based on physicochemical properties using ChemMine tool website. The ChemMine tool uses three software including Open Babel, JOELib and commander to calculate the properties. Then the resultant clusters are represented as multidimensional scaling and heat-map (hierarchical) in Fig. 2. Then, the top item from each cluster was selected as representative of the related cluster (Table 2) and was used for more investigation of the binding pocket. (Fig. 3).

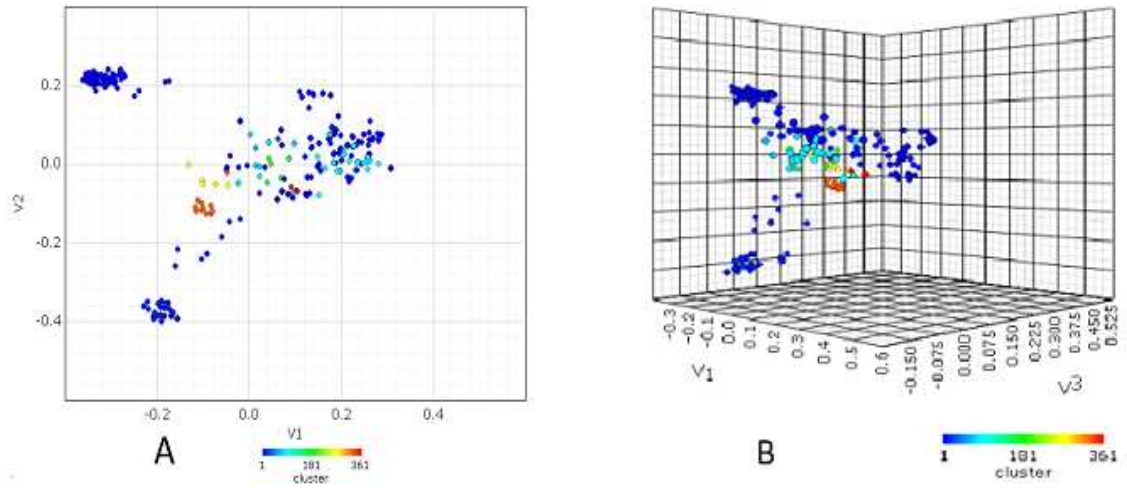

Fig. 2. A; Two-dimensional and B; three-dimensional representation of compound clustering. Color coding represents clusters.

Fig. 3. Two-dimensional representation of the best compounds. Each compound is shown in stick mode with related ZINC ID.

Table 2. Cluster representation and affinity.

Compound	Cluster size	Cluster number	Affinity (Kcal/mol)
ZINC67829151	149	1	-12.6
ZINC85531085	77	5	-12
ZINC85593824	58	11	-11.7
ZINC43335590	34	86	-10.22
ZINC85628568	10	334	-10.8
ZINC72185765	6	2	-12.6
ZINC85531741	5	264	-10.5
ZINC31425392	4	76	-10.6
ZINC29590257	3	74	-9.8
ZINC42802964	3	82	-8.8
ZINC72186671	3	156	-11.1
ZINC28530355	2	71	-9.8
ZINC49654831	2	104	-10.2
ZINC49813778	2	116	-10.5
ZINC85506909	2	193	-10.3
ZINC86039680	2	361	-10.6
ZINC03952456	1	23	-11.4
ZINC28243125	1	59	-11.3
ZINC85509032	1	195	-11.0
ZINC85594529	1	330	-10.9

Table 3. Druggability of compounds.

Compound	xlogP	Ap D	P D	H- D	H- A	Net charge	MW	R bonds	Heavy atoms
ZINC67829151	0.85	10.19	-69.7	2	13	-1	689.69	5	50
ZINC85531085	9.44	25.38	-20.26	1	8	0	794.985	5	59
ZINC85593824	1.21	14.35	-102.6	7	14	2	797.913	8	59
ZINC43335590	-3.33	1.5	-121.63	10	13	0	531.542	4	38
ZINC85628568	0.37	8.03	-119.54	8	11	2	794.94	6	59
ZINC72185765	9.15	22.94	-15.5	0	6	0	713.27	3	52
ZINC85531741	6.16	24.46	-184	4	7	3	819.123	3	61
ZINC31425392	2.38	8.61	-29.69	1	12	0	495.396	5	36
ZINC29590257	5.73	10.17	-59.51	4	13	-1	696.129	10	49
ZINC42802964	3.76	9.9	-19.38	1	9	0	556.663	6	41
ZINC72186671	1.91	9.78	-37.34	6	15	0	505.728	4	52
ZINC28530355	3.88	7.34	-26.24	3	12	0	662.497	11	44
ZINC49654831	2.87	6.84	-12.4	4	12	0	695.806	1	50
ZINC49813778	-4.34	7.68	-151.66	4	16	-1	634.522	4	45
ZINC85506909	1.78	5.23	-123.05	13	16	1	809.849	8	59
ZINC86039680	7.41	10.34	-7.74	3	5	0	602.215	1	43
ZINC03952456	5.39	1.74	-23.64	3	10	0	614.536	6	45
ZINC28243125	5.24	15.97	-31.85	0	10	0	598.995	6	43
ZINC85509032	-2.06	4.92	-113.95	8	17	1	811.821	11	59
ZINC85594529	4.95	17.79	-119.4	5	11	2	768.958	4	54

Ap D, Apolar desolvation; P D, Polar desolvation; H- D, H-bond donors; H- A, H-bond acceptors; MW, Molecular weight.

