Original Article

Design, Synthesis, In Silico Study And Preliminary Pharmacological Assessment Of New Ciprofloxacin Analogues Having Thiazole Nucleus

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Abstract

Series of ciprofloxacin derivatives (IVb_1 -IVb_4) were designed and synthesized by incorporation of thiazole ring (which has an antibacterial activity) and some of its derivatives into the secondary amine of piperazine ring at the C-7 position of ciprofloxacin nucleus. The chemical structures of the synthesized compounds were confirmed by spectral analysis, such as FT-IR and ^{1}HNMR, and some of their physicochemical properties were recorded. An in-vitro study was conducted to evaluate their antibacterial activity by using the agar-well diffusion method (AWD). It was found that the topmost inhibition zone was for the compounds IVb_1 and IVb_3 against S. aureus and the lowest inhibition zone for compound IIIb against E. coli. In Silico study was performed by Molecular Operating Environment (MOE) program and showed that all the target compounds have an affinity toward topoisomerase enzyme, and these results were consistent with the in vitro study, as the compound IVb_3 has the highest docking score value.

Keywords: In silico, Bacterial resistance, Fluoroquinolone, Thiazole ring.

INTRODUCTION

The word" antibiosis" means any biological relationship in which one living microorganism kills another to ensure its existence since most antibiotics currently used in humans are natural secretions of bacteria or fungi [1]. Bacterial resistance is considered a global security threat that affects global health. Resistant bacteria are responsible for infections that are more difficult to treat, requiring the use of drugs that are more toxic and more expensive. In some cases, bacteria have become resistant to all known antibiotics. The massive scale of antibiotic misuse accelerates the evolution of antibiotic resistance and antibiotic-resistant genes in the environment. This threat has higher morbidity and mortality rates [2,3]. Quinolones represent a large group of important antimicrobial agents that made a major impact in the field of antimicrobial therapy. Some key changes were made to the quinolone nucleus with the aim to enhance potency or spectrum of activity especially the addition of a fluorine atom on the C-6 position to enhance the bacterial cell penetration hence, the name fluoroquinolones, for example, ciprofloxacin is a second-generation fluoroquinolone which has an improved spectrum of activity. They have a wider clinical use and a broad antibacterial spectrum [4,5]. Fluoroquinolones affect both gram-positive and gram-negative bacteria by binding to DNA gyrase and topoisomerase IV by the formation of a stable complex that traps the enzyme in place [6,7]. One of the main mechanisms of resistance to fluoroquinolones is the efflux pumps that extrude the fluoroquinolones from the bacterial cell [8]. The most adaptable position for chemical changes and an area that greatly influences the fluoroquinolone potency is the C-7 position, therefore, the research is focusing on the basic groups that can be attached to it, earlier researchers have proven that large substituents placed on this position significantly modify the strength of action [9],

since, it protects the antibiotic from efflux pumps thereby decreasing bacterial resistance by different species [10,11]. Depending on this principle, new types of ciprofloxacin derivatives were synthesized by linking different thiazole derivatives on a secondary amine of piperazine ring to increase bulkiness at this position and decrease the effect of efflux pumps.

MATERIALS AND METHODS

EXPERIMENTAL

All chemicals and anhydrous solvents were of analytical grade and supplied by (Sigma-Aldrich, Central Drug House, and Merck). Melting points were recorded by using the Thomas Hover apparatus. Retention factor values were measured by using TLC to ensure the purity and progression of the reaction using (Acetone: Methanol) (1:1) as a mobile phase [12]. FT-IR was recorded at the faculty of pharmacy, the university of Kufa by using Shimadzu- Japan spectrophotometer, and determination of the spectra was performed by using KBr discs. H^1NMR recorded on Bruker 500 MHz, University of Tehran.

CHEMICAL SYNTHESIS

In the first step, ciprofloxacin was converted into methyl ester, then the secondary amine of the piperazine ring reacted with chloroacetyl chloride and then with thiourea to form a thiazole ring in which the primary amine will react with four different aromatic aldehydes to give Schiff base derivatives of ciprofloxacin, as illustrated in Scheme 1.

Synthesis of Methyl 1- cyclopropyl-6- fluoro-4- oxo- 7-(piperazin-1-yl)-1,4- dihydroquinoline-3- carboxylate Hydrochloride (Ib) [13]:

A Suspension of ciprofloxacin (5g, 15.1 mmol), in absolute methanol (50 ml), was cooled down to -15 C^0, then thionyl chloride (1.1 ml, 15.1 mmol) was added in dropwise, (the temperature was kept at -15 C^0). Then, the reaction mixture was kept at 40 C^0 for three hours, followed by refluxing for 45 hours (until HCl gas was ceased), and left at room temperature overnight. The solvent was evaporated to dryness under a vacuum, and the residue was re-dissolved in methanol and evaporated. The process was repeated several times until the complete removal of thionyl chloride. The residue was crystallized from methanol chloroform and collected as white-yellowish powder. Yield 91%. m.p. 280 ^{0}C. R_f value 0.35**.** FT-IR (cm^{-1}): (3236) (N-H) stretching vibration of secondary amine, 3024 (C-H) stretching vibration of aromatic, 2951-2927 (C-H) stretching vibration of alkane,1732 (C=O) stretching vibration of ester, 1627 (C=O) stretching vibration of quinolone, 1039 (C-F) stretching vibration.

Synthesis of Methyl 7-(4-(2-Chloroacetyl) piperazin-1-yl)-1-Cyclopropyl-6-Fluoro-4-Oxo-1,4-Dihydroquinoline-3-Carboxylate (IIb) [14]:

This compound was synthesized by a reaction of compound (Ib) with chloroacetyl chloride. Compound (Ib) (5g, 14.4mmol), was dissolved in a mixture of DMF: Chloroform (1:4) mixture (50 ml), then, TEA (1.9ml,14.4 mmol) was added, chloroacetyl chloride (1.14 ml, 14.4 mmol in 10 ml chloroform) was added in a dropwise with continuous stirring for a period of one hour, followed by refluxing the reaction mixture for five hours. the precipitate was filtered, dried, and re-crystallized from ethanol and collected as white-yellowish powder. Yield 81%. m.p. 211-213 ^{0}C. R_f value= 0.8. FT-IR (cm^{-1}): 3057 (C-H) stretching vibration of aromatic, 2970-2937 (C-H) stretching vibration of alkane, 1732 (C=O) stretching vibration of ester, 1656 (C=O) stretching vibration of amide, 1624 (C=O) stretching vibration of quinolone, 658 (C-Cl) stretching vibration.

