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Author's Affiliation:
1. Department of Pharmaceutical Chemistry, College of Pharmacy, Northern Border University, Rafha 91911 - Saudi Arabia
2. Center for Health Research, Northern Border University, Arar - Saudi Arabia
3. College of Pharmacy, Northern Border University, Rafha 91911 - Saudi Arabia
4. Faculty of Medicine, Northern Border University, Arar 91431 - Saudi Arabia
5. College of Pharmacy, Princess Nourah Bint Abdulrahman University, Riyadh 11671 - Saudi Arabia
6. College of Pharmacy, Qassim University, Qassim Region 52382 - Saudi Arabia
7. Department of Inpatient Pharmacy, Dr. Sulaiman Alhabib Hospital, Shafah Branch, Riyadh 12933 - Saudi Arabia
8. Nahdi Medical Company, Riyadh 13627 - Saudi Arabia
9. Department of Pharmacy Practice, College of Pharmacy, AlMaarefa University, Dariyah 13713, Riyadh - Saudi Arabia

Corresponding Author:
Mohd. Imran
Email: imran.pchem@gmail.com

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In-vitro and *in-silico* studies based discovery of 2-aryl-N-(4-morpholinophenyl)thiazol-4-amines as promising DNA gyrase inhibitors

Mohd. Imran[1,2*], Abdulaziz Khalaf Alshammari[3], Khattab Fahed Alfarh[3], Rakan Khalid Alanazi[4], Samr Fahed Alfrah[3], Osama Muqbil Alshammri[4], Reem Saleh Alanazi[5], Sumaih Saeed Alsaeed[6], Feras Salah Albasha [7], Hanan Furayj Alhulayfi[8], Syed Mohammed Basheeruddin Asdaq[9], Abida[1]

Abstract

Background: DNA gyrase is an important enzyme for the survival of bacteria. Many DNA gyrase inhibitors are in clinical practice. However, these inhibitors also encompass certain toxic and drug/food interactions. This warrants the development of a new template as DNA gyrase inhibitors. Therefore, this study aimed to deliver morpholine-based thiazoles (5a-5l) as safer DNA gyrase inhibitors.

Methods: The 5a-5l were prepared by reacting compound 3 with various aryl thioamides. The structures of 5a-5l were ascertained by their spectral records. The 5a-5l were subjected to their antibacterial activity potential (serial plate dilution method), DNA gyrase inhibiting activity, and toxicity analysis (MTT assay) against HepG2 & Vero cell lines. The *in-silico* studies (pharmacokinetic parameters and molecular docking) of 5a-5l were likewise performed.

Results: It was surprisingly observed that the MIC values of 5a-5l were equal to the MIC values of ciprofloxacin (12.5 µg/ml) against the tested bacteria, whereas the DNA gyrase inhibitory activity (IC_{50} in µg/ml) of 5h (3.52), 5g (3.76), 5f (3.88), 5e (4.08), 5l (4.11), 5b (4.28), 5k (4.28), 5i (4.30), and 5d (4.32) was equal/better than ciprofloxacin (4.32). The MTT assay also implied the non-cytotoxic nature of 5a-5l against HepG2 & Vero cell lines up to 200 µg/ml concentration. The docking outcomes indicated a similar binding pattern of 5a-5l and ciprofloxacin at the active site of DNA gyrase, wherein 5a-5l displayed a better binding affinity for the active site. The *in-silico* toxicity data employing the ProTox-II web server indicated no hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, or cytotoxic effect of 5a-5l. Also, the SwissADME software supported the drug-likeliness properties and high gastrointestinal absorption of 5a-5l.

Conclusion: Compounds 5h, 5g, 5f, 5e, 5l, 5b, 5k, 5i, and 5d are potent DNA gyrase inhibitors with promising safety profiles.

