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Synthesis and Characterization of Some NH-Analogues of Ciprofloxacin on Antibacterial, Antifungal, and Cytotoxic Activities

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Abstract

Present work includes synthesis of five NH-analogues of ciprofloxacin, **2-6** have been prepared to find out their medicinal assessment, for instance, antibacterial, antifungal, and cytotoxicity. The structure of the analogues has been confirmed by FT- IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral data and elemental analysis. The compounds were screened for their antimicrobial activities by the disc diffusion method. Cytotoxicity was also tested against brine shrimp lethality assay. The antimicrobial activity of the analogues compared with the parent was evaluated against three Gram-positive, five Gram-negative bacterial strains and three fungi. The synthesized compounds showed diverse antimicrobial profile among which derivatives, **2** and **3** possessed enhanced activity in contrast to the ciprofloxacin. Additionally, unlike ciprofloxacin, most of the derivatives were also found to show antifungal activity against *Candida albicans*. Regarding cytotoxicity, most of the derivatives exhibited better cytotoxic activity than ciprofloxacin.

Keywords: Ciprofloxacin derivatives; Antibacterial; Antifungal; Cytotoxicity.

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1. Introduction

Ciprofloxacin, **1** is a synthetic antibacterial related to nalidixic acid having a fluorine atom and piperazine ring at positions 6 and 7 of quinolone-3-carboxylic acid. Ciprofloxacin, first introduced in 1987, is a second-generation broad spectrum and one of the most widely used, belongs to fluoroquinolones antibiotic. Quinolones realize their effect by converting gyrases and topoisomerases IV into toxic enzymes that fragment bacterial chromosome [1-5]. Ciprofloxacin impedes the replication and transcription of bacterial DNA, leading to an increase in oxidative stress and death of bacterial cells [6]. According to the World Health Organization, it is one of the most frequently prescribed antimicrobial drugs [7]. Ciprofloxacin has been permitted to treat a lot of Gram-positive and Gram-

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negative bacterial infections [8-12]. It has a well-known anti-tumor activity against P388 leukemia [13]. Structure-activity-relationship (SAR), mechanism of action, resistance and clinical aspects of some fluoroquinolones antibacterial activity has been reported [14]. A series of nadifloxacin derivatives has been synthesized and were found to show activity against hospital infections of multi-drug-resistance and vancomycin-resistant *Staphylococcus aureus* [15]. COOH group of ciprofloxacin converted into its amide and ester derivatives and found diverse antimicrobial profile [16]. Modifications of ciprofloxacin skeletal and antimicrobial action have been studied, where some of its derivatives possessed antifungal properties [17]. Ciprofloxacin has been incorporated into a new series of Schiff base of 1,2,4-triazole via Mannich reaction and got comparable antibacterial results with ciprofloxacin [18]. NH-derivatives of ciprofloxacin have been prepared and showed enhanced activities against Gram-negative bacteria compared to ciprofloxacin [19]. Metal complexes continue a significant resource for creating a chemical range in the fields of pharmaceutical chemistry as antitumor and antimicrobial agents and are permitted to treat with drug-resistant bacteria and a range of viral diseases [20-24]. Earlier we produced ciprofloxacin- *p*-nitro benzoyl derivative, and its transition metal complexes for biological evaluation. The compounds showed diverse antimicrobial profile amongst which most compounds possessed a comparable or better activity in comparison to the ciprofloxacin and some of the derivatives were also found to demonstrate antifungal property [25]. Most of the ciprofloxacin biological research has been focused on the functionality at the C-7 position or other functional groups but the SAR reveals that the C-7 substituent is the most adaptable site for chemical change and is an area that determines the strength and target predilection. An initiative has been taken to substitute the H-atom of the NH group of the piperazine moiety of ciprofloxacin with N-methylpyrrolidone, camphor, 1-cyanoguanidine, 1-cyanonaphthalene, and dimethylsulfate respectively to obtain derivatives **2-6** for biological evaluation (Scheme 1).

In the present study, the synthesis, structure conformation and evaluation of biological activities, i.e., antibacterial, antifungal and cytotoxicity of some ciprofloxacin derivatives will be reported.