Synthesis of Methyl 7-(4-(2-aminothiazol-4-yl) piperazine-1-yl)-1-Cyclopropyl-6-Fluoro-4-Oxo-1,4-Dihydroquinoline-3-Carboxylate (IIIb) [15]:

The compound IIb (5g, 11.85 mmol) was dissolved in 50 ml of absolute ethanol 99%, and thiourea (0.9 g, 11.85 mmol) was added with continuous shaking until dissolved. The reaction mixture was refluxed for four hours, the solvent was

evaporated and the product was recrystallized by diethyl ether and collected as white-yellowish powder. Yield 86%. m.p 126-128 ^{0}C. R_f value 0.6. FT-IR (cm^{-1}): 3348, 3269 (N-H) stretching vibration of primary amine, 3170 (C-H) stretching vibration of aromatic, 2980 (C-H) stretching vibration of alkane, 1732 (C=O) stretching vibration of ester, 1608 (C=O) stretching vibration of quinolone.

SYNTHESIS OF SCHIFF BASE DERIVATIVES (IVB_1-IVB_4):

These compounds were synthesized by an aldehyde-amine condensation reaction to form imine [16]:

Into a mixture of compound III (5 g, 11.3 mmol) and (11.3 mmol) of a suitable aldehyde (their quantities mentioned in table number 1) dissolved in 50 ml of absolute ethanol, a few drops of glacial acetic acid were added, then the reaction mixture was refluxed for 24 hours, then the solvent was evaporated by using a rotary evaporator and the residue was collected and washed by using diethyl ether and ethanol. Four types of aromatic aldehydes were used which are 4-hydroxy benzaldehyde, 4-bromo benzaldehyde, 4- methyl benzaldehyde, 4- nitro benzaldehyde.

Methyl 1-cyclopropyl-6-fluoro-7-(4-(2-((4-hydroxybenzylidene) amino) thiazol-4-yl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (IVb_1):

Yellow powder. Yield 61%. m.p. 246-248. R_f value 0.7. FT-IR (cm^{-1}) (KBr): 3404 (O-H) Broad stretching vibration of -OH, 3184 (C-H) stretching vibration of aromatic, 2951 (C-H) stretching vibration of alkane, 1722 (C=O) stretching vibration of ester, 1625 (C=O) stretching vibration of quinolone,1496-1462 (C=C) stretching vibration of aromatic overlap with (C=N) of imine. M.p= 248 ^{0}C d .^{1}HNMR ($C_{28}H_{26}FN_5O_4S$) (500MHz, DMSO):1.33 (m, 4H, $CH_2$of cyclopropyl), 2.8-3.7 (m, 8H, $CH_2$of piperazine), 3.9 (s, 3H, CH_3 of ester), 4.2 (m, 1H, CH of cyclopropyl), 7 (s, 1H, H of aromatic), 7.3-7.7 (t, 3H, H of aromatic overlap with H of thiazole), 7.8 (d, 2H, H of aromatic), 8.4(s, 1H, H of aromatic), 8.7 (s,1H, H of imine), 8.9 (s, 1H, H of alkene), 9.5 (s, 1H, H of OH).

Methyl 7-(4-(2-((4-bromobenzylidene) amino) thiazol-4-yl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (IVb_2):

Yellow powder. Yield 55%. m.p. 212-214. R_f value 0.5. FT-IR (cm^{-1}) (KBr)**:** 3182 (C-H) stretching vibration of aromatic, 2949 (C-H) stretching vibration of alkane,1722 (C=O) stretching vibration of ester, 1624 (C=O) stretching vibration of quinolone, 1498-1463 (C=C)stretching vibration of aromatic overlap with (C=N) of imine. M.p= 212-214 ^{0}C. ^{1}HNMR ($C_{28}H_{25}BrFN_5O_3S$) (500MHz, DMSO):1.4 (m, 4H, $CH_2$of cyclopropyl), 2.8-3.4 (m, 8H, $CH_2$of piperazine), 3.9 (s, 3H, $CH_3$of ester), 4.3 (m, 1H, CH of cyclopropyl), 7.1 (s, 1H, H of aromatic), 7.6 (s, 1H, CH of thiazole), 7.7(d, 2H, H of aromatic), 7.9 (d, 2H, H of aromatic), 8.7 (s, 2H, H of aromatic overlap with CH of imine), 9.5 (s, 1H, CH of alkene).

Methyl 1-cyclopropyl-6-fluoro-7-(4-(2-((4-nitrobenzylidene) amino)thiazol-4-yl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (IVb_3):

Yellow powder. Yield 58%. m.p. 242-244. R_f value 0.6. FT-IR (cm^{-1}) (KBr): 3178 (C-H) stretching vibration of aromatic, 2953 (C-H) stretching vibration of alkane, 1728 (C=O) stretching vibration of ester, 1624 (C=O) stretching vibration of quinolone, 1502-1463 (C=C)stretching vibration of aromatic overlap with (C=N) of imine. M.p= 244 ^{0}C d. ^{1}HNMR ($C_{28}H25FN_6O_5S$) (500MHz, DMSO): 1.4 (m, 4H, CH_2 of cyclopropyl), 2.9-3.5 (m, 8H, CH_2 of piperazine), 3.9 (s, 3H, CH_3 of ester), 4.2 (m, 1H, CH of cyclopropyl), 7 (s, 1H, H of aromatic), 7.5 (s, 1H, CH of thiazole), 7.8(s, 1H, H of aromatic), 8.3(d, 2H, H of aromatic), 8.6(d, 2H, H of aromatic), 8.8 (s, 1H, CH of imine), 9.4(s, 1H, CH of alkene).

Methyl 1-cyclopropyl-6-fluoro-7-(4-(2-((4-methylbenzylidene) amino) thiazol-4-yl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (IVb_4):

White-yellowish powder. Yield 67%. m.p. 201-203. R_f value 0.5. FT-IR (cm^{-1}) (KBr): 3030 (C-H) stretching vibration of aromatic, 2943 (C-H) stretching vibration of alkane, 1726 (C=O) stretching vibration of ester, 1622 (C=O) stretching vibration of quinolone, 1502-1463(C=C) stretching vibration overlap with (C=N) of imine. M.p= 201-203 ^{0}C. ^{1}HNMR (500MHz, DMSO): 1.4 (m, 4H, CH_2 of cyclopropyl),2.3(s, 3H,aromatic CH_3), 2.8-3.5 (m, 8H, CH_2 of piperazine), 3.9 (s, 3H, CH_3 of ester), 4.2 (m, 1H, CH of cyclopropyl), 7 (s, 1H, H of aromatic),7.3- 7.6 (t, 1H, CH of thiazole overlap with H of aromatic), 7.8(d, 2H, H of aromatic), 8.5(s, 2H, H of aromatic), 9 (s, 1H, H of imine), 9.5(s, 1H, CH alkene).

ANTIBACTERIAL STUDY

In vitro antibacterial activities of the synthesized target compounds were evaluated against both gram-positive and gram-negative bacteria S. aureus and E. coli respectively, by using agar- well diffusion method**.** this method involves the use of Brain Heart Infusion Agar (BHIA). The antimicrobial agents were dissolved in dimethyl sulfoxide, afterward, 1ml of spore suspension of each bacterium was spread evenly on the sterile solid media. Wells of 6mm were made in the plates filled with 0.1 ml of each concentration. The plates were incubated at 37 for 24 hours. The zones of inhibition were observed and measured to determine the antibacterial activity [17,18]. The antibacterial study was performed at Al- Ameen center for advanced research and biotechnologies. The calculated doses are shown in table (1).