Introduction

DNA gyrase is an indispensable enzyme for bacterial viability as it is responsible for the topology maintenance and supercoiling of the DNA. It is an important antimicrobial target because it is essential for bacteria, takes part in DNA replication and cell division, has bacterial specificity, encompasses multiple target sites and its inhibition causes bactericidal effects [1]. Many DNA gyrase inhibitors are in clinical practice, for example, ciprofloxacin, ofloxacin, levofloxacin, delafloxacin, and moxifloxacin [2]. However, these fluoroquinolone-based DNA gyrase inhibitors can also cause unwanted peripheral neuropathy, tendon rupture, and CNS effects. These drugs can also make insoluble complexes with divalent metals (Ca^{++}), which lead to increased incidences of drug-drug and drug-food interactions and ultimately subtherapeutic effects of the drug [2]. Accordingly, the development of new chemical templates as DNA gyrase inhibitors is warranted.

In past years, the morpholino phenylamine -based Schiff bases [3], carbamates & sulfonamides [4], triazoles [5], 1,3-thiazolan-4-ones [6], and oxadiazoles [7] have been reported as promising antibacterial agents (Figure 1).

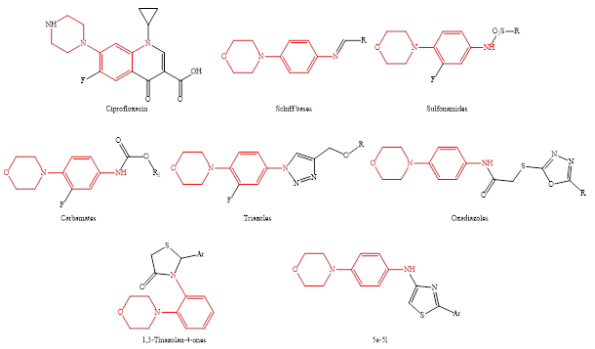

Figure 1: Structures of ciprofloxacin, morpholinophenylamine moiety-based antibacterial agents reported in the literature and the titled compounds (5a-5l) [3-7].

The antibacterial potential of the thiazole ring is also well-established [8]. Accordingly, the author planned to discover 2-aryl-N-(4-morpholinophenyl)thiazol-4-amines (5a-5l) (Figure 1) as antibacterial agents, establish their mechanism of action, and evaluate their safety and *in-silico* pharmacokinetic profile.

Methods

Sigma Aldrich (USA) supplied all of the chemicals and solvents. The R_f values were calculated using a combination of ethanol and acetic acid (7:3). This was based on the solubility of the compounds. The Gallenkamp apparatus, Shimadzu 440 spectrometer, Varian Gemini 500/125 MHz spectrometer, GCMS/QP 1000 Ex mass spectrometer (70 eV), and VARIO El Elementer apparatus were used to obtain the melting point (°C), FTIR spectra (KBr, ν_{max} in cm^{-1}), ^{1}H-NMR (DMSO-d_6, 500 MHz, δ in ppm), ^{13}C-NMR (DMSO-d_6, 125 MHz, δ in ppm), mass spectra (MS, m/z), and the elemental analysis (EA, provided as Calcd. (Found)), respectively.

Preparation of intermediates (1-3) and compounds (5a-5l)

The intermediates (1-3) used for the preparation of 5a-5l were prepared according to Figure S1 (Supplementary data). The preparation of 1 and 2 from 1-bromo-4-nitrobenzene is disclosed in the literature [9]. The preparation of 2-bromo-N-(4-morpholinophenyl)acetamide (3) from 2 is also provided in prior publications [7]. The compounds 5a-5l were prepared utilizing compound 3 and different aryl thioamides 4 (Figure S2) (Supplementary data). The structures of 5a-5l were established based on their spectral data (Supplementary data).

In vitro antimicrobial activity

It was done by utilizing the well-known serial plate dilution process against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae* employing nutrient agar medium, which was prepared according to the reported method [10]. The serial dilutions (200 to 6.25 µg/mL) of 5a-5l and ciprofloxacin were formulated in sterile DMSO. The DMSO deprived of drugs was utilized as a control. The plates were prepared with the agar medium, microorganisms were spread on them, cups were made and formulations of test/control/standard compounds were supplemented to the cups. The MIC (minimum inhibitory concentration) was ascertained (Table 1).