2. Materials and Methods

2.1. *General*

Gonoshasthaya Antibiotic Ltd, Savar, Dhaka, Bangladesh gifted ciprofloxacin hydrochloride. All the synthetic works were carried out by using laboratory reagents and analytical grade solvents whenever necessary. The solvents and reagents were purified and dried according to a standard procedure. The progress of all reactions was monitored by TLC, which was performed on aluminum sheets pre-coated with silica gel 60F254 to a thickness of 0.25 mm (Merck, Germany). The mobile phase was acetonitrile: conc. NH_3 solution: CH_3OH: CH_2Cl_2 (10: 20: 40: 40). The chromatograms were visualized under ultraviolet light, 254 nm or iodine vapors. The purity of the compound was examined by

HPLC on an LC-20 AT liquid chromatography equipped with UV detector SPD-20A at 278 nm and column oven CTO-10ASvp, using a mobile phase of acetonitrile and phosphoric acid (2.45 g/L solution) in the ratio 13:87 and pH adjusted at required pH 3.0 with triethylamine. HPLC column was 250 × 4.6 mm length with 10 µL injection system. The column temperature was maintained at 40 °C during analysis, with a flow rate of 1.5 mL/min. The compounds were purified by recrystallization using suitable solvents. The melting points of the synthesized compounds were determined in open capillaries using Veego VMP-1 apparatus and expressed in °C and were uncorrected. The IR spectra of the compounds were recorded on a Shimadzu FT-IR-8400s spectrometer using KBr pellet technique. ^{1}H-NMR and ^{13}C- NMR spectra were recorded on a Bruker DRX-400 (400 MHz FT-NMR) using dimethyl sulfoxide (DMSO) solvent and tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained using a Shimadzu LC-MS (ESI) 2010A spectrophotometer. Either protonated ions $(M + H)^+$ or sodium adducts $(M + Na)^+$ were used for empirical formula confirmation at the Department of Nano Fusion Technology, Organic Optoelectronic Material Lab., Pusan National University, South Korea. Elemental analyses (C, H, and N) were obtained using a Carlo Erba NA-1500 analyzer. The *in vitro* antimicrobial activities of the analogues were carried out by the disc diffusion method, and all the bacterial and fungal strains were collected as a pure culture from Vaccine Research Laboratory, Gonoshasthaya Kendra, Savar, Dhaka. Cytotoxicity measured by the brine shrimp lethality assay from the Department of Chemistry, Jahangirnagar University, Savar, Dhaka, Bangladesh.

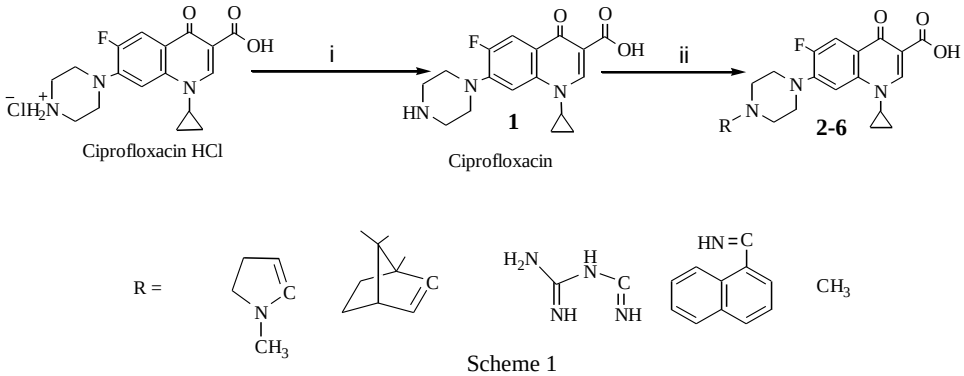
2.2. *Regeneration of ciprofloxacin and synthesis of ciprofloxacin derivatives*

A solution of ciprofloxacin hydrochloride (10 g, 27.19 mmol) in water (50 mL) was treated with 5 % aqueous sodium carbonate solution at pH, 7.0 resulting in the formation of white precipitates, filtered through suction filter and left to dry as a neutral ciprofloxacin, **1** (8.2 g, 91 %). These precipitates were used as starting material for all the reactions without purification. Generally, ciprofloxacin was converted to its enamine, cyanoguanidine, cyanonaphthalene, and N-methylation derivatives by reaction with N-methylpyrrolidone, camphor, 1-cyanoguanidine, 1-cyanonaphthalene, and dimethylsulfate respectively to obtain derivatives **2-6** (Scheme 1).

