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ORIGINAL RESEARCH

In-silico design, synthesis and evaluation of novel DNA-gyrase B inhibitors

Umesh Shiroya · Milan Patel

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Abstract 2-Quinolones are an important class of compounds, isomeric to 4-quinolones. They may become promising candidates for exploiting more useful therapeutically active molecules. DNA-gyrase has drawn much attention as a selected target for finding potent anti-bacterial agents against multi-drug resistant strains such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *enterococci*, and penicillin-resistant *Streptococci pneumonia*. The objective of the present study was to study the molecular docking simulations on 2-quinolone analogs as probable candidates for inhibiting DNA gyrase subunit-B of *S. aureus*. In the present study, docking simulations were carried out on the reported inhibitors of DNA-gyrase subunit A and B using docking software. Based on it, series of 2-quinolone analogs (compound **1–8**) were designed, synthesized, characterized, and evaluated for their anti-bacterial activity against *S. aureus* and *E. coli*. Out of the eight test compounds, compound-**2** showed good anti-bacterial activity against *S. aureus* and *E. coli* as compared with the rest of the other compounds. The rational approach to lead discovery has prompted a better insight into developing more specific 2-quinolones as potential antibacterial agents.

Keywords Antibacterial activity · Docking simulations · DNA-gyrase · 2-quinolones

U. Shiroya (✉)
17/Gopi Park Society, Hirawadi Road, Bapunagar, Ahmedabad 382350, Gujarat, India
e-mail: u_shiroya@yahoo.com

U. Shiroya · M. Patel
Department of Pharmaceutical Chemistry, L.B.Rao Institute of Pharmaceutical Education and Research, Bethak Road, Khambhat 388620, Gujarat, India

Introduction

The increasing use of anti-bacterial agents such as beta-lactams, macrolides, vancomycin, or quinolones has resulted in the emergence of multi-drug resistant pathogens, especially gram-positive bacteria (Tanitame *et al*., 2004a, b). Widespread emergence of bacterial resistance to present drugs represents a serious problem in treatment of bacterial infections. Modern approaches toward development of new potential inhibitors are based on knowledge of structure and function of proteins specific to bacteria. One of them is DNA gyrase, a characteristic and essential bacterial enzyme the inactivation of which leads to bacterial death. For this reason, gyrases have been chosen as targets for antibacterial agents (Gradisar *et al.,* 2007). DNA-gyrase has drawn much attention as a selected target for finding potent anti-bacterial agents against multi-drug resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE), and penicillin-resistant *S. pneumonia* (PRSP). DNA-gyrase, a typical type-II topoisomerase, has been known to cause DNA replication, transcription, and recombination. DNA-gyrase catalyzes the ATP-dependent introduction of negative supercoils into bacterial DNA as well as the decatenation and unknotting of DNA. The DNA-gyrase enzyme consists of two subunits, A and B, of molecular mass 97 and 90 kDa, respectively, with the active enzyme being an A_2B_2 complex (Liu *et al.,* 2009, Brvar *et al.,* 2010).

The structure activity relationships of compounds based on nalidixic acid have led to a large group of synthetic antibacterial agents known collectively as quinolones. The 4-quinolones such as ciprofloxacin, ofloxacin, lomefloxacin, enoxacin are established synthetic antibacterial agents which act as DNA gyrase inhibitors (Mitscher *et al.*, 1986).

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The benzopyrone ring in compounds of coumarins and flavones is isosteric to quinolones and has shown DNA gyrase inhibition (Gradisar *et al.,* 2007). The 2-quinolones, also called carbostyrils or 1-aza coumarins, are isosteric to coumarins and isomeric to 4-quinolones and could become a potential candidate for antibacterial activity (Thomas *et al.*, 2010). Reports are available on DNA gyrase inhibition by quinolones, coumarins (chlorobiocin, novobiocin), and cyclothialidines (Wentland *et al*. 1988, Boehm *et al*. 2000, Gray *et al.,* 2003, Foroumadi *et al.,* 2003, Tanitame *et al*., 2005). Quinolones are known to inhibit the DNA breakage–reunion cycle by binding to the subunit A and by blocking the gyrase–DNA complex, whereas the latter acts on subunit B. More recently, GyrB inhibitors from various chemical classes have been reported including indazole, pyrazole, benzimidazole, phenol, and indolinone (Tanitame *et al*. 2004a, 2004b, Angehrn *et al.,* 2004, Liu *et al.,* 2008, Brvar *et al.,* 2010, Ronkin *et al.,* 2010).

In the present study, docking simulations were carried out on the reported inhibitors of DNA-gyrase subunit A and B using docking software. Based on it, series of 2-quinolone analogs (Compound 1 to 8) were designed, synthesized, characterized, and evaluated for their anti-bacterial activity against *S. aureus* and *E. coli*.

Materials and methods

Chemistry

The chemical structure and three-dimensional (3D) structures were drawn using Structure Builder (Marvin-Sketch version-5.2, ChemAxon). Docking studies were done using the facilities of Molecular Modeling (Arguslab 4.0.1, Mark Thompson and Planaria Software LLC). The melting point was determined using a melting point apparatus (DBK Precision melting point apparatus) and found to be uncorrected. The reactions were monitored by thin-layer chromatography (TLC), and the R_f values were determined using TLC plates with the solvent system (Toluene : Methanol (4:2)). The λ_{max} values for the synthesized compounds were established by ultraviolet (UV)–visible spectrometer (UV-1700 Pharmaspec, Shimadzu Corporation, Kyoto). The IR studies were done with FT-IR (DRS-8400, Shimadzu Corporation, Japan). The NMR study was carried out by the instrument NMR using DMSO-d6 solvent (Bruker Avance-II 500 MHz FT-NMR, TOPSPIN 1.3 Version), and the mass spectral studies were done using LC–MS (MDS SCIEX API 2000 LC–MS/MS).