Table 4. Binding statistics of selected compounds.

Compound	H-bond	Non-polar bond	IUPAC name
ZINC28243125	2	17	(4S)-4-Ethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-
ZINC42802964	0	14	(3R)-10-Cyclohexyl-3-ethyl-N-[2-(4-morpholinyl)ethyl]-1,13-dioxo-
ZINC86039680	0	13	12-Chloro-2-isopropenyl-14b,14c,17,17-tetramethyl-10-methylene-

For investigating druggability of the selected compounds, Lipinski's rule was considered. Results showed that only five molecules, including ZINC85509032, ZINC85593824, ZINC43335590, and ZINC85506909 have more than one mismatch with these rules and must be excluded from the study (Table 3).

Biological activity of compounds was calculated using Molinspiration web server. This activity comprises of binding to GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. Results demonstrated that two compounds (ZINC ID: ZINC49813778 and ZINC86039680) could bind to GPCR receptor and the following items have enzyme inhibitory activity: ZINC28243125, ZINC31425392, ZINC42802964, ZINC43335590 and ZINC86039680. Eventually, three molecules including ZINC28243125, ZINC42802964 and ZINC86039680 (Fig. 4) which follow Lipinski's rule were more analyzed as potent candidates for DNA gyrase inhibition. Figs. 4 and 5 show the binding pocket of the inhibitors onto the active site where the ASP87, Ala117, Asp115, Gln94, Ile112, Ser111, Phe96, Gly114, Gln267, Tyr266, Ser97, Ser172, Leu98, Asn169, His45, Lys42, Arg91, Ser116 and Val90 are directly involved in binding with the ZINC28243125 as the best inhibitor. The binding statistics of these compounds are summarized in Table 4.

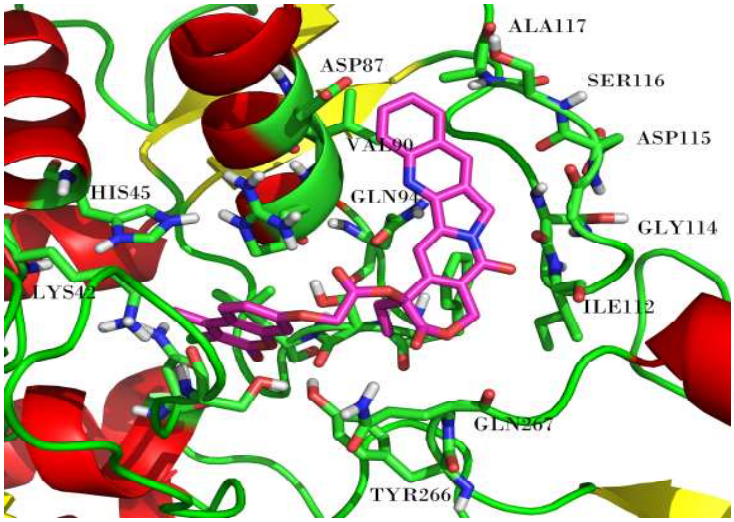

Fig. 4. The binding pocket in three-dimensional representations. The binding pocket and interacting residues are shown in stick mode.

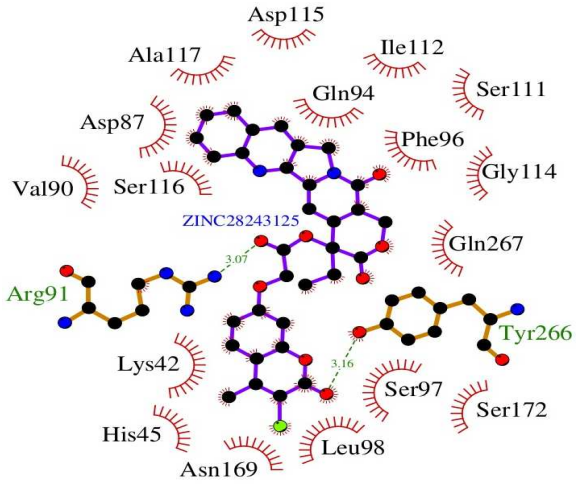

Fig. 5. Two-dimensional representations of interactions between ligand and receptor; the hydrogen and hydrophobic bonds shown by dashed line and an arc with spokes, respectively.