DNA gyrase inhibitory activity

The assay kit of *E. coli* DNA gyrase supercoiling was obtained from TopoGEN, Inc. (Cat. No. TG1003, Port Orange, FL). The instructions provided by the supplier were followed to perform the assay [11]. In short, the dilutions (0.1-50 µM) of 5a-5l and ciprofloxacin were formulated in DMSO along with the specified quantities of other ingredients (buffer, DTT, KCl, $MgCl_2$, spermidine, acetylated BSA, glycerol, ATP, album, and pBR322 substrate). The DMSO dilution (3 µL) of the desired compound was mixed with 2 U of *E. coli* DNA gyrase and kept at 37°C for 30 min. The reaction was ended by adding 3X gel-loading buffer (10 ml). The resultant mixture (20 ml) was packed on agarose-TAE gel (1%) and run (3h, 60 V). The gel was blotted with ethidium bromide (0.5 mg/l in TAE) for a half-hour and then destained with water for 20 min. The fluorescent images were obtained at 300 nm employing a UV

transilluminator imaging system. The fluorescence intensity of the supercoiled plasmid reaction product was quantitated utilizing ImagQuant software (Molecular Dynamics, Sunnyvale, CA, USA). The experiments were run in triplicate for each sample, and the IC_{50} values were calculated by nonlinear regression analysis (Table 1).

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

The MTT test was employed to assess the toxicity profile of 5a-5l concerning HepG2 cell (HCL) and Vero cell (VCL) lines [12,13]. The VCL (10^4 cells/well) and HCL (5 x 10^3 cells/well) were positioned in well plates and incubated (37°C) for 24 h. The working formulations of the test and standard (200, 150, 100, and 50 µg/ml) were formulated in Dulbecco's Modified Eagle's Medium (DMEM). The blank formulation deprived of standard/test compounds was formulated. The working/standard/blank formulation was supplemented to wells encompassing VCL and HCL. The VCL (72 h) and HCL (24 h) well plates were incubated. The MTT component (50 µl, 2 mg/ml) was supplemented to well plates and incubated (4h). The sterile DMSO (50 µl) was supplemented to each well to liquefy formazan crystals. The OD (optical density) of the wells was quantified at 540 nm employing an Elisa reader. The %cell viability (test's OD x 100 / blank's OD), and %cell inhibition (100 - %cell viability) were determined. The curve fitting program was utilized to calculate the CC_{50} (smallest concentration required for 50% cell killing) values (Table 1).

Molecular docking

It was accomplished by MOE software utilizing the chain A of DNA gyrase protein (PDB ID: 6F86) [14,15]. The selected chain was prepared for docking exercising MOE's Quickprep functionality. The ligands (5a-5l and ciprofloxacin) were made and reserved (mdb files). The docking was executed by MOE's default docking programming (10 poses for each compound). The DS (docking score, kcal/mol), and the RMSD (root mean square deviation) of the compounds are supplied in Table 2.

In silico toxicity prediction

It was performed by utilizing the ProTox-II web server [16,2]. The molefiles of 5a-5l and ciprofloxacin were uploaded to the software. The toxicity prediction button was pressed, and the toxicity data of 5a-5l and ciprofloxacin were recorded (Table 3).

In silico drug-likeliness and ADME predictions

These parameters were determined by the Swiss web server [17]. The molefiles of 5a-5l and ciprofloxacin were uploaded in the software, the run button was pressed, and the data were collected (Table 4).

Statistical analysis

It was performed utilizing SPSS software (version 20, Chicago, IL, USA), wherein *p-value* < 0.05 represents a statistically significant result.

Results

The compounds 5a-5l were prepared utilizing compound 3 and different aryl thioamides 4 (Figure S2) (Supplementary data). The antibacterial activity of 5a-5l was assessed against *S. aureus*, *B. subtilis*, *E. coli*, and *K. pneumoniae* (Table 1).