In silico approaches

One of the most important considerations in docking simulations is the selection of appropriate docking and scoring algorithm. There are several algorithms and number of scoring functions available today for docking. Roughly, the scoring functions are categorized into knowledge based and empirical categories. In the present study, an attempt was made to work with Arguslab for the docking calculations.

The crystal structure of 24-kDa N-terminal fragment of DNA-gyrase B complex with inhibitor, 4-methyl-5(3-(Methylsulfanyl)-1*H*-pyrazol-5-yl)-2-thiophen-2-yl-1,3-thiazole (PDB code: 3G75, www.rcsb.org) was retrieved from protein data bank in PDB format as starting point. All water molecules from protein structure were deleted. Protein structure consists of two chains A and B; among them chain-B was deleted. In protein structure, missing bond orders, hybridization states, charges, and angles were assigned, and explicit hydrogen molecules were added using the different parameters. The protein structure was energy minimized by Line search method using geometry optimization method. The Validation of docking algorithms (Fig. 1) was performed by changing different docking parameters until the lowest RMSD value was obtained. By using the Grid resolution 0.8 Å and Lamarckian genetic algorithms dock engine, while other parameters were set as default, the RMSD value of best pose of ligand was obtained as 1.62 Å.

All molecular docking simulations were carried out using Arguslab Docker (ALD) version 4.0.1 installed on Intel Pentium-4 machine. All the ligand structures were constructed using Chemsketch software version-5.2

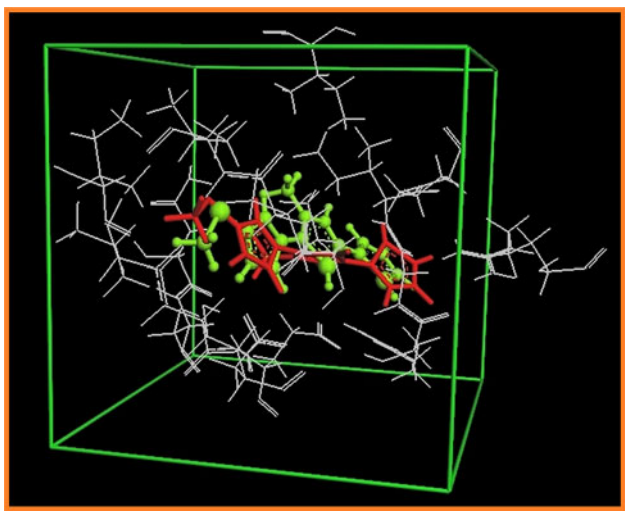


Fig. 1 Showing the validation of docking algorithm using protein structure 3G75

installed on Intel Pentium-4 machine. The ligands were saved in MDL-Mol file. The ligands were then imported in Mervin-view software version-5.2 installed on Intel Pentium-4 machine, and explicit hydrogen atoms were added and converted to 3D conformations using Mervin-view software. The ligands were then saved in a single MDL-Mol file. The ligands were energy minimized by Line search method using geometry optimization function in Argus-lab, and then lower energy conformations were selected for further studies. Different docking parameters were taken as per validation of docking algorithms (Table 1). All docking calculations were carried out using grid based A-Score function with a Grid resolution of 0.8 Å. The binding site on the receptor was defined as extending in X, Y, and Z directions around dock molecule with binding site box size of around 15 Å. Lamarckian genetic dock algorithm with maximum of 1,000 generations was used through the calculations keeping all other parameters as default. The lowest binding free energy pose of conformation was displayed and saved in pdb format. Then pdb file was imported into the Molegro virtual viewer version 2010 2.0.0 for analyzing different interactions such as hydrogen bonding, hydrophobicity, electrostatic interactions, docking view etc.

Design of synthesized test compounds and their docking

Molecular docking studies offer precise information for studying the interaction between the ligand and the protein residue and aids as a guide for the drug design. In the present study, the key feature was focused on the optimization of the amino group attached to C-7 of 2-quinolone. Initially, Molecular docking simulations were carried out using 7-amino-4-methylquinolin-2(1H)-one as reported in the Thomas *et al.*. Then side chain of 7-amino-4-methylquinolin-2(1H)-one was extended and the substituted phenyl group and heterocycles were chosen for their steric, hydrophobic, and van der Waal interactions with the protein residues. Secondly, the carboxylic acid group was selected as the hydrogen bond donor to be attached to the phenyl ring. A total of eight acid derivatives were designed for molecular docking study. The docking studies (compound-**A**) of 7-amino-4-methylquinolin-2(1H)-one (Fig. 2) revealed the interaction of N-1 of 2-quinolone with Ser-55 and the hydrogen bonding between C-7 amino of molecule and Thr-173. The docking studies (Fig. 3) of 4-((4-methyl-2-oxo-1,2-dihydroquinolin-7yl)amino)benzoic acid with a 24-kDa fragment of DNA-gyrase B from *S.aureus* (Compound-2) revealed the one hydrogen bonding between carboxylic acid group of molecule and Ser-129 residue and