Table (1): Calculated doses of ciprofloxacin derivatives

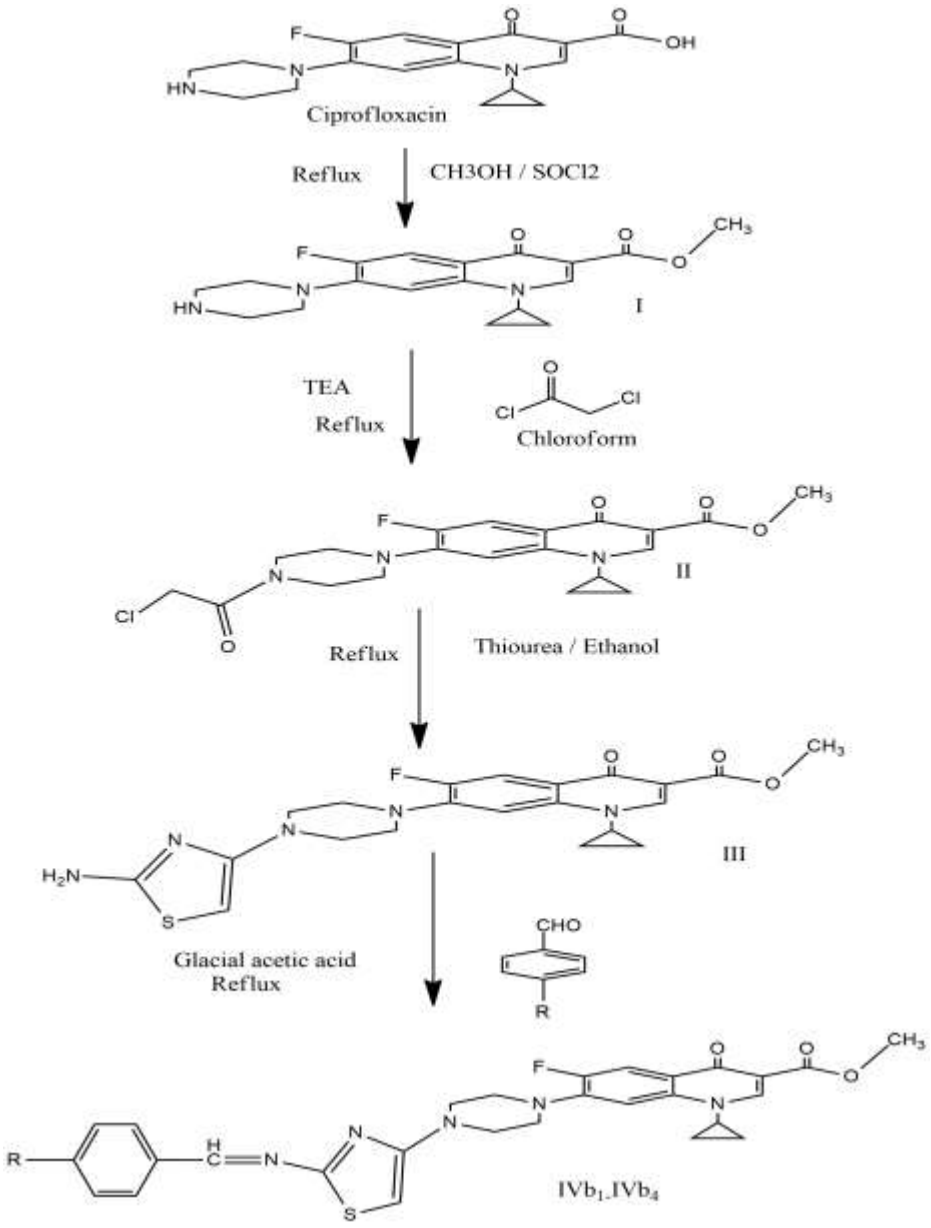
Compound	**Molecular weight (g/mol)**	**Dose (µg/ml)**
IIIb	443.49	4.43
IVb_1	547.6	5.47
IVb_2	610.5	6.11
IVb_3	576.6	5.76
IVb_4	545.6	5.45
Ciprofloxacin	331.3	3.31

DOCKING STUDY

The molecular docking study was conducted by using the Molecular Operating Environme (MOE) software program 2015.10. The process of docking involves protein preparation and ligand preparation. Ligand preparation involves the protonation of a three-dimensional structure in MOE software program, partial charge addition, and energy minimization. The protein 2xct was retrieved from the PDB website (www.rcsb.org) and prepared by removing the solvent molecules (water) and other sites on topoisomerase enzyme II (DNA gyrase) to facilitate the interaction of only ligands and the selected receptor, followed by the addition of protons that were deleted to facilitate the upload and download of the protein from PDB, then the addition of broken bond and fixation of the potential of the protein molecule. Finally, the active site of the topoisomerase was selected in MOE software program, and the amino acids of this site were determined [19].

RESULTS AND DISCUSSION

The target compounds were synthesized according to scheme 1:



IVb_1 if R=OH

IVb_2 if R=Br

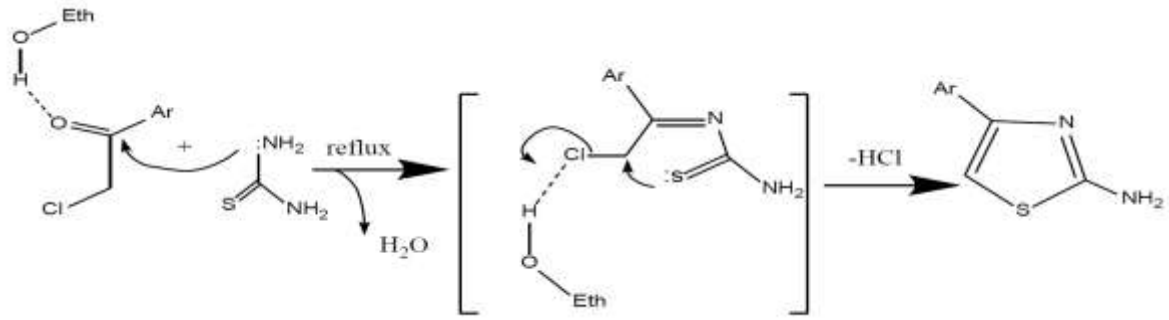
IVb_3 if R=NO2

IVb_4 if R= CH3

Scheme (1): Synthesis of the final target compounds and their intermediates

CHEMISTRY

The broadband above 3000 cm^{-1} disappeared and a sharp band of C=O was shifted from (1708 cm^{-1}) to (1732 cm^{-1}), this is considered evidence for the conversion of the carboxyl group in ciprofloxacin to methyl ester derivative. N-acetylation of the secondary amine of the piperazine ring of ciprofloxacin was achieved by using chloroacetyl chloride to get 2- chloro- acetamide derivative. In this reaction, the chloroacetyl chloride was converted to amide involving the tetrahedral intermediate via nucleophilic acyl substitution reaction [20]. Selectivity via excessive nucleophilic reactivity toward acid chlorides of nucleophilic substitution was induced at the α- carbon atom of chloroacetyl chloride. Differences in the electrophilicity between the two carbon atoms in chloroacetyl chloride led to this selectivity. Differences in electronic factors and steric factors act in this selection [21]. The thiazole ring was synthesized by a reaction of the acyl derivative of ciprofloxacin ester with thiourea. The hydrogen bond with the carbonyl oxygen of the acetyl chloride enhances the electrophilicity of this group leading to the formation of a thiazole ring via attack of the amino nitrogen of thiourea and the sulfur of chloromethyl carbon and subsequently via removing of an HCl molecule [22], as described in scheme 2.