2.2.1. *Reaction of ciprofloxacin with N-methyl pyrrolidone, 1-cyclopropyl-6-fluoro-7-(4-(1-methyl-4, 5-dihydro-1H-pyrrol-2-yl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, **2***:

Ciprofloxacin (1.5 g, 4.52 mmol) was dissolved in a mixture of methanol (10.0 mL) and dichloromethane (5.0 mL) to which equal ratio of N-methyl-2-pyrrolidone (0.448 g, 4.52 mmol) was added with vigorous stirring. One drop of conc. HCl was added to make the solution clear. The reaction mixture was warmed at 40 °C with stirring for 90 min and organic solvents were removed by distillation. To the mixture was added 50 mL of water,

after which the crystalline product deposited. It was collected by filtration, washed with 60 % aqueous ethanol and dried under vacuum in a desiccator. The spectral data and other findings are given in section 3.1.1.



Scheme 1

Scheme 1. Synthesis of ciprofloxacin derivatives, **2-6** by alkylation reaction.

2.2.2. *Reaction of ciprofloxacin with camphor, 1-cyclopropyl-6-fluoro-4-oxo -7-[4-(1, 7, 7-trimethyl -bicyclo [2.2.1] hept-2-en-2-yl)-piperazin-1-yl]-1, 4-dihydro-quinoline-3-carboxylic acid, **3***:

Ciprofloxacin (1.5 g, 4.52 mmol) was dissolved in a mixture of methanol (10.0 mL) and dichloromethane (5.0 mL) to which equal ratio of camphor (0.69 g, 4.52 mmol) was added with vigorous stirring. One drop of conc. HCl was added to make the solution clear. The reaction mixture was warmed at 40 °C with stirring for 90 min and organic solvents were removed by distillation. To the mixture was added 50 mL of water after which the crystalline product deposited. It was collected by filtration, washed with 60 % aqueous ethanol and dried under vacuum in a desiccator. The spectral data and other findings are presented in section 3.1.2.

2.2.3. *Reaction of ciprofloxacin with 1-cyanoguanidine, 1-cyclopropyl-6-fluoro-7-[4-(guanidino-imino-methyl)–piperazin -1-yl]-4-oxo-1, 4-dihydro-quinoline-3-carboxylic acid, 4*:

An equal proportion of ciprofloxacin (1.00 g, 3.01 mmol) and 1-cyanoguanidine (0.254 g, 3.02 mmol) were mixed and heated to 140-145 °C to make a homogeneous mass. It was heated for an additional 10 min at the same temperature and allowed to cool at room temperature for solidification. 50 mL of water was added when yellow crystals deposited. It was filtered off, washed with 60 % aqueous ethanol and dried under vacuum in a desiccator. The results are given in section 3.1.3.

2.2.4. *Reaction of ciprofloxacin with 1-cyanonaphthalene, 1-cyclopropyl-6-fluoro-7-(4-(imino (naphthalen-1-yl) methyl) piperazin-1-yl)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid,* ***5***:

An equal proportion of ciprofloxacin (1.00 g, 3.01 mmol) and 1-cyanonaphthalene (0.462 g, 3.02 mmol) were mixed and heated to 140-145 °C to make a homogeneous mass. It was heated for an additional 10 min at the same temperature and allowed to cool at room temperature for solidification. 50 mL of water was added when yellow crystals deposited. It was filtered off, washed with 60 % aqueous ethanol and dried under vacuum in a desiccator. The spectral, physical, and others analytical results are given in section 3.1.4.

2.2.5. *N-methylation of ciprofloxacin, 1-cyclopropyl-6-fluoro-7-(4-N-methyl–piperazin -1-yl)-4-oxo-1, 4-dihydro-quinoline-3-carboxylic acid, 6*:

Ciprofloxacin (1.5 g, 4.52 mmol) was dissolved in 5 % aqueous NaOH solution and it was added in dimethyl sulfate (1.0 mL) with vigorous stirring. The reaction mixture was warmed at 60 °C with stirring for 90 min and pH was adjusted to 4.50. A crystalline product deposited. It was filtered off, washed with 60 % aqueous ethanol and dried under vacuum in a desiccator. All results are shown in section 3.1.5.

2.3. *Antimicrobial activity (in-vitro)*

2.3.1. *Antibacterial studies*

The antimicrobial activity of the derivatives was determined by the disc diffusion method [26-28] against Gram-positive, Gram-negative bacteria and antifungal strains. The organisms were accumulated as pure cultures. The experiments were carried out in triplicate using ciprofloxacin as standard and the results have been shown as mean ± SD. For the antibacterial study, 100 µg/mL stock solution of ciprofloxacin and its derivatives were prepared in hot methanol. Commercially available filter paper discs were drenched in the prepared drug and analogues solution, dried and applied on the surface of solid culture media (Nutrient agar), which had been streaked with standardized bacterial inoculums and incubated at 37 °C for 24 h. This method is based on the determination of an inhibited zone comparative to the bacterial susceptibility to the antibacterial present in the disc. The compounds were screened for their antibacterial activity and compared with the parent against three different Gram-positive strains, i.e., *Staphylococcus aureus, Streptococci, Bacillus* spp, and five Gram-negative strains, i.e., *E. coli, Klebsiella pneumoniae, V. cholerae, Salmonella* spp, and *Shigella dysenteriae.* The results are presented in Tables 1 and 2.