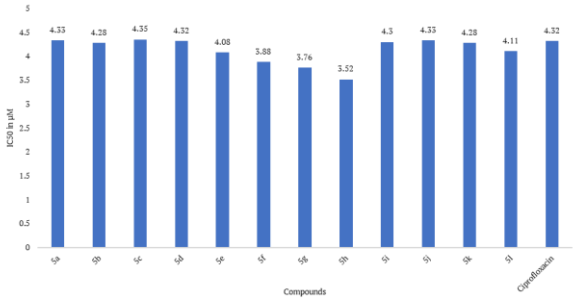

Figure 2: The IC_{50} (µg/ml) of ciprofloxacin and 5a-5l against DNA gyrase.

The docking of 5a-5l was executed to understand the reason for the excellent antibacterial activity of 5a-5l in comparison to ciprofloxacin (Table 2). The RMSD value of all ligands was < 1.5, whereas the DS ranged from -6.78 to -7.42 kcal/mol. An RMSD value < 2 and a higher negative DS indicates a strong binding of the molecule with the selected protein, and a strong inhibitory effect of the molecule, respectively [18]. The toxicity profile of 5a-5l was assessed by the ProTox-II webserver (Table 3). This web server provides the possible toxicity profile of a molecule based on the reported toxicity profile of similar compounds [15,16]. The LD_{50} of the prepared compounds ranged from 681-900 mg/kg (class 4, 300 < LD_{50} ⩽ 2000), except 5j which had an LD_{50} value of 222 mg/kg (class 3, 50 < LD50 ⩽ 300) [2]. The SwissADME software was utilized to predict drug-likeliness and pharmacokinetic parameters of 5a-5l (Table 4) [17].

Discussion

It was surprisingly observed that the MIC values of 5a-5l were equal to the MIC values of ciprofloxacin (12.5 µg/ml) against the tested bacteria (Table 1). This observation reflected an equipotency of 5a-5l with ciprofloxacin. The IC_{50} values of 5a-5l against DNA gyrase increased as 5h < 5g < 5f < 5e < 5l < 5b < 5k < 5i < 5d < ciprofloxacin < 5c < 5a < 5j. This demonstrates 5h, 5g, 5f, 5e, 5l, 5b, 5k, 5i, and 5d as more potent DNA gyrase inhibitors than ciprofloxacin.

Compound	Zone of inhibition in mm (MIC in µg/ml)				DNA GyrB Inhibitory activity (IC_{50} in µg/ml)	MTT assay data (CC_{50} in µg/ml)	
	S. aureus (ATCC 25923)	*B. subtilis* (ATCC 6633)	*E. coli* (ATCC 25922)	*K. pneumonia* (ATCC 700603)		HCL	VCL
5a	19.5±0.22 (12.5)	18.22±0.33 (12.5)	20.32±0.38 (12.5)	17.40±0.42 (12.5)	4.33	> 200	> 200
5b	22.3±0.34 (12.5)	20.12±0.16 (12.5)	21.22±0.18 (12.5)	20.66±0.22 (12.5)	4.28	> 200	> 200
5c	22.9±0.10 (12.5)	20.5±0.50 (12.5)	21.14±0.28 (12.5)	20.55±0.15 (12.5)	4.35	> 200	> 200
5d	22.8±0.18 (12.5)	20.44±0.10 (12.5)	21.10±0.11 (12.5)	21.85±0.25 (12.5)	4.32	> 200	> 200
5e	23.6±0.16 (12.5)	22.88±0.43 (12.5)	23.33±0.44 (12.5)	21.90±0.19 (12.5)	4.08	> 200	> 200
5f	23.5±0.50 (12.5)	22.44±0.14 (12.5)	23.16±0.18 (12.5)	22.88±0.16 (12.5)	3.88	> 200	> 200
5g	25.1±0.25 (12.5)	23.20±0.11 (12.5)	27.10±0.13 (12.5)	25.75±0.41 (12.5)	3.76	> 200	> 200
5h	28.3±0.17 (12.5)	24.4±0.34 (12.5)	28.15±0.46 (12.5)	25.58±0.28 (12.5)	3.52	> 200	> 200
5i	23.3±0.31 (12.5)	22.28±0.15 (12.5)	21.11±0.29 (12.5)	19.46±0.15 (12.5)	4.30	> 200	> 200
5j	22.8±0.22 (12.5)	21.23±0.36 (12.5)	20.90±0.19 (12.5)	20.15±0.16 (12.5)	4.33	> 200	> 200
5k	21.2±0.44 (12.5)	21.22±0.10 (12.5)	21.05±0.04 (12.5)	20.44±0.11 (12.5)	4.28	> 200	> 200
5l	24.4±0.38 (12.5)	22.22±0.45 (12.5)	27.14±0.17 (12.5)	23.15±0.24 (12.5)	4.11	> 200	> 200
Ciprofloxacin	22.5±0.15 (12.5)	21.30±0.22 (12.5)	24.40±0.28 (12.5)	22.5±0.12 (12.5)	4.32	> 200	> 200