Table 1 Showing the docking parameters used for the docking simulations

Different docking parameters taken for docking simulation	
Grid resolution = 0.8 Å	Cross over rate = 0.8
Genetic algorithms dock engine	Elitism = 5
Calculation type = DOCK	Mutation rate = 0.2
Scoring function = A Score	Convergence = 0.0015936
Local search rate = 0.06	Binding site box size = 15 × 15 × 15 Å
Local search convergence = 0.000159 Å	Local search steps = 20
Population size = 50	Maximum generations = 1,000

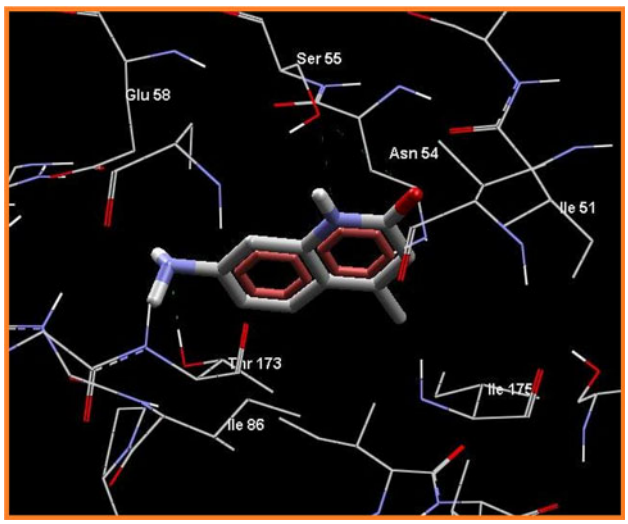


Fig. 2 Showing the docking studies of 7-amino-4-methylquinolin-2(1H)-one (Compound-A) revealed the interaction of N-1 of 2-quinolone with Ser-55 and the hydrogen bonding between C-7 amino of molecule and Thr-173

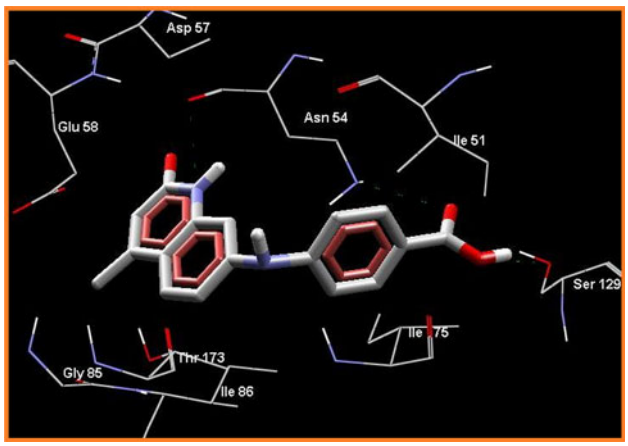


Fig. 3 Showing the docking studies of 4-((4-methyl-2-oxo-1,2-dihydroquinolin-7yl)amino)benzoic acid (Compound-2) revealed the hydrogen bonding between carboxylic acid group of molecule and Ser-129, the hydrophobic interaction between substituted phenyl ring and Ileu-175 residue and the hydrogen bonding between N-1 of 2-quinolone and Asn-54 residue

the hydrophobic interaction between substituted phenyl ring and Ileu-175 residue and one hydrogen bonding between N-1 of 2-quinolone and Asn-54 residue. Molecular docking was carried out for total eight derivatives of 2-quinolones; among them Compound-2 and Compound-7 showed better binding interactions within active site of DNA-gyrase B subunit.

Synthesis

The chemicals and reagents used in the project work were of AR and LR grade, procured from s.d.fine Chem. Ltd., National Chemicals, Qualigens Fine Chemicals, Ranbaxy fine chemicals limited, Shital Chemical Industries, and Finar reagents. The chemicals were used without further purification.

Based on the molecular docking simulations studies, it was decided to synthesize 2-quinolone and its derivatives. The present study utilizes Conrad-Limpach synthesis for the preparation of 7-amino-4-methyl-2-quinolone (Furniss and Hannaford, 2005). It was obtained by the reaction of m-phenylene diamine with ethylacetoacetate to form the amido-ketone by cyclization at high temperature. The nature of substituted anilines, the temperature, and the solvent used greatly influence the synthesis.

Synthesis of 7-amino-4-methylquinolin-2(1*H*)-one (compound-**A**, Fig. 4) included 10.8 g (0.1 mol) of m-phenylenediamine and 12.64 ml (0.1 mol) of ethylacetoacetate, which were taken together in a 250 ml round bottomed flask. The reaction mixture was refluxed at 150 °C for 18 h on an oil bath. At the end of the reaction period, 200 ml of water was added to the flask, and the contents were heated on a hot plate to the boiling temperature of water. The mixture was then filtered; the filtrate was chilled in refrigerator till the precipitation appeared. The precipitate was retrieved, dried in air, recrystallized from methanol, and was characterized.