Ar=Ciprofloxacin methyl ester

Scheme (2): Mechanism of thiazole ring synthesis

Finally, the primary aromatic amine of the thiazole ring was reacted with different aromatic aldehydes by condensation reactions to form the target imines which was confirmed by the disappearance of N-H bands above 3000 cm^{-1} and the appearance of imine bands around 1624 cm^{-1} in FT-IR. In the ^{1}H NMR spectra, the C-H peak of the imine appeared (above 8.6 deltas), and the aromatic protons area (6.5-8 delta) was broadened. The observations from FT-IR and ^{1}HNMR spectra provided us with a strong confirmation for the synthesized compounds.

ANTIBACTERIAL STUDY

In vitro antibacterial activity of the target compounds were evaluated by using agar- well diffusion method. The antibacterial activity was shown to be with the topmost inhibition zone for the compounds IVb_1, and IVb_3, against staphylococcus aureus and the lowest inhibition zone with compound IIIb against E. coli as shown in the table (2):

Table (2): The antibacterial Activity of synthesized compounds

Compound	**Inhibition zone of bacterial growth (average in mm)**	
	Staphylococcus aureus (G+ve)	**E. coli (G-ve)**

Ciprofloxacin	30	16
IVb_1	32	16
IVb_2	28	16
IVb_3	32	16
IVa_4	30	16

DOCKING STUDY

Docking study was performed by using Molecular operating environment program (MOE 2015.10) to study the docking process of the target synthesized compounds with topoisomerase enzyme II (DNA gyrase) which has the symbol (2xct) which was obtained from the PDB website. The synthesized compounds showed high affinity toward the enzyme in which the compounds IVb_1 and IVb_3 showed high docking scores which is compatible with antibacterial study results against S. aureus. Ciprofloxacin displayed high affinity toward DNA gyrase through its oxygen atom present in the carboxyl group which forms hydrogen bond with Ser. amino acid and phenyl ring which forms Vander Waals bonds with Arg. and Phe. amino acids. Interestingly, the target compounds (IVb_1-IVb_4) have different interaction patterns compared to ciprofloxacin with higher docking scores in ΔG (Kcal/mol). This finding can be explained by the higher number of interactions of each derivative with the active site of the enzyme and comparable pose of these compounds with ciprofloxacin which was indicated by RMSD values of the target compounds compared with ciprofloxacin (all of the target compounds have RMSD value less than that of ciprofloxacin). Additionally, the presence of thiazole ring seems to play an important role in the interaction with the active site of enzyme by orienting the bulky imine moieties and consequently, the inhibitory activity of these derivatives. The results of docking study are shown in table 3 and figures 1-10.

Table (3): Docking results of the target compounds and ciprofloxacin

Compound	Docking-scores in ΔG (Kcal/mol)	RMSD	No. of binding sites	Amino acids
Ciprofloxacin	-5.08	2.19	2	Ser B1084, Arg B458
IVb_1	-6.21	1.74	4	Glu. B435, Ala. B439, Gly. B584, Glu. B477
IVb_2	-5.98	1.55	3	Phe. A1123, Met. A1121, Glu B435
IVb_3	-6.5	1.98	3	Arg. B471, Asn. B476, Arg. A1122
IVb_4	-6.24	1.65	4	Gly. B584, Ala B439, Ser. B438, Gly. B436

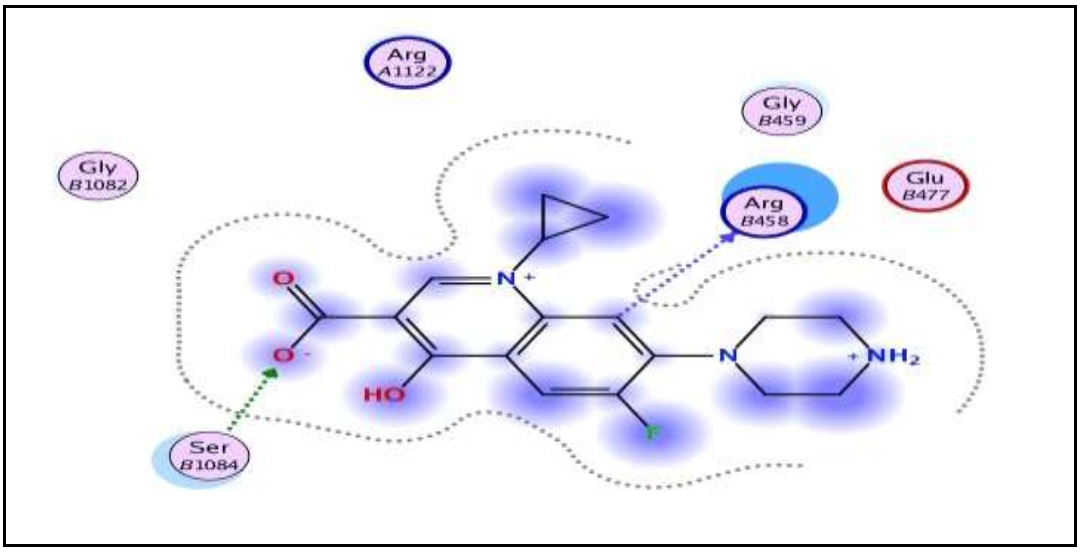


Figure (1): 2D image representing ciprofloxacin interaction with the amino acid residues of the active site of DNA gyrase (PDB ID: 2xct)

