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Design, Synthesis, Antibacterial Screening and Cytotoxicity Measurement of new N-4 piperazinyl Derivative of Ciprofloxacin

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Abstract: A New ciprofloxacin derivative **1** was synthesized, characterized and screened for *in vitro* antibacterial activity against Gram-positive strain *Staphylococcus aureus ATCC 6538* and Gram-negative strains *E. coli ATCC 25922 and Klebsiella pneumoniae ATCC* using the standard agar cup diffusion method. The *in vitro* antibacterial screening showed the higher potency of compound **1** against *Staphylococcus aureus ATCC 6538* with MIC value of **1.70** µg/mL than parent ciprofloxacin with MIC value of 5.49 µg/mL.
Keywords: Ciprofloxacin; Antibacterial; topoisomerases and gyrase enzyme.

1 Introduction

Ciprofloxacin is a second-generation fluoroquinolone with broad spectrum antibacterial activity and excellent safety profile[1]. It is producing its antibacterial activity *via* inhibition of bacterial topoisomerase II (DNA gyrase) and/or topoisomerase IV enzymes resulted in inhibition of DNA replication, recombination, and repair [2-4]. Recent investigations have discovered that substituting a lipophilic substituent for the *N*-4 piperazinyl ciprofloxacin decreases zwitter-ion effect, improves physiochemical parameters and increase activity against Gram-positive bacteria and antimycobacterial activity [5, 6]. As a result, the aim of this work is to synthesize new *N*-4-piperazinyl ciprofloxacin derivative and to evaluate its antibacterial activity against both Gram-negative and Gram-positive bacterial strains.

2 Results and Discussion

2.1. Chemistry

2.1.1 Synthesis of the Target Compound 1

The target compound **l** was synthesized as outlined in **Scheme 1**. Butryl ciprofloxacin **1** was synthesized through acylation of ciprofloxacin with chlorobutryl chloride in dichloromethane in presence of potassium carbonate as a base [7].

2.2. Biological Investigations

2.2.1Screening of Antibacterial Activities.

The antibacterial activities of compound **1** was evaluated *in vitro* against Gram-positive bacteria *Staphylococcus aureus* ATCC 6538, and Gram-negative bacteria such as *Klebsiella pneumoniae* ATCC *10031* and *Escherichia coli ATCC 25922* and using standard agar cup diffusion method [8] using ciprofloxacin as reference drug. Results of the antibacterial screening showed that compound **1** exhibited more potent activity against *Staphylococcus aureus ATCC 6538* and comparable activity to ciprofloxacin towards gram negative strains *Klebsiella pneumoniae ATCC 10031* and *Escherichia coli ATCC 25922*, as shown in **Table 1**. The results emphasize the fact that *N*-4-piperazinyl substitution enhancing the activity against gram positive and decreasing activity against Gram negative bacterial strains [9].

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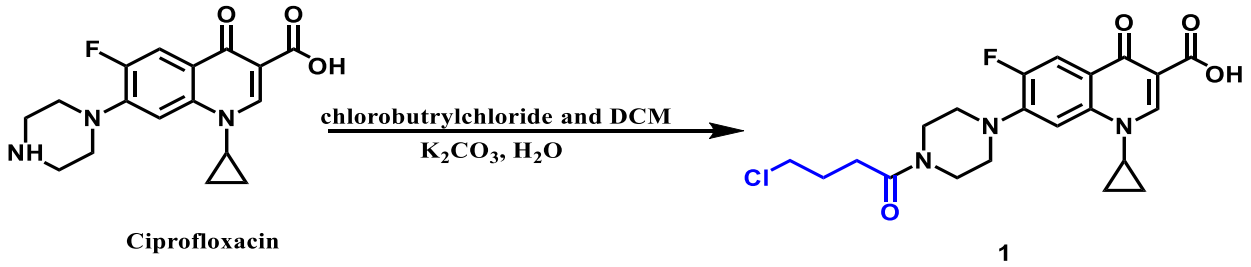

Scheme 1: Synthesis of 7-(4-(4-chlorobutanoyl) piperazin-1-yl)-1- cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

Table 1: The MICs of antibacterial activities of the ciprofloxacin derivative 1 and ciprofloxacin (µM).