All data had *p-value < 0.05*; N = 3; Mean±SD.

Table 1: Biological activity data of 5a-5l.

Ligand	DS (kcal/mol)	RMSD	Common interacting residues between 5a-5l and ciprofloxacin	Reported DS and interacting residues of ciprofloxacin	Additional interacting residues
5a	-6.88	1.44	Asp 73, Glu 50, Arg 76, Arg 136, Gly 77, Thr 165, Asn 46, Pro 79, Val 71, Ile 94, Ala 47, Val 43, Val 167, Ile 78	-6.9 Asp 73, Glu 50, Arg 76, Gly 77, Thr 165, Asn 46, Pro 79, Ile 94, Ala 47, Ile 78 [1]	Val 120
5b	-7.41	1.27			-
5c	-7.05	1.44			Val 120
5d	-6.89	1.06			Val 120
5e	-6.80	1.45			-
5f	-6.87	0.84			Val 120
5g	-6.89	1.27			Met 95, Leu 132, Val 120
5h	-6.99	1.26			-
5i	-6.80	1. 34			-
5j	-7.35	1.45			-
5k	-7.03	1.39			-
5l	-7.24	1.45			-
Ciprofloxacin	-6.78	1.41			-

Table 2: The docking results of 5a-5l with chain A of 6F86.

Compound	LD_{50} (mg/kg)	Toxicity class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
5a	700	4	No	No	No	No	No
5b	700	4	No	No	No	No	No
5c	681	4	No	No	No	No	No
5d	700	4	No	No	No	No	No
5e	700	4	No	No	No	No	No
5f	700	4	No	No	No	No	No
5g	900	4	No	No	No	No	No
5h	900	4	No	No	No	No	No
5i	700	4	No	No	No	No	No
5j	222	3	No	No	No	No	No
5k	700	4	No	No	No	No	No
5l	750	4	No	No	No	No	No
Ciprofloxacin	2000	4	No	No	No	Yes	No

LD = Lethal dose.

Table 3: Toxicity prediction of 5a-5l by Protox-II.

Compounds	Drug likeness (Lipinski's rule violations)	Pharmacokinetics								
		GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (cm/s)
5a	Yes (Zero)	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-5.35
5b	Yes (Zero)	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-6.09
5c	Yes (Zero)	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-6.11
5d	Yes (Zero)	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-6.11
5e	Yes (Zero)	High	No	Yes	Yes	Yes	Yes	Yes	Yes	-5.92
5f	Yes (Zero)	High	No	Yes	Yes	Yes	Yes	Yes	Yes	-5.92
5g	Yes (Zero)	High	No	Yes	Yes	Yes	Yes	Yes	Yes	-5.70
5h	Yes (Zero)	High	No	Yes	Yes	Yes	Yes	Yes	Yes	-5.53
5i	Yes (Zero)	High	No	Yes	Yes	No	Yes	Yes	Yes	-6.85
5j	Yes (Zero)	High	No	Yes	Yes	No	Yes	Yes	Yes	-6.55
5k	Yes (Zero)	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	-6.12
5l	Yes (Zero)	High	No	Yes	Yes	Yes	Yes	Yes	Yes	-5.61
Ciprofloxacin	Yes (Zero)	High	No	Yes	No	No	No	No	No	-9.09