Synthesis of derivatives (Fig. 4)

A mixture of 5.77 g (0.166 mol) 7-amino-4-methylquinolin-2(1H)-one, 0.026 mol of different halo-acids (o-chlorobenzoic acid, p-chlorobenzoic acid, m-bromobenzoic acid, 2-chloropyridine-3-carboxylic acid, 4-chloropyridine-3-carboxylic acid, 6-chloropyridine-3-carboxylic acid, 3-chloropyrazine-2-carboxylic acid, and 5-chloropyrazine-2-carboxylic acid), 0.82 g of K_2CO_3, and 0.2 g of copper foil and 0.02 g tin powder was heated under gentle reflux for 2–3 h in a 250 ml round bottomed flask. The mixture was cooled, and to the semi solid mass, a mixture of 5 ml of concentrated hydrochloric acid and 5 ml of water

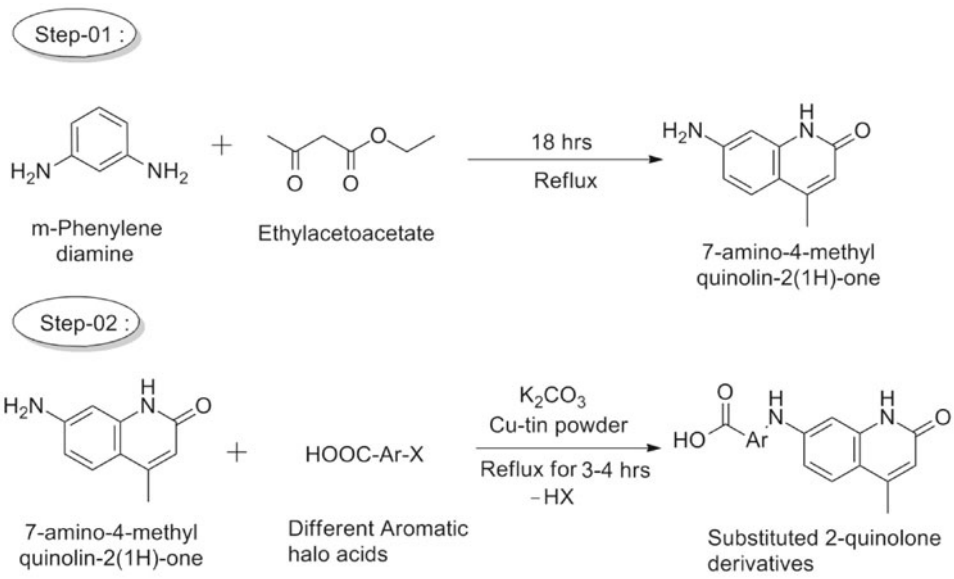


Fig. 4 Showing the general scheme of synthesis for substituted 2-quinolone analogs

Compound Code	-Ar
Compound-1	o-chlorobenzoic acid
Compound-2	p-chlorobenzoic acid
Compound-3	m-bromobenzoic acid
Compound-4	2-chloropyridine-3-carboxylic acid
Compound-5	4-chloropyridine-3-carboxylic acid
Compound-6	6-chloropyridine-3-carboxylic acid
Compound-7	5-chloropyrazine-2-carboxylic acid
Compound-8	3-chloropyrazine-2-carboxylic acid

were added. The resulting mixture was shaken vigorously, filtered, and the residue was dissolved in 4 ml of 4 N NaOH. Then, decolorizing carbon was added and boiled for 2–3 min and filtered the hot mixture. The mixture was cooled, and the filtrate was acidified with conc. hydrochloric acid and filtered. The residue was washed with about 100 ml of water and dried at 100 °C.

Spectral studies

*Compound-**A** (7-amino-4-methylquinolin-2(1H)-one)*

The yield was 75 %, the melting point 265–268 °C, and the R_f value 0.60. Spectroscopic analysis showed IR (KBr) cm^{-1}: NH_2 (str) 3,300–3,500, NH (str) 2 amide 3,200–3,300, NH (b) amide 1,550–1,600, C=O (str) 1,630–1,660, C=C (str) aromatic 1,400–1,450, CH (str) aromatic in methyl 1,250–1,300, CH (str) aromatic 2,850–3,000. MASS m/e ratio: 175.08 $[M+1]^+$, 147.08 $[M–CHO]^+$, 107.05 $[M–C_4H_5O]^+$, 93.00 $[M–C_4H_6NO]^+$. ^{1}H-NMR (500 MHz, DMSO-d6): 2.42 (s, 3H, CH_3), 6.05 (d, 1H, Ar–H), 6.27 (s, 2H, Ar–NH_2), 6.35 (s, 1H, Ar–H), 6.90 (s, 1H, Ar–H), 7.12 (d, 1H, Ar–H), 8.05 (s, 1H, Ar–NH). ^{13}C-NMR (500 MHz, DMSO-d6): 19, 107, 109, 110, 121, 127, 138, 146, 148, 162.

*Compound-**1** 2-((4-methyl-2-oxo-1,2-dihydroquinolin-7yl)amino)benzoic acid*

The yield was 72 %, the melting point 140–143 °C, and the R_f value 0.49. Spectroscopic analysis showed IR (KBr) cm^{-1}: NH (str) 2 amine 3,100–3,300, NH (str) 2 amide 3,200–3,300, NH (b) amide 1,550–1,600, C=O (str) 1,630–1,660, C=C (str) aromatic 1,400–1,450, CH (str) aromatic 2,850–3,000, CH (str) aromatic in methyl 1,250–1,300, C=O (str) in carboxylic acid 1,700–1,750, OH (str) in carboxylic acid 2,500–2,800, C–O (str) in carboxylic acid 1,200–1,300. MASS m/e ratio: 295.10 $[M+1]^+$, 267.11 $[M–CHO]^+$, 227.07 $[M+1]$ $[M–C_4H_5O]^+$, 122.03 $[M+1]$ $[M–C_{10}H_{10}ON_2]^+$, 107.05 $[M+2]$ $[M–C_{11}H_{10}O_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d6): 2.42 (s, 3H, CH_3), 4.05 (s, 1H, Ar–NH), 6.35 (s, 1H, Ar–H), 6.90 (s, 1H, Ar–H), 7.02 (t, 1H, Ar–H), 7.11 (d, 1H, Ar–H), 7.21 (d, 1H, Ar–H), 7.54 (t, 1H, Ar–H), 7.85 (d, 1H, Ar–H), 8.05 (s, 1H, Ar–NH), 8.42 (d, 1H, Ar–H), 11.0 (s, 1H, Ar-COOH). ^{13}C-NMR (500 MHz, DMSO-d6): 19, 110, 112, 113, 118, 121, 128, 131, 135, 138, 162, 169.