Compound	MIC (µM)		
	Gram-positive	Gram-negative	
	Staphylococcus aureus ATCC 6538	*E. coli ATCC 25922*	*Klebsiella pneumoniae ATCC 10031*
1	**1.70**	**0.60**	**1.20**
Ciprofloxacin	**5.49**	**0.04**	**0.10**

2.2.2 in Vitro Cytotoxicity Screening

Compound **1** was tested at a single concentration of 10 µM against 60 cancer cell lines in National Cancer Institute (NCI). Results showed that compound **1** exhibited no significant cytotoxic activity against tested cell lines as shown in **Figure 1**. As a result, compound **1** has selective antibacterial activity without toxicity to the mammalian cells.

2.2.3. Docking Study

compounds **1** and the parent ciprofloxacin were docked into the active site of gyrase enzyme (PDB: 2XCT) to recognize the possible binding interactions resulting from the structural modification of ciprofloxacin by introduction *N*-4-pipreazinyl substitution. Docking studies were carried out using MOE 2014 software. the docked compound **1** has high affinity for the active site of gyrase enzyme (PDB: 2XCT) with binding free energy (ΔG) value of -13.78 compared to -9.93 for ciprofloxacin as shown in Table 1. Binding scores have negative values considering good evidence for the spontaneous binding of new ciprofloxacin derivatives with the active site of gyrase enzyme. Both target compound **1** and parent ciprofloxacin make chelation with Mn^{++} through the C-3 carboxylic group and C-4 carbonyl functionality of quinolone nucleus. Compound **1** showed extra hydrogen bonding with amino acid residues SER 1084 and ASN 475 and hydrophobic interactions with nucleotide bases of DNA.

Table 2: Interactions, energy scores (kcal/mol) and binding of compound **1** and **ciprofloxacin** docked into the active site *S. aureus* DNA gyrase (PDB: 2XCT).

Compound	Energy score (kcal/ mol)	Ligand interaction		
		Amino acid residue	Interaction type	Length
Ciprofloxacin	-9.9348	GLU 435 MN 2000 MN 2000	H-bond Metal interaction Metal interaction	3.07 1.80 1.84
1	-13.78	SER 1084 ASN 475 MN 2000 MN 2000 DA 13 DG5 9	H-bonding H-bonding Metal interaction Metal interaction H-Pi Pi-Pi	2.98 3.30 1.57 1.86 3.91 3.91