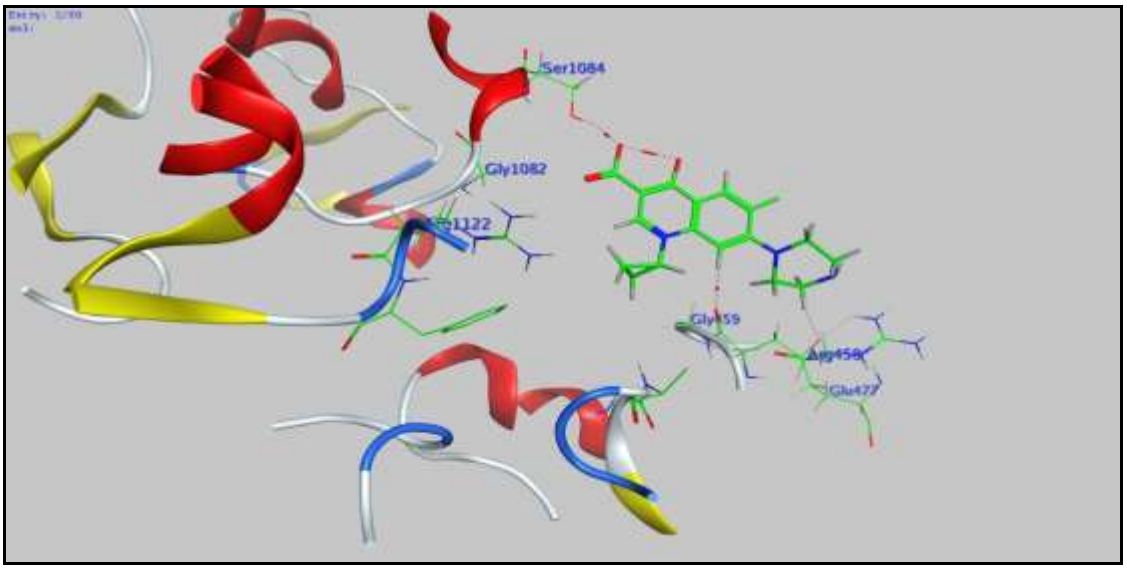


Figure (2): 3D image representing ciprofloxacin interaction with the amino acid residues of the active site of DNAgyrase (PDB ID: 2xct)

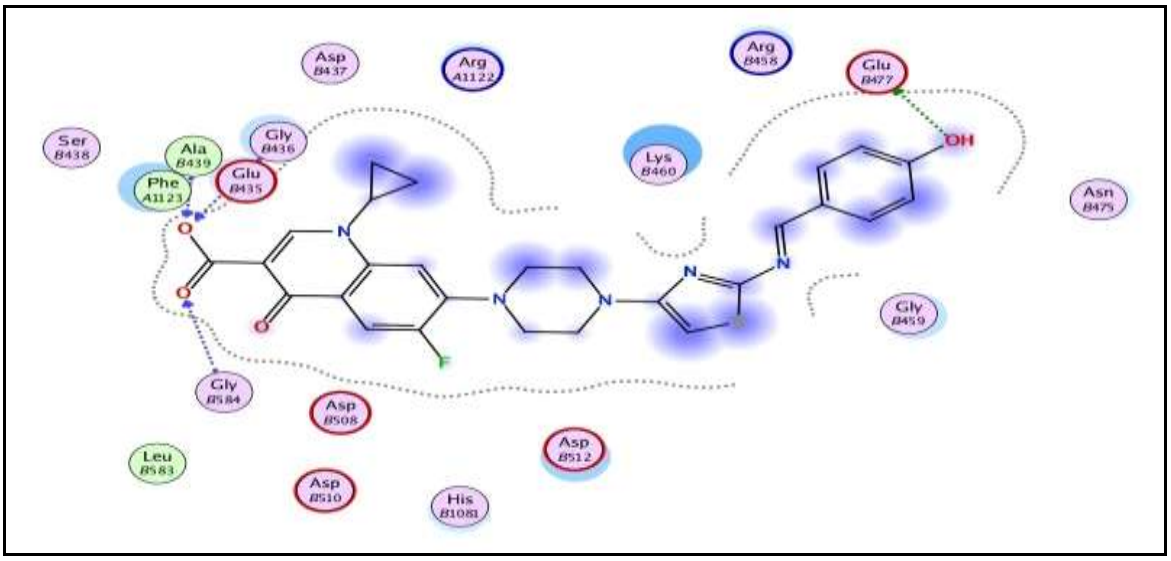


Figure (3): 2D image representing IVb_1 interaction with the amino acid residues of the active site of DNAgyrase (PDB ID: 2xct)

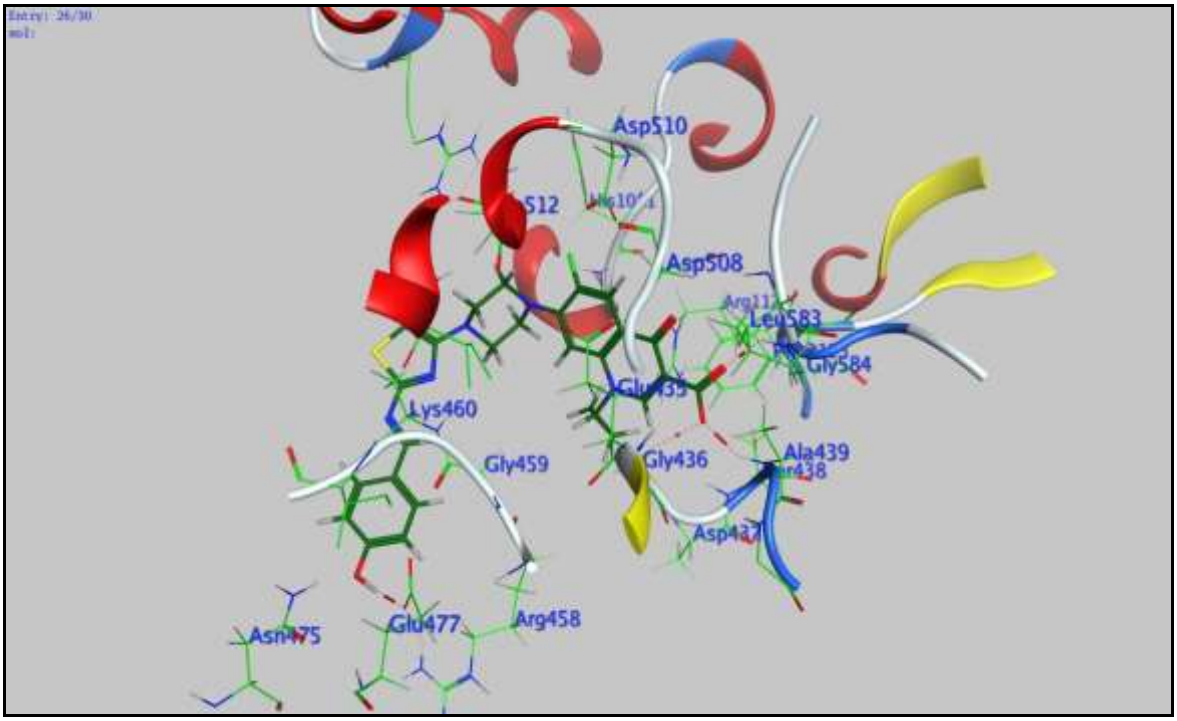


Figure (4): 3D image representing IVb_1 interaction with the amino acid residues of the active site of DNAgyrase (PDB ID: 2xct)