*Compound-**2** 4-((4-methyl-2-oxo-1,2-dihydroquinolin-7yl)amino)benzoic acid*

The yield was 78 %, the melting point 194–197 °C, and the R_f value 0.45. Spectroscopic analysis showed IR (KBr) cm^{-1}: NH (str) 2 amine 3,100–3,300, NH (str) 2 amide 3,200–3,300, NH (b) amide 1,550–1,600, C=O (str) 1,630–1,660, C=C (str) aromatic 1,400–1,450, CH (str) aromatic 2,850–3,000, CH (str) aromatic in methyl 1,250–1,300, C=O (str) in carboxylic acid 1,700–1,750, OH (str) in carboxylic acid 2,500–2,800, C–O (str) in carboxylic acid 1,200–1,300. MASS m/e ratio: 295.10 $[M+1]^+$, 267.11 $[M–CHO]^+$, 227.07 $[M + 1]$ $[M–C_4H_5O]^+$, 122.03 $[M+1]$ $[M–C_{10}H_{10}ON_2]^+$, 107.05 $[M+2]$ $[M–C_{11}H_{10}O_3]^+$. ^{1}H-NMR (500 MHz, DMSO-d6): 2.44 (s, 3H, CH_3), 4.08 (s, 1H, Ar–NH), 6.34 (s, 1H, Ar–H), 6.89 (s, 1H, Ar–H), 7.14 (d, 1H, Ar–H), 7.21 (d, 1H, Ar–H), 7.73 (d, 2H, Ar–2H), 7.81 (d, 2H, Ar–2H), 8.05 (s, 1H, Ar–NH), 11.0 (s, 1H, Ar–COOH). ^{13}C-NMR (500 MHz, DMSO-d6): 19, 110, 111, 121, 122, 128, 131, 138, 148, 162, 169.

*Compound-**3** 3-((4-methyl-2-oxo-1,2-dihydroquinolin-7yl)amino)benzoic acid*

The yield was 80 %, the melting point 178–180 °C, and the R_f value 0.52. Spectroscopic analysis showed IR (KBr) cm^{-1}: NH (str) 2 amine 3,150–3,300, NH (str) 2 amide 3,100–3,300, NH (b) amide 1,550–1,615, C=O (str) 1,630–1,660, C=C (str) aromatic 1,400–1,450, CH (str) aromatic 2,850–3,000, CH (str) aromatic in methyl 1,250–1,300, C=O (str) in carboxylic acid 1,700–1,750, OH (str) in carboxylic acid 2,500–2,800, C–O (str) in carboxylic acid 1,200–1,300. MASS m/e ratio: 295.30 $[M+1]^+$, 267.65 $[M–CHO]^+$, 227.02 $[M+1]$ $[M–C_4H_5O]^+$, 122.32 $[M+1]$ $[M–C_{10}H_{10}ON_2]^+$, 107.15 $[M+2]$ $[M–C_{11}H_{10}O_3]^+$ ^{1}H-NMR (500 MHz, DMSO-d6): 2.42 (s, 3H, CH_3), 4.01 (s, 1H, Ar–NH), 6.35 (s, 1H, Ar–H), 6.90 (s, 1H, Ar–H), 7.11 (d, 1H, Ar–H), 7.21 (d, 1H, Ar–H), 7.27 (s, 1H, Ar–H), 7.41 (t, 1H, Ar–H), 7.57 (d, 1H, Ar–H), 7.97 (d, 1H, Ar–H), 8.02 (s, 1H, Ar–NH), 11.05 (s, 1H, Ar–COOH). ^{13}C-NMR (500 MHz, DMSO-d6): 19, 110, 111, 117, 120, 121, 126, 128, 129, 134, 137, 138, 139, 148, 162, 166.

*Compound-**4** 2-((4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)amino)nicotinic acid*

The yield was 68 %, the melting point 155–158 °C, and the R_f value 0.44. Spectroscopic analysis showed IR (KBr) cm^{-1}: NH (str) 2 amine 3,100–3,300, NH (str) 2 amide 3,200–3,300, NH (b) amide 1,550–1,600, C=O (str) 1,630–1,660, C=C (str) aromatic 1,400–1,450, CH (str) aromatic 2,850–3,000, CH (str) aromatic in methyl 1,250–1,300, C=O (str) in carboxylic acid 1,700–1,750, OH (str) in carboxylic acid 2,500–2,800, C–O (str) in carboxylic acid 1,200–1,300. C=N (str) aromatic 1,450–1,515. MASS m/e ratio: 296.30 $[M+1]^+$, 267.13 $[M–CHO]^+$, 227.06 $[M+1]$ $[M–C_4H_5O]^+$, 159.05 $[M+1]$ $[M–C_7H_8ON_2]^+$, 124.03 $[M+1]$ $[M–C_{10}H_{10}ON_2]^+$, 107.15 $[M+2]$ $[M–C_{11}H_{10}O_3]^+$

^{1}H-NMR (500 MHz, DMSO-d6): 2.42 (s, 3H, CH_3), 4.00 (s, 1H, Ar–NH), 6.38 (s, 1H, Ar–H), 6.95 (s, 1H, Ar–H), 7.14 (d, 1H, Ar–H), 7.21 (d, 1H, Ar–H), 7.25 (t, 1H, Ar–H), 7.96 (d, 1H, Ar–H), 8.02 (s, 1H, Ar–NH), 8.28 (d, 1H, Ar–H), 11.04 (s, 1H, Ar–COOH). ^{13}C-NMR (500 MHz, DMSO-d6): 19, 106, 108, 110, 112, 113, 121, 127, 138, 139, 142, 148, 151, 161, 162, 169.