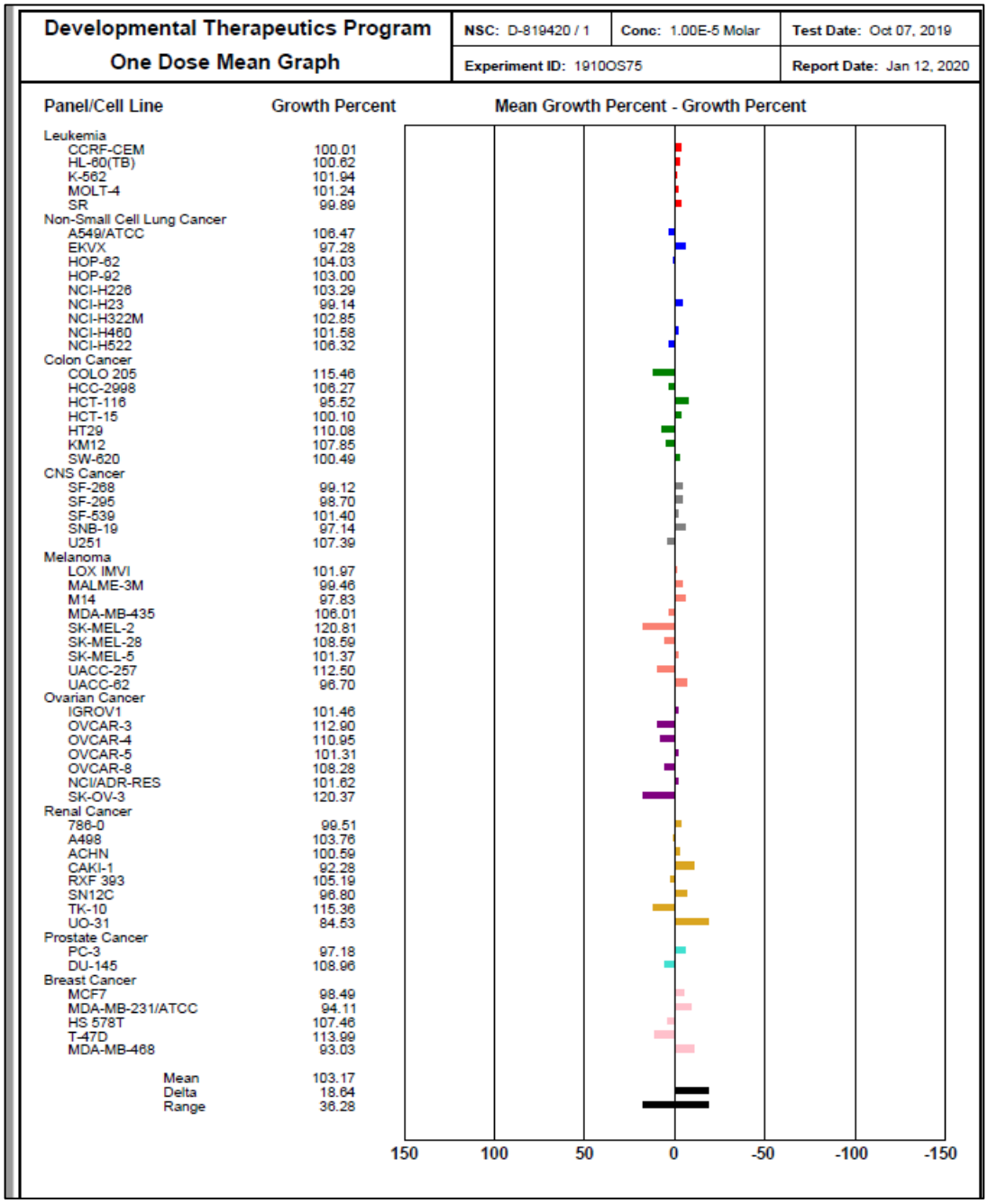

Fig. 1: One dose growth (%) and mean graph for compound **1.**

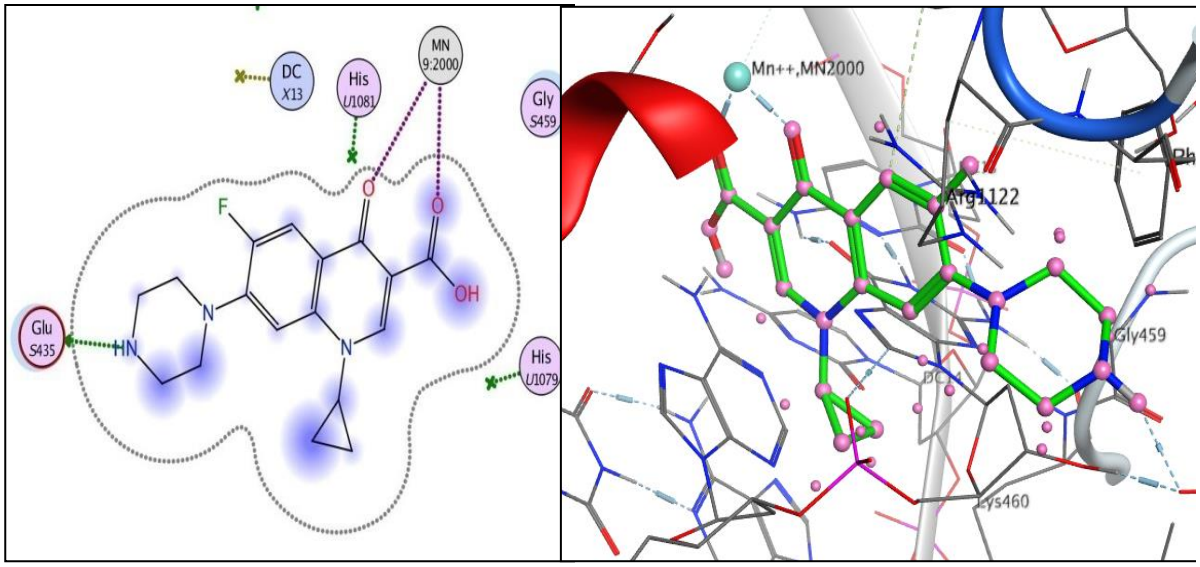

Fig. 2: 2D and 3D diagram of **ciprofloxacin** docked into the active site of DNA gyrase enzyme (PDB: 2XCT).