BBB = Blood-brain barrier; GI = Gastrointestinal.

Table 4: Pharmacokinetic (ADME and drug-likeliness) predictions of 5a-5l by Swiss-ADME.

This reflection also implies that the presence of 2-alkypyridin-4-yl provides potent DNA gyrase inhibitors, wherein the DNA gyrase inhibitory activity of compounds improves with the size of the alkyl group increases (5f < 5g < 5h). The unsubstituted pyridin-2-yl (5b) and pyridin-4-yl (5d) rings also provide potent

DNA gyrase inhibitors. The presence of thiophene ring (5l) provides better inhibitors of DNA gyrase than pyrrole (5k) and pyrazine (5i) based compounds. The replacement of the pyridin-2-yl ring (5b) with pyridin-3-yl (5c) or pyrimidin-2-yl ring (5j) causes a decrease in activity. The MTT assay implied the non-cytotoxic nature of 5a-5l against HCL and VCL up to a concentration of 200 µg/ml.

The docking results indicate that 5a-5l and ciprofloxacin bind to the same active pocket of DNA gyrase. However, 5a-5l had a better binding affinity with the amino acids of the active site of DNA gyrase. This is evident from the DS of 5a-5l. This may be the reason that 5a-5l provided equal or better IC_{50} values than ciprofloxacin against DNA gyrase. The 2D interaction of 6F86 with ciprofloxacin and three compounds (5f, 5g, and 5h) with least IC_{50} values against DNA gyrase has been depicted in Figure S3, Figure S4, Figure S5, and Figure S6, respectively (Supplementary file). Some of the compounds displayed additional interaction with the residual amino acids, for example, 5a (Val 120), 5c (Val 120), 5d (Val 120), 5f (Val 120), and 5g (Met 95, Leu 132, Val 120). However, the presence of these additional interactions does not have any influence on the binding affinity of 5a, 5c, 5d, 5f, and 5g in the active site of DNA gyrase.

The toxicity data indicated no hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxic effect of 5a-5l. These results supported our MTT assay results, wherein 5a-5l displayed a non-toxic effect on HCl and VCl up to a concentration of 200 µg/ml. However, the author recommends *in vivo* toxicity studies to ascertain the non-toxic nature of 5a-5l.

The drug-likeliness determination is based on the physicochemical parameters of a molecule that would make it orally active [19]. The data of Table 4 indicate that 5a-5l possess all drug-likeliness properties, and also have a high gastrointestinal absorption. This is also evident from the bioavailability radar of 5f, 5g, and 5h (Figure S7, Supplementary data) that were obtained from SwissADME software. This observation suggests an orally active nature of 5a-5l. Most of the prepared compounds have been predicted as inhibitors of some metabolizing enzymes, unlike ciprofloxacin. Accordingly, these compounds might interact with the metabolism of other drugs, especially those metabolized by CYP3A4, CYP1A2, CYP2C9, and CYP2D6. However, for the clarity of such interaction, further studies need to be carried out.