*Compound-**5** 4-((4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)amino)nicotinic acid*

The yield was 73 %, the melting point 180–182 °C, and the R_f value 0.54. Spectroscopic analysis showed IR (KBr) cm^{-1}: NH (str) 2 amine 3,100–3,350, NH (str) 2 amide 3,225–3,300, NH (b) amide 1,550–1,600, C=O (str) 1,630–1,660, C=C (str) aromatic 1,400–1,450, CH (str) aromatic 2,850–3,000, CH (str) aromatic in methyl 1,250–1,300, C=O (str) in carboxylic acid 1,700–1,750, OH (str) in carboxylic acid 2,500–2,800, C–O (str) in carboxylic acid 1,200–1,340. C=N (str) aromatic 1,450–1,550. MASS m/e ratio: 296.29 $[M+1]^+$, 280.06 $[M–CH_3]^+$, 267.10 $[M–CHO]^+$, 227.16 $[M+1]$ $[M–C_4H_5O]^+$, 159.28 $[M+1]$ $[M–C_7H_8ON_2]^+$, 124.16 $[M+1]$ $[M–C_{10}H_{10}ON_2]^+$, 107.15 $[M+2]$ $[M–C_{11}H_{10}O_3]^+$ ^{1}H-NMR (500 MHz, DMSO-d6): 2.42 (s, 3H, CH_3), 4.01 (s, 1H, Ar–NH), 6.34 (s, 1H, Ar–H), 6.92 (s, 1H, Ar–H), 7.05 (d, 1H, Ar–H), 7.12 (d, 1H, Ar–H), 7.20 (d, 1H, Ar–H), 8.02 (s, 1H, Ar–NH), 8.71 (d, 1H, Ar–H), 8.73 (s, 1H, Ar–H), 11.0 (s, 1H, Ar–COOH). ^{13}C-NMR (500 MHz, DMSO-d6): 19, 106, 108, 110, 120, 127, 137, 141, 148, 151, 152, 157, 162, 169.

*Compound-**6** 6-((4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)amino)nicotinic acid*

The yield was 74 %, the melting point 167–170 °C, and the R_f value 0.46. Spectroscopic analysis showed IR (KBr) cm^{-1}: NH (str) 2 amine 3,100–3,300, NH (str) 2 amide 3,200–3,300, NH (b) amide 1,550–1,600, C=O (str) 1,630–1,660, C=C (str) aromatic 1,400–1,450, CH (str) aromatic 2,850–3,000, CH (str) aromatic in methyl 1,250–1,300, C=O (str) in carboxylic acid 1,700–1,750, OH (str) in carboxylic acid 2,500–2,800, C–O (str) in carboxylic acid 1,200–1,300. C=N (str) aromatic 1,475–1,550. MASS m/e ratio: 296.32 $[M+1]^+$, 267.10 $[M+1]$ $[M–CHO]^+$, 227.16 $[M+1]$ $[M–C_4H_5O]^+$, 183.07 $[M+1]$ $[M–C_5H_6O_3]^+$, 124.16 $[M+1]$ $[M–C_{10}H_{10}ON_2]^+$, 107.15 $[M+2]$ $[M–C_{11}H_{10}O_3]^+$.^{1}H-NMR (500 MHz, DMSO-d6): 2.41 (s, 3H, CH_3), 4.03 (s, 1H, Ar–NH), 6.35 (s, 1H, Ar–H), 6.82 (d, 1H, Ar–H), 6.90 (s, 1H, Ar–H), 7.11 (d, 1H, Ar–H), 7.21 (d, 1H, Ar–H), 7.72 (d, 1H, Ar–H), 8.01 (s, 1H, Ar–NH), 8.17 (s, 1H, Ar–H), 11.0 (s, 1H, Ar–COOH). ^{13}C-NMR (500 MHz, DMSO-d6): 19, 106, 107, 110, 112, 120, 127, 137, 140, 142, 148, 150, 158, 161, 166.

*Compound-**7** 5-((4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)amino)pyrazine-2 carboxylic acid*

The yield was 71 %, the melting point 205–207 °C, and the R_f value 0.50. Spectroscopic analysis showed IR (KBr) cm^{-1}: NH (str) 2 amine 3,100–3,300, NH (str) 2 amide 3,200–3,300, NH (b) amide 1,550–1,600, C=O (str) 1,630–1,660, C=C (str) aromatic 1,400–1,450, CH (str) aromatic 2,850–3,000, C–N (str) aromatic 1,080–1,200, CH (str) aromatic in methyl 1,250–1,300, C=O (str) in carboxylic acid 1,700–1,750, OH (str) in carboxylic acid 2,500–2,800, C–O (str) in carboxylic acid 1,200–1,300, C=N (str) aromatic 1,500–1,550. MASS m/e ratio: 297.09 $[M+1]^+$, 269.10 $[M–CHO]^+$, 254.07 $[M+1]$ $[M–C_2H_4O]^+$, 229.06 $[M+1]$ $[M–C_4H_5O]^+$, 125.04 $[M+1]$ $[M–C_{10}H_{10}ON_2]^+$, 106.05 $[M+1]$ $[M–C_9H_9O_3N_2]^+$. ^{1}H-NMR (500 MHz, DMSO-d6): 2.40 (s, 3H, CH_3), 4.02 (s, 1H, Ar–NH), 6.38 (s, 1H, Ar–H), 6.91 (s, 1H, Ar–H), 7.10 (d, 1H, Ar–H), 7.24 (d, 1H, Ar–H), 8.36 (s, 1H, Ar–H), 8.61 (s, 1H, Ar–H), 8.01 (s, 1H, Ar–NH), 11.13 (s, 1H, Ar–COOH). ^{13}C-NMR (500 MHz, DMSO-d6): 19, 107, 108, 110, 120, 127, 133, 136, 138, 142, 146, 148, 160, 162, 165.