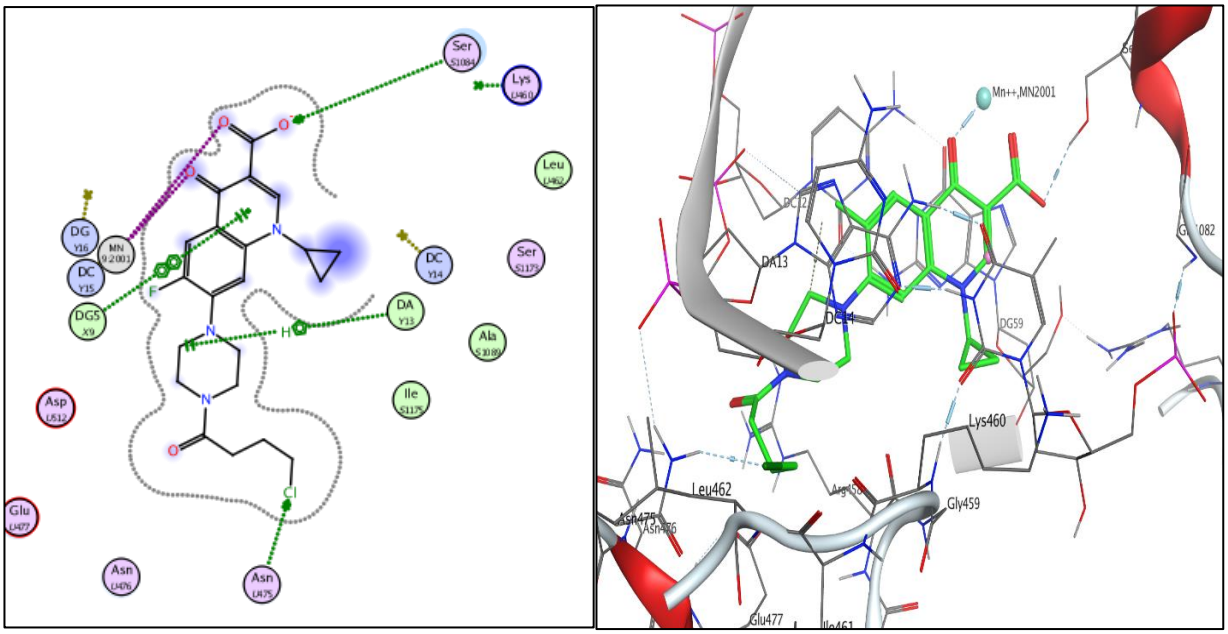

Fig. 3: 2D and 3D diagram of **Compound 1** docked into the active site of DNA gyrase enzyme (PDB: 2XCT).

3 Experimental Sections

3.1 Chemistry

Reactions were monitored by TLC, using Merk 9385 pre-coated aluminum plate silica gel (Kieselgel 60) 5cm x 20 cm plates (eluent: methylene chloride 95: methanol 5 V/V), the spots were detected by exposure to UV- lamp at ƛ = 254 nm. Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific Co.) and were uncorrected. IR spectra are recorded as KBr disks on a Shimaduz 408 instrument Spectrophotometer at the Faculty of Science, Sohag University. NMR Spectra were taken on a Bruker AM NMR (400 MHz) spectrometer at Faculty of Science, Sohag University. All numbers referring to NMR data obtained are in parts per million (ppm).

3.1.2 Procedure of synthesis of butryl ciprofloxacin 1

At 0–5 °C, to a stirred solution of ciprofloxacin (1 mmol) in DCM (30 mL) and potassium carbonate (1.1 mmol) in

distilled water (30 mL) solution chlorobutryl chloride (1.1 mmol) in DCM (20 mL) was gently added over 30 minutes. Stirring was continued for 2 hours at 0–5 °C, then for an additional 12 hours at room temperature. The organic layer was collected in a separating funnel and washed with distilled H2O (2X 25 mL), 1N HCl (2X 25 mL), distilled H2O (2X 25 mL), dried over sodium sulphate anhydrous, flittered off, and dried to yield compound 1.[7].