Ciprofloxacin (Figure 1) is a well-known DNA gyrase inhibitor. In its chemical structure, the carbonyl group and the carboxylic acid group are at ortho-position. A similar position of these two groups is also present in the chemical structures of enoxacin, fleroxacin, lomefloxacin, norfloxacin, ofloxacin, pefloxacin, rufloxacin, levofloxacin, sparfloxacin, tosufloxacin, gatifloxacin, and moxifloxacin. These two groups bind with the bivalent metal, for example, magnesium, that is needed for the activity of DNA gyrase [20]. These groups also bind with food/drugs containing the bivalent metal, for example, milk and calcium gluconate. This interaction causes a decrease in the gastrointestinal absorption of ciprofloxacin and its structurally related DNA gyrase inhibitors [20-22]. The chemical structures of 5a-5l (Figure S2, (Supplementary data) neither possess the carbonyl group nor the carboxylic acid group. Therefore, the author believes that 5a-5l must not pose an interaction with food/drugs containing bivalent metals like calcium, unlike ciprofloxacin.

In conclusion, this study provides morpholine-based thiazole derivatives (5a-5l) as DNA gyrase inhibitors possessing promising safety and efficacy profiles. The compounds demonstrated equal MIC values along with ciprofloxacin (12.5 µg/ml) against *S. aureus*, *B. subtilis*, *E. coli*, and *K. pneumoniae*. The 5h, 5g, 5f, 5e, 5l, 5b, 5k, 5i, and 5d displayed potent DNA gyrase inhibitory activity than ciprofloxacin. This observation was supported by the molecular docking data of 5a-5l. The MTT assay and ProTox-II webserver data indicated the non-toxic nature of 5a-5l. The SwissADME software further indicated the drug-likeliness properties and high gastrointestinal absorption of 5a-5l. The 5h, 5g, 5f, 5e, 5l, 5b, 5k, 5i, and 5d have been identified as potent DNA gyrase inhibitors with promising safety and efficacy profiles. However, detailed toxicity studies are also recommended to establish the non-toxic nature of 5a-5l.

Supplementary Data

Supplementary data is available on demand.

Author Contributions

Mohd Imran, Abida, and Basheeruddin Asdaq conceptualized, supervised, and edited the article. All other members performed in the literature search, and experiments (silico studies, preparation, and in *vitro studies*), and participated in first manuscript draft writing.

Conflict of Interest

The author declare that there is no conflict of interest regarding the publication of this paper.

References

1. Fisher LM, Pan XS. Methods to assay inhibitors of DNA gyrase and topoisomerase IV activities. Methods in Molecular Medicine, (2008); 142: 11-23.