*Compound-**8** 3-((4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)amino)pyrazine-2-carboxylic acid*

The yield was 76 %, the melting point 210–213 °C, and the R_f value 0.52. Spectroscopic analysis showed IR (KBr) cm^{-1}: NH (str) 2 amine 3,100–3,300, NH (str) 2 amide 3,225–3,300, NH (b) amide 1,550–1,600, C=O (str) 1,630–1,660, C=C (str) aromatic 1,400–1,450, CH (str) aromatic 2,850–3,000, C–N (str) aromatic 1,080–1,200, CH (str) aromatic in methyl 1,250–1,300, C=O (str) in carboxylic acid 1,700–1,750, OH (str) in carboxylic acid 2,500–2,800, C–O (str) in carboxylic acid 1,200–1,300, C=N (str) aromatic 1,450–1,550. MASS m/e ratio: 297.28 $[M+1]^+$, 269.10 $[M–CHO]^+$, 254.07 $[M+1]$ $[M–C_2H_4O]^+$, 229.06 $[M+1]$ $[M–C_4H_5O]^+$, 184.07 $[M+1]$ $[M–C_5H_6O_3]^+$, 125.04 $[M+1]$ $[M–C_{10}H_{10}ON_2]^+$, 106.05 $[M+1]$ $[M–C_9H_9O_3N_2]^+$. ^{1}H-NMR (500 MHz, DMSO-d6): 2.42 (s, 3H, CH_3), 4.01 (s, 1H, Ar–NH), 6.35 (s, 1H, Ar–H), 6.91 (s, 1H, Ar–H), 7.11 (d, 1H, Ar–H), 7.25 (d, 1H, Ar–H), 7.84 (d, 1H, Ar–H), 8.02 (s, 1H, Ar–NH), 8.40 (d, 1H, Ar–H), 11.03 (s, 1H, Ar–COOH). ^{13}C-NMR (500 MHz, DMSO-d6): 19, 107, 108, 110, 121, 127, 136, 138, 142, 148, 157, 162, 168.

Anti-bacterial activity

Antimicrobial activity was determined by the agar diffusion method. All eight synthesized test compounds were tested against two species of bacteria, namely, *S. aureus* (gram-positive) and *E. coli* (gram-negative). Stock solutions of synthesized test compounds and standard drug were prepared in DMSO. Ciprofloxacin was used as standard at a concentration of 1,500 μM. The test compounds were used at a concentration of 1,500 μM. Nutrient agar medium (peptic digest of animal tissue 5 gms/liter, beef extract 1.50 gms/liter, yeast extract 1.50 gms/liter, sodium chloride 5 gms/liter, and agar 15 gms/liter in distilled water at a pH 7.4 ± 0.2 and 25 °C, sterilized by autoclave at 15 lb pressure at 121 °C for 15 min) was used for the agar diffusion method. The petri-dishes were thoroughly washed and sterilized in a hot air oven at 160 °C for 1 h. The inoculum was added to the medium, which was poured into sterile petri-dishes for solidifying. Wells (bores) were made in the medium using a sterile borer after solidification. To the respective bores, 0.1 ml of the test and standard solutions were added. A control bore containing only DMSO was maintained in each plate. The petri-dishes were kept at room temperature for 30 min for diffusion to take place, then incubated at 37 °C for 24 h. The zone of inhibition was observed and measured using a scale.

Result and discussion

The designing of a theoretical molecule before arriving at the New Chemical Entities is a promising approach. This can be achieved by the rational approach to new drug discovery. In the present study, docking has generated an insight into developing an ideal DNA gyrase inhibitor from 2-quinolone class. Thus, in the present study, an approach of designing 2-quinolone derivatives by docking simulations, followed by the synthesis, characterization, and biological evaluation was carried out.