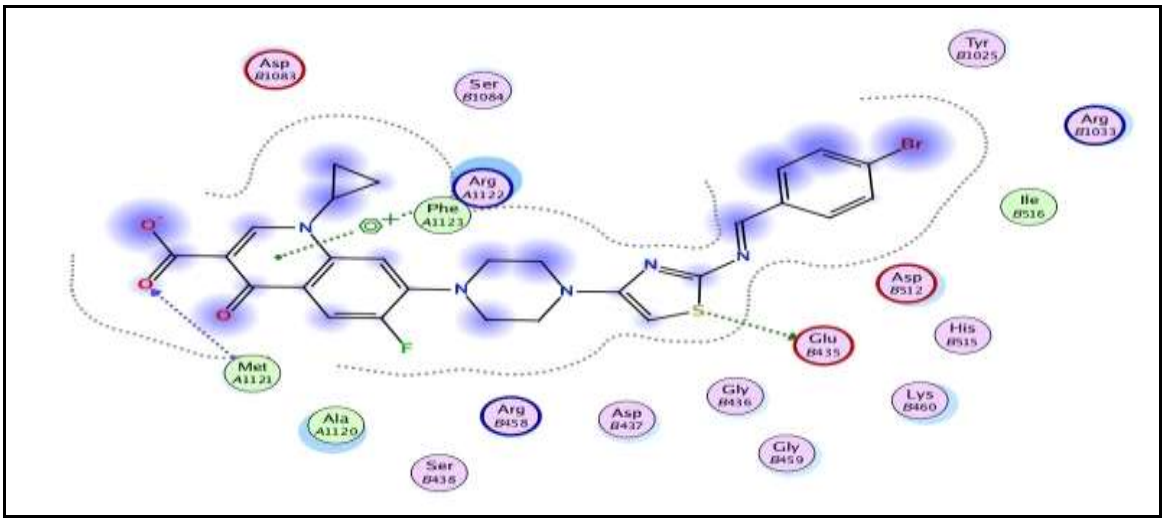


Figure (5): 2D image representing IVb_2 interaction with the amino acid residues of the active site of DNAgyrase (PDB ID: 2xct)

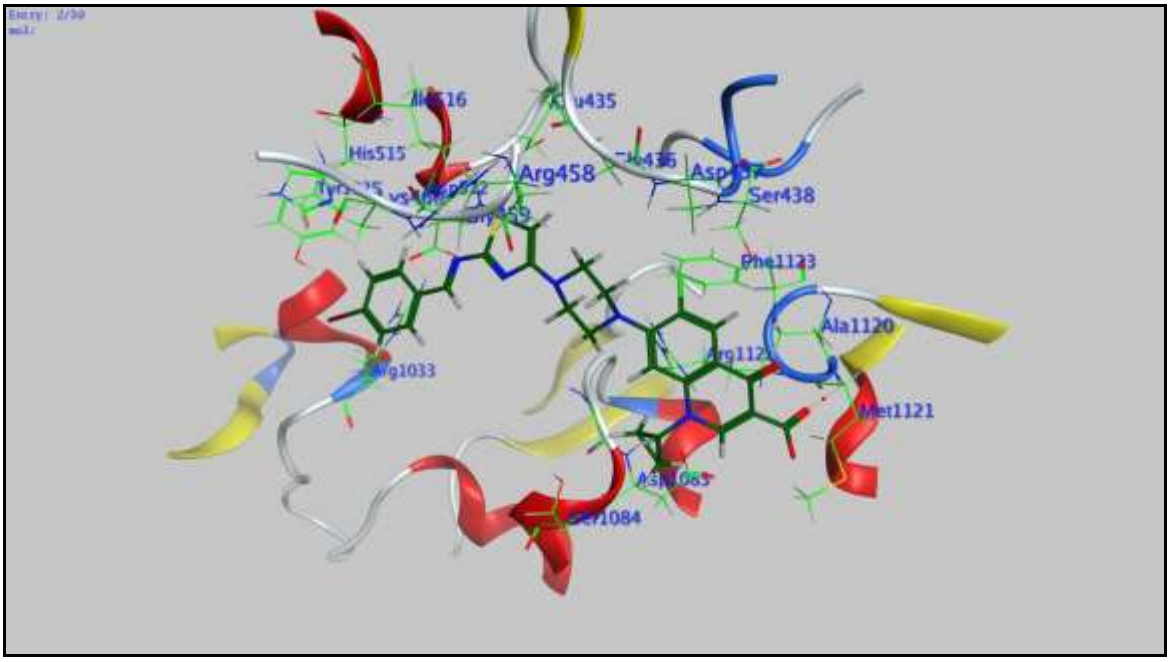


Figure (6): 3D image representing IVb_2 interaction with the amino acid residues of the active site of DNAgyrase (PDB ID: 2xct)

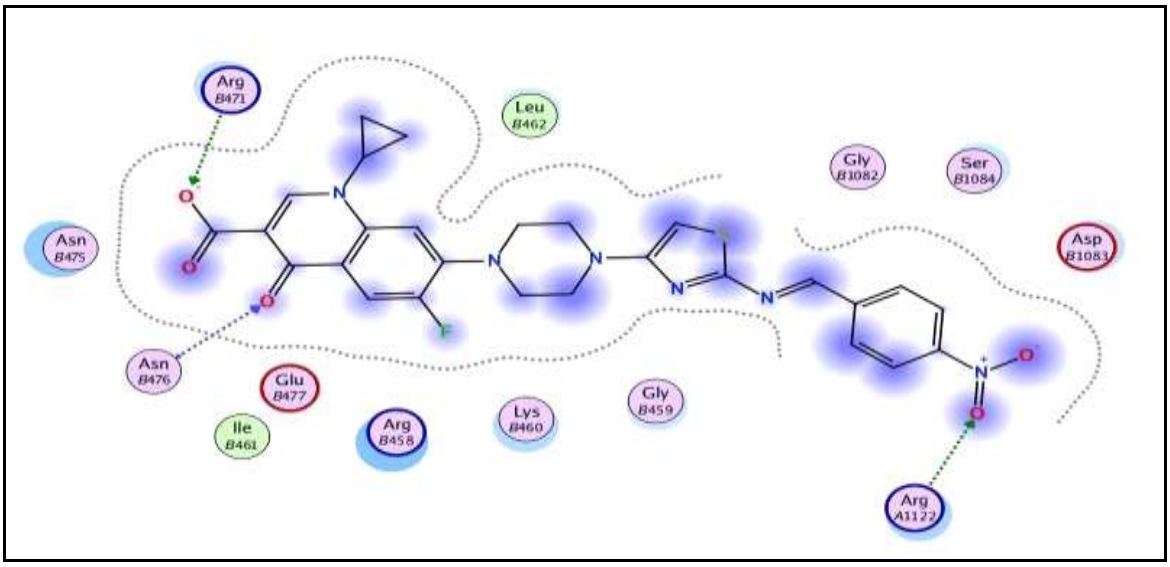


Figure (7): 2D image representing IVb_3 interaction with the amino acid residues of the active site of DNAgyrase (PDB ID: 2xct)