2. Eyler RF, Shvets K. Clinical pharmacology of antibiotics. Clinical Journal of the American Society of Nephrology, (2019); 14(7): 1080-1090.
3. Panneerselvam P, Nair RR, Vijayalakshmi G, Subramanian EH, Sridhar SK. Synthesis of Schiff bases of 4-(4-aminophenyl)-morpholine as potential antimicrobial agents. European Journal of Medicinal Chemistry, (2005); 40(2): 225-229.
4. Janakiramudu DB, Rao DS, Srikanth C, Madhusudhana S, Murthy PS, et al. Sulfonamides and carbamates of 3-fluoro-4-morpholinoaniline (linezolid intermediate): synthesis, antimicrobial activity and molecular docking study. Research on Chemical Intermediates, (2018); 44(1): 469-489.
5. Narsimha S, Nukala SK, Jyostna TS, Ravinder M, Rao MS, et al. One-pot synthesis and biological evaluation of novel 4-[3-fluoro-4-(morpholin-4-yl)]phenyl-1 H-1,2,3-triazole derivatives as potent antibacterial and anticancer agents. Journal of Heterocyclic Chemistry, (2020); 57: 1655– 1665.
6. Nagaraj A, Ravi G, Kumar SS, Raghaveer S, Rana N. Synthesis and antimicrobial evaluation of novel 1,3-thiazolan-4-one derivatives. Research & Reviews: Journal of Chemistry, (2014); 3(2): 17-21.
7. Gul S, Aziz-ur-Rehman, Abbasi MA, Khan KM, Nafeesa K, et al. Synthesis, antimicrobial evaluation and hemolytic activity of 2-[[5-alkyl/aralkyl substituted-1,3,4-oxadiazol-2-yl]thio]-N-[4-(4-morpholinyl)phenyl]acetamide derivatives. Journal of Saudi Chemical Society, (2017); 21(1): 425-433.
8. Kashyap A, Adhikari N, Das A, Shakya A, Ghosh SK, et al. Review on synthetic chemistry and antibacterial importance of thiazole derivatives. Current Drug Discovery Technologies, 2018; 15(3): 214-228.
9. Rai SK, Patil RI, Roy SS, Sharma K, Dwivedi R, et al. Novel apoptosis signal-regulating kinase 1 inhibitors. PCT International Patent Application Publication Number WO2020261294A1, 2020. Available at https://patents.google.com/patent/WO2020261294A1/en?oq=WO2020261294A1.
10. Ansari MI, Khan SA. Synthesis and antimicrobial activity of some novel quinoline-pyrazoline-based coumarinyl thiazole derivatives Medicinal Chemistry Research, (2017); 26: 1481-1496.
11. Mohi El-Deen EM, Abd El-Meguid EA, Karam EA, Nossier ES, Ahmed MF. Synthesis and biological evaluation of new pyridothienopyrimidine derivatives as antibacterial agents and Escherichia coli Topoisomerase II inhibitors. Antibiotics (Basel), (2020); 9(10): 695.
12. Kamiloglu S, Sari G, Ozdal T, Capanoglu E. Guidelines for cell viability assays. Food Frontiers, (2020); 1: 332–349.
13. Ammerman NC, Beier-Sexton M, Azad AF. Current Protocols in Microbiology, (2008); Appendix 4: Appendix 4E.
14. Imran M, Mohd AA, Nayeem N, Alaqel SI. In silico studies-assisted design, synthesis, and discovery of biologically active isatin derivatives. Tropical Journal of Pharmaceutical Research, (2023); 22(6): 1263-1269.
15. Eswaramoorthy R, Hailekiros H, Kedir F, Endale M. In silico Molecular Docking, DFT Analysis and ADMET Studies of Carbazole Alkaloid and Coumarins from Roots of Clausena anisata: A Potent Inhibitor for Quorum Sensing. Advances and Applications in Bioinformatics and Chemistry, (2021); 14: 13-24.
16. Mohd AA, Imran M, Alnaser NY, Altimyat SS, Al-Otaibi NM, et al. Discovery of new isoniazid derivatives as anti-tubercular agents: In silico studies, synthesis, and in vitro activity evaluation. Oriental Journal of Chemistry, (2023); 39(6): 1510-1520.
17. Imran M, Mohd AA, Nayeem N, Al-Otaibi NM, Homoud M, et al. Synthesis and cyclooxygenase-2 inhibitory activity evaluation of some pyridazine derivatives. Oriental Journal of Chemistry, (2023); 39(5): 1113-1119.
18. Imran M. 2-Butyl-6-phenyl-4,5-dihydropyridazin-3(2H)-one: Synthesis, In Silico Studies and In Vitro Cyclooxygenase-2 Inhibitory Activity. Molbank, (2020); 2020(3): M1155.
19. Khan A, Diwan A, Thabet HK, Imran M, Bakht MA. Discovery of novel pyridazine-based cyclooxygenase-2 inhibitors with a promising gastric safety profile. Molecules, (2020); 25(9): 2002.
20. Walden DM, Khotimchenko M, Hou H, Chakravarty K, Varshney J. Effects of magnesium, calcium, and aluminum chelation on fluoroquinolone absorption rate and bioavailability: A computational study. Pharmaceutics, (2021); 13(5): 594.
21. Kozieł R, Zabłocki K, Duszyński J. Calcium signals are affected by ciprofloxacin as a consequence of reduction of mitochondrial DNA content in Jurkat cells. Antimicrobial Agents and Chemotherapy, (2006); 50(5): 1664-1671.
22. Pham TDM, Ziora ZM, Blaskovich MAT. Quinolone antibiotics. MedChemComm, (2019); 10(10): 1719-1739.