2.3.2. *Antifungal studies*

For the antifungal assay, 100 µg/mL stock solution of ciprofloxacin and its derivatives were prepared in hot methanol. The stock solutions were diluted to three different concentrations, i.e. 20, 40 and 60 µg/mL. Commercially available filter paper discs were impregnated with the prepared solutions of the drugs and its derivatives, dried and applied on the surface of the agar plate over which a culture of microorganism was already streaked. After 48 h of incubation at 37 °C, the clear zone of inhibition around the disc was determined; this is proportional to the fungal susceptibility for the fungal agent present in the disc. The results have been shown as mean ± SD. Ciprofloxacin and its derivatives were screened for their antifungal activity against the fungi; *Candida albicans*, *Fusarium solani* and *Aspergillus fumigatus* and compared with the parent as well as an antifungal drug miconazole nitrate. The results of antifungal activity are given in Table 3.

Table 1. Zone of inhibition (mm) of the compounds (100 µg/mL) against bacteria.

Compound no.	Gram-positive bacteria		
	Staphylococcus aureus	*Streptococci*	*Bacillus* spp
1	18.02±0.02	14.10±0.01	16.30±0.02
2	23.12±0.01	17.30±0.03	16.84±0.02
3	24.80±0.04	15.94±0.02	18.02±0.01
4	23.12±0.01	14.06±0.02	15.62±0.02
5	8.52±0.02	9.02±0.02	8.01±0.12
6	18.10±0.02	13.94±0.11	16.32±0.08

Table 2. Zone of inhibition (mm) of the compounds (100 µg/mL) against bacteria.

Compound	Gram-negative bacteria				
no.	a	b	c	d	e
1	12.22 ±0.04	24.24±0.08	21.68 ±0.01	26.20±0.01	26.12±0.02
2	14.20 ±0.05	15.44±0.04	24.20±0.04	26.48±0.04	15.62±0.05
3	18.24±0.08	26.28±0.04	27.82±0.03	27.38±0.04	28.36±0.06
4	-	13.32±0.06	14.20±0.04	19.82±0.03	10.12±0.06
5	-	12.52±0.04	12.42±0.12	20.24±0.02	10.18±0.02
6	8.82±0.12	22.38±0.05	21.60±0.08	21.32±0.01	11.66±0.02

a = *E. coli*, b= *Klebsiella pneumoniae*, c = *V. cholerae*, d = *Salmonella* spp and e = *Shigella dysenteriae*

2.4. *Cytotoxicity bioassay (in-vitro)*

The cytotoxic activity of the synthesized compounds was measured by brine shrimp lethality assay [29,30]. For determining cytotoxic activity 4.0 mg of each compound was dissolved in 10 mL of DMSO to get the first concentration 400 µg/mL and diluted to 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 and 0.0390 µg/mL using DMSO with the help of micropipette. An equal amount of the vincristine sulfate was dissolved in DMSO to get a preliminary concentration of 400 µg/mL from which solution with decreasing concentration was made by serial dilutions using DMSO to get 200, 100, 50, 25, 12.5,

6.25, 3.125, 1.563, 0.781 and 0.039 µg/mL. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conically shaped vessel (1 L), filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48 h. After hatching, active nauplii free from eggshells were collected from a brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 4.5 mL of brine solution. In each experiment, 0.5 mL of the sample was added to 4.5 mL of brine solution and maintained at 25 °C for 24 h under the light and surviving larvae were counted. The median lethal concentration LC_{50} of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration. The best-fit line was obtained from the graph. The readings were taken in triplicate. The anticancer drug vincristine sulfate was used as the positive control and DMSO as the negative control for the experiment. LC_{50} results of the compounds are shown in Table 4.

Table 3. Zone of inhibition (mm) of the compounds against various fungi.