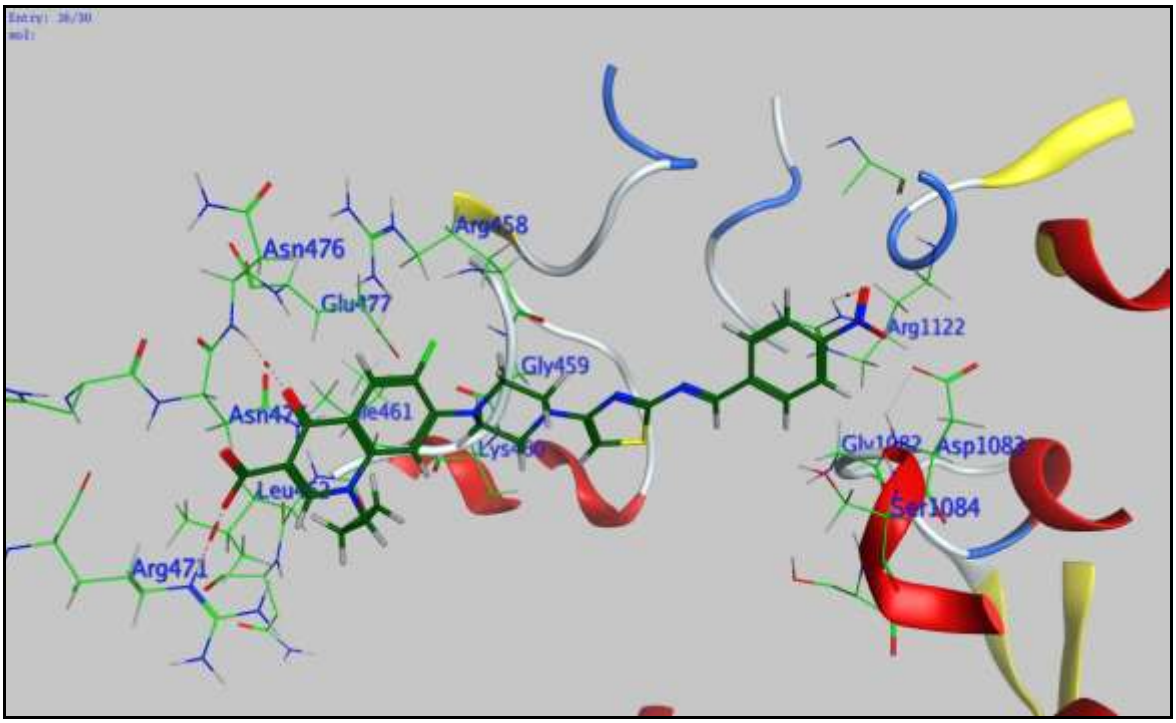


Figure (8): 3D image representing IVb_3 interaction with the amino acid residues of the active site of DNAgyrase (PDB ID: 2xct)

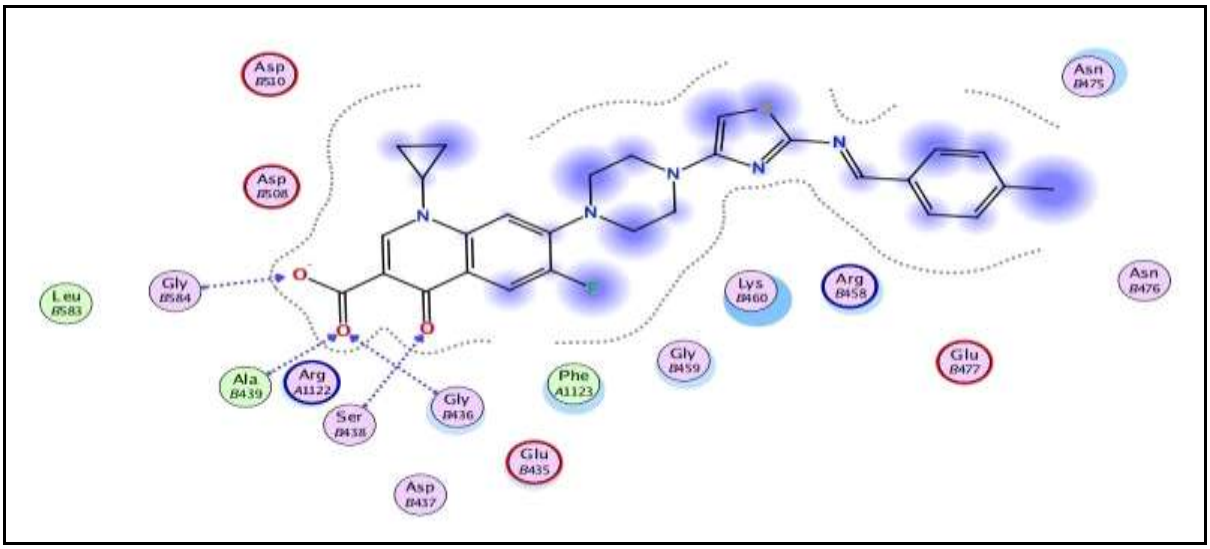


Figure (9): 2D image representing IVb_4 interaction with the amino acid residues of the active site of DNAgyrase (PDB ID: 2xct)

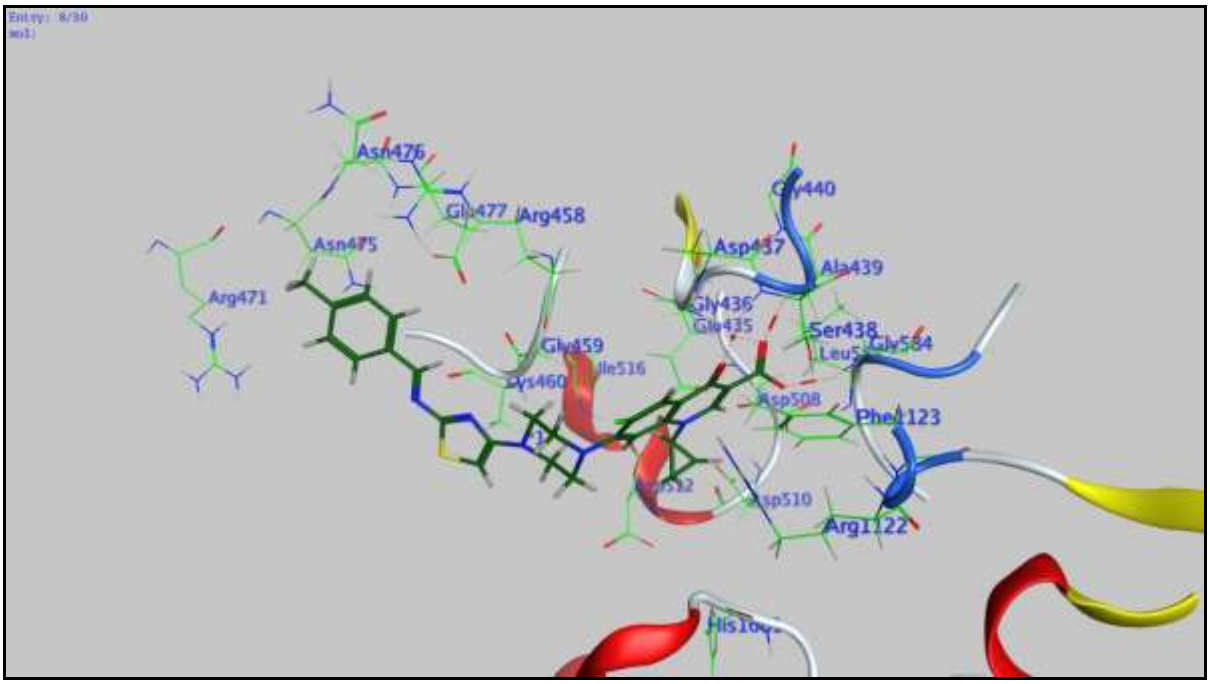


Figure (10): 3D image representing IVb_4 interaction with the amino acid residues of the active site of DNAgyrase (PDB ID: 2xct)