Molecular docking was carried out for total eight derivatives of 2-quinolones. Among them, compound-**2** and compound-**7** showed better binding interactions within active site of DNA-gyrase B subunit. The starting materials for the synthesis of 7-amino-4-methyl-2-quinolone were m-phenylene diamine and ethylacetoacetate. The m-phenylene diamine was treated with β-ketoester (ethylacetoacetate) at high temperature to give 7-amino-4-methylquinolin-2(1H)-one (Compound-**A**), the parent compound. It was purified by recrystallization from methanol, which gave pale brown colored needle-shaped crystals. The test compound was characterized by melting point determination and thin layer chromatography. Further, eight acid derivatives (Compound **1 to 8**) of the parent compound (Compound-**A**) were synthesized. The purity of test compounds was checked by melting point determination and thin layer chromatography, and structures were established by IR, ^{1}H-NMR, and mass spectral studies. The yields of final test compounds (Table 2) were in the range of 65–80 %. The λ_{max} values of the synthesized compounds (Table 2) were found to be in the range of 265–295 nm. The substitution on the parent molecule decreased the λ_{max}. Out of the eight test compounds synthesized and tested for their antibacterial activity (Table 3), compound-**2** showed activity better than rest of the other test compounds against *E. coli* and *S. aureus*. Further, from the docking simulation studies, it was found that compound-7 showed good interactions within active site of the target enzyme DNA gyrase. However, in reality when tested against the organisms, it did not generate any useful data, which might be due to impurity profile or improper diffusion of test compounds into the agar medium. Based on the in silico approaches, the parent test compound 7-amino-4-methyl-2-quinolone (Compound-**A**) and its derivatives (Compound **1** to **8**) were synthesized. All the test compounds were characterized by TLC (Toluene: methanol 4:2), melting point determination, UV, IR, ^{1}H-NMR, and mass spectral data. The test compounds were obtained in the yield of 65–80 %. The presence of a singlet peak at 6.27 (NH_2) in ^{1}H-NMR of compound-**A** and its absence, with presence of a new singlet peak at 4.05 (NH) and the presence of a new singlet peak at 11.0 (COOH) confirmed the characterization of compound**-A** and its derivatives, respectively. The mass spectra of all the test compounds were characterized by the presence of molecular ion peak. The UV absorption characteristics of the test compounds were then determined by their λ_{max} values. Out of the eight test compounds synthesized and tested for their antibacterial activity, compound**-2** showed activity better than rest of the other test compounds against *E. coli* and *S. aureus.*

Conclusion

The rational approach to lead discovery has prompted a better insight into developing more specific 2-quinolones analogs as potential antibacterial agents. The data showing docking have definitely generated useful parameters needed for an ideal bacterial DNA gyrase inhibitor. The rational approach to lead discovery presents a direct correlation between an ideal therapeutically built up molecule and the synthesized test compound. However, the present study revealed that this method was not solely responsible for generating functional activities of test compounds in reality. This approach has also paved the way for generating more useful 2-quinolone analogs in future studies as bacterial DNA gyrase inhibitors.

Table 2 Showing the melting point, yield, R_f value, and λ_{max} of synthesized test compounds

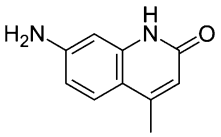
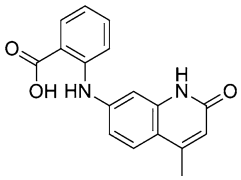
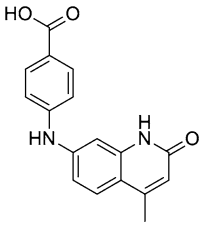
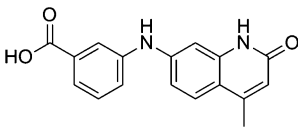
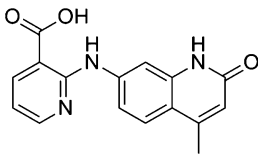
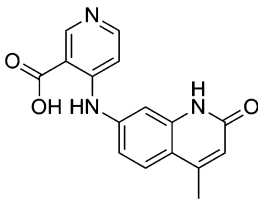
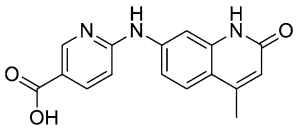
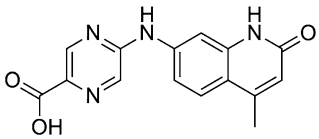
Compound code	Synthesized compounds	Molecular formula	Molecular weight	Melting point (°C)	Yield (%)	R_f value	λ_{max} (nm)
Compound-A		$C_{10}H_{10}N_2O$	174.20	265–268	75	0.60	357.0
Compound-1		$C_{17}H_{14}N_2O_3$	294.30	140–143	72	0.49	276.0
Compound-2		$C_{17}H_{14}N_2O_3$	294.30	194–197	78	0.45	265.5
Compound-3		$C_{17}H_{14}N_2O_3$	294.30	178–180	80	0.52	271.0
Compound-4		$C_{16}H_{13}N_3O_3$	295.29	155–158	68	0.44	268.0
Compound-5		$C_{16}H_{13}N_3O_3$	295.29	180–182	73	0.54	273.5
Compound-6		$C_{16}H_{13}N_3O_3$	295.29	167–170	74	0.46	287.0
Compound-7		$C_{15}H_{12}N_4O_3$	296.09	205–207	71	0.50	267.5

Table 2 continued

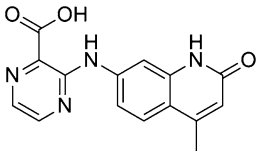
Compound code	Synthesized compounds	Molecular formula	Molecular weight	Melting point (°C)	Yield (%)	R_f value	λ_{max} (nm)
Compound-8		$C_{15}H_{12}N_4O_3$	296.09	210–213	76	0.52	294.0

Table 3 Showing the antibacterial activity of the synthesized test compounds

Sr. no.	Compound code	Zone of inhibition (mm)	
		S. aureus	*E. coli*
1	Compound-**A**	5.5	4.0
2	Compound-**1**	8.0	7.0
3	Compound-**2**	13.5	12.8
4	Compound-**3**	7.0	7.0
5	Compound-**4**	5.0	4.2
6	Compound-**5**	9.0	7.0
7	Compound-**6**	3.0	–
8	Compound-**7**	9.0	8.5
9	Compound-**8**	7.5	8.0
10	Ciprofloxacin	15.0	14.0

S. aureus Staphylococcus aureus, *E. coli* Escherichia coli

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