3.1.2.1 7-(4-(4-chlorobutryl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid 1

White powder; yield: 0.400 g (91.96 %); mp: 262 °C, IR (KBr) ύ (cm^{-1}): 1714 (carboxylic C=O), 1683 (amidic C=O) and 1626 (4-keto C = O); ^{1}H-NMR (400 MHz, DMSO-d6) δ ppm: 1.20–1.25 (2 H, m, cyclopropyl-2H), 1.31–1.37 (2H, m, cyclopropyl-2H), 2.00 (2H, m, Br–$CH_2C\underline{H_2}$–CH_2), 2.55 (2H, m, *J* = 8.0 Hz, Cl–$CH_2CH_2C\underline{H_2}CO$), 3.30-3.36 (4H, m, piperazinyl-H), 3.69 (2H, t, *J* = 8.0 Hz, Cl–$C\underline{H_2}CH_2C\underline{H_2}CO$), 3.70–3.75 (4H, m, piperazinyl-H), 3.80-3.85 (1 H, m, cyclopropyl-H), 7.59 (1H, d, $J_{H\text{-}F}$ = 6.8 Hz, H8), 7.91(1H, d, $J_{H\text{-}F}$ = 13.6 Hz, H5), 8.66 (1 H, s,H2), 15.11 (1 H, s, COOH); ^{13}C-NMR (100 MHz, DMSO-d_6): 8.06, 27.86, 29.81, 36.33, 42.95, 44.94, 45.48, 49.63, 50.02, 106.99, 107.29, 111.54 (d, *J* = 23 Hz), 119.21, 139.60, 145.29, 148.43, 153.35 (d, $J_{C\text{-}F}$ = 248 Hz), 166.23, 170.32, 176.86.

3.2 Biological Investigations

3.2.1 Screening of antibacterial activity.

The antibacterial activity of compound **1** and ciprofloxacin were determined according to the standard agar cup diffusion method at Department of Molecular Biotechnology, Faculty of science, Assuit University, Assuit, Egypt.

3.2.1.1. Microbial strains and culture conditions:

Three bacterial species representing both Gram-positive and Gram-negative strains and were used to test the antibacterial activity of the new compounds. Standard strains of *Staphylococcus aureus ATCC 6538, Klebsiella pneumoniae ATCC 10031* and *Escherichia coli ATCC 25922* were obtained from microbiological resource center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. All isolates were maintained at -70 ºC in Trypticase Soya Broth (TSB, Becton and Dickinson) with 10 % glycerol. Prior to inoculation, all isolates were sub cultured at 37 ºC for 24 h on Trypticase Soya Agar (TSA, Becton and Dickinson) and TSB, respectively

3.2.1.2. Determination of the minimum inhibitory concentration (MIC)

From all the tested bacteria 0.5 mL of 1×108 CFU/mL (0.5 McFarland turbidity) were plated in sterile petri dishes, then 20 mL of Mueller Hinton Agar media (Oxoid) was added to each petri dish. The plates were rotated slowly to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. After solidification, four equidistant and circular wells of 10 mm diameter were carefully punched using a sterile cork bore. Two folds serial dilutions of the tested compounds using DMSO were performed. An equal volume of 100µL of each dilution was applied separately to each well in three replicates using a micropipette. All plates were incubated at 37 ºC for 24 h. The inhibition zones were measured, and their average was calculated. The MIC was calculated by plotting the natural logarithm of the concentration of each dilution of the tested compounds against the square of zones of inhibition and a regression line was drawn through the points then the antilogarithm of the intercept on the logarithm of concentration axis gave the MIC value.

3.2.2. Screening of Cytotoxicity

The cytotoxicity screening of the target compound **1** was evaluated at the national cancer institute (NCI) against 60 cell lines of different cell types according to NCI protocol [10].

3.2.3. Docking Studies.

compounds **1** and the parent compound ciprofloxacin were docked into the active site of gyrase enzyme (PDB: 2XCT) to recognize possible mechanism of action and obtain possible binding modes between the compounds and the enzyme active site. Docking experiments were done using Molecular Operating Environment (MOE®) version 2014. X-ray crystallographic structure of the ligand-enzyme complex were downloaded from protein data bank (www.rcsb.org); gyrase enzyme (PDB: 2XCT). The energy was reduced until an RMSD (Root Mean Square Deviations) gradient of 0.01 Kcal/mol and RMS (Root Mean Square) gap of 0.1 Aº with MMFF-94X (Merck Molecular Force Field 94x) force–field and the partial charges were automatically measured using the builder interface of the MOE software.