CONCLUSION

Series of ciprofloxacin derivatives were designed, synthesized, characterized and evaluated by in silico and in vitro studies compared with ciprofloxacin. The antibacterial study showed higher zones of inhibition of the target compounds than ciprofloxacin. These findings were achieved by the incorporation of thiazole ring into a secondary amine of ciprofloxacin piperazine ring which improves the antibacterial activity of the synthesized compounds. These

findings were consistent with the results of the in-silico study of the synthesized compounds which showed higher affinity of the target compounds (IVb_{1-4}) than ciprofloxacin toward DNA gyrase (PDB ID:2xct).

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS CONTRIBUTION

HAJ conducted the synthesis and wrote the manuscript, NHN, designed the final compounds and the scheme of synthesis, AHA conducted the in-silico study and revised the manuscript, SAH, performed the invitro study for the compounds.

REFERENCES

1. Durand GA, Raoult D, Dubourg G. Antibiotic discovery: history, methods and perspectives. Int J Antimicrob Agents. 2019;53(4):371–82.

2. Serwecińska L. Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. Water (Switzerland). 2020;12(12).

3. Bottery MJ, Pitchford JW, Friman VP. Ecology and evolution of antimicrobial resistance in bacterial communities. ISME J. 2021;15(4):939–48.

4. Mohammed HHH, Abuo-Rahma GE-DAA, Abbas SH, Abdelhafez E-SMN. Current Trends and Future Directions of Fluoroquinolones. Curr Med Chem. .2018;26(17):3132–49.

5. Pallo-Zimmerman LM, Byron JK, Graves TK. Fluoroquinolones: Then and now. Compend Contin Educ Vet. 2010;32(7):1–9.

6. Singh K. Inhibition of the CmeABC efflux pump by antisense peptide nucleic acids reduces the emergence of spontaneous fluoroquinolone resistant mutants in Campylobacter jejuni. Lowa State Univ. 2020;1–86.

7. Valenzuela M V., Domenech M, Mateos-Martínez P, González-Camacho F, de la Campa AG, García MT. Antibacterial activity of a DNA topoisomerase I inhibitor versus fluoroquinolones in Streptococcus pneumoniae. PLoS One. 2020;15(11):1–16.

8. Geetha PV, Aishwarya KVL, Mariappan S, Seka U. Fluoroquinolone Resistance in Clinical Isolates of Klebsiella oxytoca. Chemotherapy. 2020;12(2):121–5.

9. Eugene-Osoikhia TT, Obodozie JC, and Ayeni F. Synthesis, Characterization and Antimicrobial Studies of Fe (II) and Cu(II) Complexes of Acetylated and Benzoylated Derivatives of Ciprofloxacin. SELL J. 2020;25(1):25–43.

10. Mahdi MF, Naser NH, Alfadhel SM, Abd Razik BM, Osman H. Design, synthesis, and acute anti-inflammatory assessment of new ketoprofen analogs having 4-thiazolidinone nucleus. Int J Res Pharm Sci. 2017;8(4):576–84.

11. A.A. H, Abbas SH, Hassan HA, Abuo-Rahma GEDA. Recent updates of fluoroquinolones as antibacterial agents. Arch Pharm (Weinheim). 2018;351(9):1–14.

12. Billah MM, Rana SMM, Hossain MS, Saifuddin AKM, Islam SKMA, Naim Z, et al. Determination of the presence and pharmacokinetic profile of ciprofloxacin by TLC and HPLC method respectively in broiler chicken after single oral administration. J Antibiotics (Tokyo). 2014;67(11):745–8.

13. Sithole B. Peptide-Antibiotic Conjugates as Novel Developments for Increased Antimicrobial Transport and Efficiency. Univ York. 2015;1–121.

14. Naser NH. Design, synthesis and hydrolysis study of gatifloxacin-NSAIDs as mutual prodrugs. Asian J Chem. 2018 ;30(1):195–200.

15. Doregiraee A, Kermani ET, Khabazzadeh H, Pouramiri B. Synthesis of new 1,3-thiazole derivatives; using 1-(4-carbamoylphenyl)-3-methylthiourea and 1-methyl-3-(quinolin-8-yl) thiourea as starting materials. J Chil Chem Soc. 2015;60(3):3021–3023.

16. Hu G, Wang G, Duan N, Wen X, Cao T, Xie S, et al. Design, synthesis and antitumor activities of fluoroquinolone C-3 heterocycles (IV): s-triazole Schiff–Mannich bases derived from ofloxacin. Acta Pharm Sin B. 2012;2(3):312–7.

17. Rajeshkumar S, Malarkodi C. In Vitro Antibacterial Activity and Mechanism of Silver Nanoparticles against Foodborne Pathogens. Bioinorg Chem Appl. 2014; 2014:1–10.

18. Skogman ME, Kanerva S, Manner S, Vuorela PM, Fallarero A. Flavones as quorum sensing inhibitors identified by a newly optimized screening platform using chromobacterium violaceum as reporter bacteria. Molecules. 2016; 21(9):2–11.

19. Attique SA, Hassan M, Usman M, Atif RM, Mahboob S, Al-Ghanim KA, et al. A molecular docking approach to evaluate the pharmacological properties of natural and synthetic treatment candidates for use against hypertension. Int J Environ Res Public Health. 2019;16(6):1–17.

20. Dewick P.M.: Essentials of Organic Chemistry (1st ed.). John Wiley & Sons. Ltd, 2006; Chapter 7: Nucleophilic Reactions of Carbonyl Groups (Nitrogen as a Nucleophile: Amides): pp. 262-266.

21. Bruckner, R.: Advanced organic chemistry (Reaction mechanisms), (1st ed.). Academic press, Elsevier, 2008; Chapter 6: Nucleophilic Substitution Reaction: pp. 221-256.

22. Kishor S.J., Jitender B.B., Muthu K.K., Vikas K.R., Gajanan S.W., Nitin A.L. and Satish N.D.: An efficient and rapid synthesis of 2-amino- 4-arylthiazoles employing microwave irradiation in water. Green and Sustainable Chemistry 2011; 1: pp. 36-40.