DISCUSSION

The bacterial DNA gyrase has been exploited as an antibacterial target since it is essential in bacteria but is lacking in humans (1). Unfortunately, overzealous use of gyrase inhibitors such as fluoroquinolones appears to impose a selective pressure on bacteria and drive the evolution of multidrug resistance in many pathogens. For instance, the incidence of fluoroquinolone-resistant *Shigella* strains has risen dramatically, presumably due to the extensive use of these drugs. This kind of resistance has been related to the mutations in gyrA83 and/or gyrA87 (15–18) and, interestingly, docking calculations have illustrated that Asp87 and Ser83 mutations affects binding of fluoroquinolones to gyrA, whilst the mutation of Arg121 does not have the same effects.

Among the DNA gyrase inhibitors, SD8 inhibits both ciprofloxacin-resistant mutants and wild-type DNA gyrase (II, I) (18–21), and importantly, this inhibition is performed through a unique mechanism of action. While the fluoroquinolones trap DNA in a covalent complex with the enzyme, SD8 is a competitive inhibitor of DNA binding to the enzyme. In fact, SD8 binds to sites on DNA gyrase that are distinct from the fluoroquinolones binding site (18,20,22). Therefore, designing new compounds based on SD8 structure will probably be helpful to eliminate the infections caused by the fluoroquinolone-resistant strains.

In this study, structural similarity search and molecular docking were implemented to identify new molecules, based on SD8 structure, for DNA gyrase inhibition. The compounds, ZINC28243125, ZINC42802964 and ZINC86039680 exhibited sufficient potency in this regard and, also, were compatible with drug-like criteria of Lipinski's rule in terms of biological activity and physiochemical properties. Among the various residues constituting the binding pocket, Arg91, Tyr266, Asn169, Ala117, Asp87 and Ser116 were directly involved in the binding with these new inhibitors (Fig. 4). From these results, it is evident that Ser83 is excluded from the binding pocket residues and it is likely that *S. flexneri* strains with codon mutations in GyrA 83 will be possibly susceptible to our proposed new compounds.

The compounds ZINC86039680 and ZINC28243125 contain coumarin region, and therefore in this respect they are similar to SD8 (Fig. 3). The SD8-ligand crystal structure reveals distinct binding pockets for the coumarin and the benz[a]anthracene system, both of which are required for maximum inhibition. Consequently, the compounds having similarity to SD8 structure especially with coumarin and the benz[a]anthracene moieties can be proposed as gyrase inhibitors. Moreover, it has been demonstrated in the literature that quinoline ring plays a pivotal role in the gyrase inhibitory activity of molecules and recently, Mitton-Fry and coworkers designed novel quinoline derivatives for the inhibition of *Staphylococcus aureus* DNA gyrase (23). Surprisingly, the compounds ZINC28243125 and ZINC42802964 bear quinoline moiety in their backbones, which may confer them a gyrase inhibitory effect.

In addition to our study, there are a couple of studies suggesting new gyrase inhibitors based on SD8 structure and binding pattern. For example, in a study by Verghese and colleagues (22), a set of flavone-based analogues were investigated as DNA gyrase inhibitors that act mechanistically like SD8. They suggested the bicyclic ring of the flavone as an isosteric replacement for the coumarin moiety of SD8. Flavone-based analogs were also designed by Verghese and colleagues based on SD8's catalytic inhibition mechanism and two out of ten compounds inhibited DNA gyrase (22). However, these compounds also inhibit the human topoisomerase II, which is an important drawback. In the other study by Gaskell and colleagues (24) 16 compounds were synthesized based on SD8 structure in order to determine the pharmacophore required for SD8 binding to DNA gyrase. According to their results, a minimum pharmacophore could be determined including a coumarin scaffold bonded to a carboxylic acid group through an approximately 15 Å hydrocarbon linker. The structure of compounds in the above mentioned reports are

somehow similar to the three potent compounds introduced in the present study.

The structural features of gyrase inhibitors required for efficient interaction with DNA gyrase in the binding pocket has been the subject of some studies in the literature. In an effort by Huang and coworkers to extract the bioactive molecular fingerprints from a data set of 61 gyrase inhibitors, they suggested a pharmacophore model consisting one hydrophobic feature and three hydrogen bond acceptor features (25). In our present study, the compound ZINC28243125 has two hydrogen bond acceptor features and 17 hydrophobic features, which approximately match with the composition of pharmacophore features proposed by Huang and colleagues.

CONCLUSION

Based on the SD8 structure and through computational methods, three potential candidate molecules for inhibition of bacterial DNA Gyrase were identified. The compound ZINC28243125 indicated highest activity and has a great potential of being developed as an efficient drug for *S. flexneri* DNA gyrase inhibition. However, further studies are needed to confirm that this compound display much better clinical efficiency toward resistant bacteria than existing drugs.

ACKNOWLEDGEMENTS

We are thankful to the System Biology Laboratory in Molecular Biology Research Center of Baqiyatallah University of Medical Sciences for financial support of this project.

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