The enzyme was prepared for docking studies by removing the ligand, testing the atom relation, and adding hydrogen atoms automatically. The receptor's potential was then set and the modelled compounds were docked into the 3D structure of the catalytic site of the obtained poses. The poses that revealed the most effective ligand-enzyme interactions were chosen and saved for energy calculations.

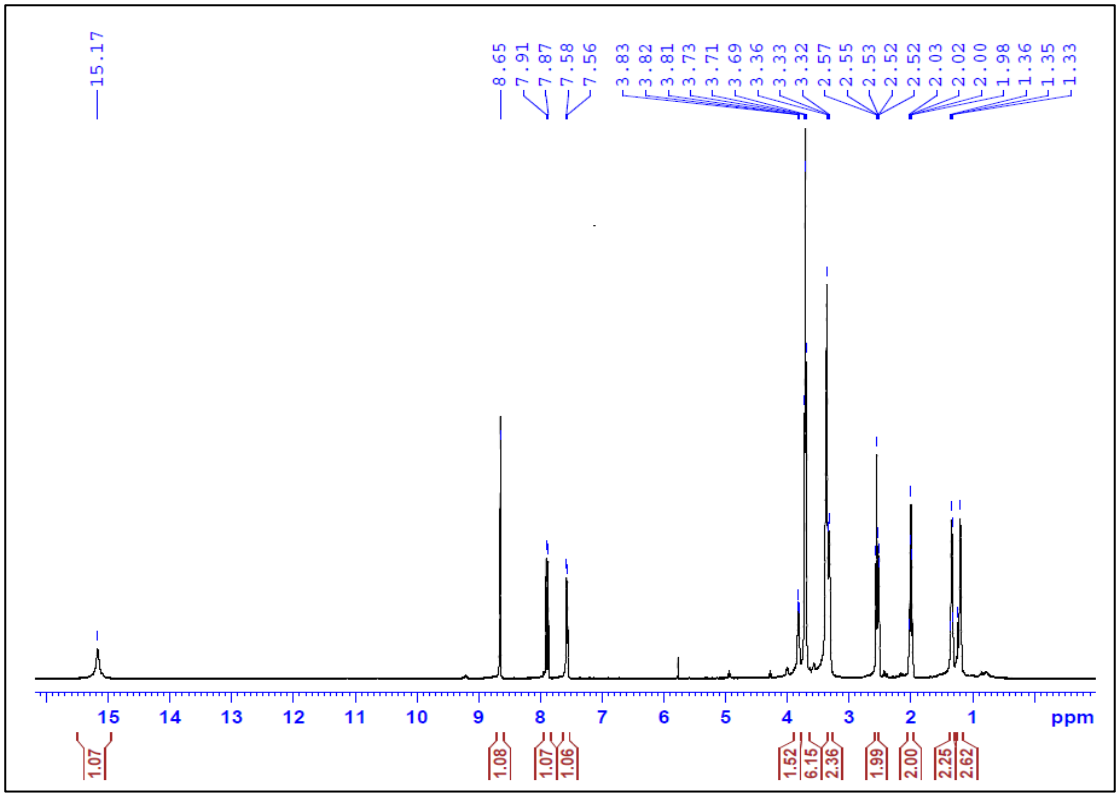

Fig. 4: [1]HNMR spectrum of compound **1** (400 MHz, DMSO-d_6).

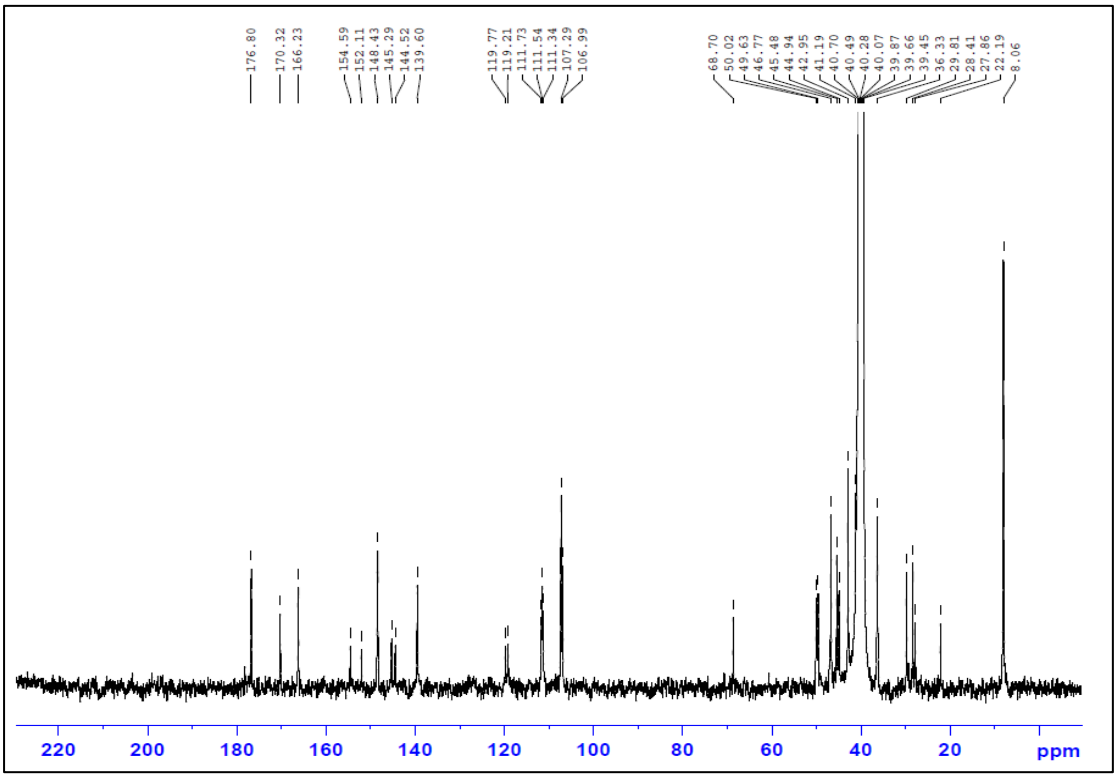

Fig.5: [13]CNMR spectrum of compound **1**(100 MHz, DMSO-d_6).

4 Conclusions

New *N*-4-piperazinyl butyryl ciprofloxacin derivative was synthesized, characterized by various spectroscopic techniques and evaluated for their antibacterial activity. screening of antibacterial activity showed that new synthesized compound has more potent activity against *Staphylococcus aureus ATCC 6538* without toxicity on human cells. Also, compound **1** showed activity against G ram negative strains, *Klebsiella pneumoniae ATCC 10031* and *Escherichia coli ATCC 25922* but lower than parent ciprofloxacin. The antibacterial activity of compound **1** is in line with the fact that *N*-4-piperazinyl substitution of ciprofloxacin enhances activity against gram positive strains.

References

[1] Bisacchi, G.S.J.J.o.m.c., Origins of the quinolone class of antibacterials: an expanded "discovery story" miniperspective., **58(12)**, 4874-4882, 2015.

[2] Pommier, Y., DNA topoisomerases and cancer. 2012: Springer.

[3] Wang, J.C.J.N.r.M.c.b., Cellular roles of DNA topoisomerases: a molecular perspective., **3(6)**, 430-440, 2002.

[4] Anderson, V.E. and N.J.C.p.d. Osheroff, Type II topoisomerases as targets for quinolone antibacterials turning Dr. Jekyll into Mr. Hyde., 7(5), 337-353, 2001.

[5] Abou Rahma, G., et al., New N-4 piperazinyl derivatives of norfloxacin: design, synthesis, and correlation of calculated physicochemical parameters with antibacterial activity., **42**, 1072-1085, 2018.

[6] Aziz, H.A., et al., New fluoroquinolones/nitric oxide donor hybrids: design, synthesis and antitubercular activity., **28(8)**, 1272-1283, 2019.

[7] Aziz, H.A., et al., New norfloxacin/nitric oxide donor hybrids: Synthesis and nitric oxide release measurement using a modified Griess colorimetric method., **8(2)**, 119-124, 2017.

[8] Bonev, B., J. Hooper, and J.J.J.o.a.c. Parisot, Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method., **61(6)**, 1295-1301, 2008.

[9] Aziz, H.A., et al., Thiazolidine-2, 4-dione-linked ciprofloxacin derivatives with broad-spectrum antibacterial, MRSA and topoisomerase inhibitory activities.,1-17, 2021.

[10] The methodology of the NCI anticancer screening has been described in details: (http://www.dtp.nci.nih.gov), Bethesda, USA.