Compound no.	*Candida albicans* (µg/mL)			*Fusarium solani* (µg/mL)			*Aspergillus fumigatus* (µg/mL)		
	20	40	60	20	40	60	20	40	60
1	-	8.06 ±0.01	9.92 ±0.02	-	-	-	-	-	-
2	12.30 ±0.02	12.86 ±0.02	14.20 ±0.04	-	8.04 ±0.01	8.42 ±0.02	-	8.02 ±0.04	10.10 ±0.01
3	12.02 ±0.01	13.62 ±0.01	15.04 ±0.02	-	8.06 ±0.08	8.26 ±0.04	-	8.04 ±0.01	8.96 ±0.02
4	10.34 ±0.01	11.08 ±0.02	12.64 ±0.04	-	8.42 ±0.02	9.12 ±0.02	-	9.04 ±0.04	10.46 ±0.04
5	9.08 ±0.01	10.16 ±0.01	12.04 ±0.02	-	9.24 ±0.02	10.38 ±0.01	-	-	-
6	-	-	9.40 ±0.01	-	9.22 ±0.01	10.72 ±0.02	-	8.06 ±0.04	10.00 ±0.12
MN	23.46 ±0.14	28.08 ±0.01	34.00 ±0.02	22.04 ±0.02	26.68 ±0.01	29.86 ±0.01	20.24 ±0.02	26.14 ±0.01	28.36 ±0.04

MN = Miconazole nitrate

Table 4. LC_{50} of the compounds against brine shrimps.

Compound no.	**1**	**2**	**3**	**4**	**5**	**6**	VS
LC_{50} (µg/mL)	36.42	36.02	20.72	18.02	08.46	36.12	0.78

VS = Vincristine sulfate

3. Results and Discussion

3.1. *Chemistry*

Ciprofloxacin has, besides other groups, the secondary amino group which can be readily converted to various derived products. To study the antibacterial, antifungal, and

cytotoxicity, the five derived products of ciprofloxacin based on secondary amino group, **2-6** were synthesized and characterized by m.p., TLC, HPLC, FT-IR, ^{1}H-NMR, ^{13}C-NMR, ESI-MS, and elemental analysis.

*Derivative, **2***: Ciprofloxacin was converted to its enamine derivative, **2** with N-methyl-2-pyrrolidone with 69 % yield. The distinct melting point and TLC R_f value give introductory information about the formation of derivative, **2**. It was 98.64 % pure as determined by HPLC. The infrared spectrum showed a new stretching absorption band at 2856 cm^{-1} for C-H of the CH_3 group and absence of N-H stretching band at 3350 cm^{-1} of ciprofloxacin that confirmed with the proposed structure, **4**. Further, the enamine derivative is confirmed by the ^{1}H and ^{13}C-NMR spectra. The ^{1}H-NMR spectrum showed four new bands at δ 3.33 (s, 1H, H-6'), 2.93 (s, 3H, N-CH_3), 2.69 (s, 1H, H-4') and 2.23 (s, 1H, H-5') that confirmed the introduction of an N-methyl pyrrole moiety in the derivative. ^{13}C spectrum is also consistent with this finding, enamine carbon C-2' appeared at δ 163.63, C-6' at δ 73.1, C-4' and N-methyl carbons appeared at δ 50-52. Finally, the ESI-MS showed the (M+ H^+) peak at 416.1848 appropriate for $C_{22}H_{25}FN_4O_3$ and the elemental analysis results (% C, H, and N) gave the satisfactory results which also established molecular formula and structure of the derivative (section 3.1.1).

*Derivative, **3***: Ciprofloxacin was converted to its enamine derivative, **3** using camphor. It was obtained in 68.60 % yield. The melting point and R_f value primarily confirmed the derivative of ciprofloxacin. It was 98.80 % pure as determined by HPLC. The IR spectrum displayed most of the bands like ciprofloxacin excluding one at 3350 cm^{-1} (N-H str.) and showed a new band at 2856 cm^{-1} (C-H str., CH_3) which indicated the methyl group present in the molecule and confirmed 2° amino group of ciprofloxacin had enamine formation with camphor. That ciprofloxacin has reacted with camphor is revealed by the ^{1}H-NMR. The new peak in the aliphatic region was a very broad multiplet δ 1.19-1.54 (H-11, 12, 13, 14 & 14') along with two peaks at δ 2.26 (br s, 1H, H-10) and 5.02 (d, 1H, H-9) for allylic and vinylic protons that confirmed the enamine formation in ciprofloxacin. ^{13}C spectrum is also consistent with this finding, enamine carbon C-2' appeared at δ 158.62, C-3' at δ 104.15, C-4' C-7' & C-8' at δ 50-56, δ 36-25 for C-5', C-6', and δ 8-14 (C-9' C-10' and C-11') that over again confirmed a camphor moiety in the derivative. The ESI-MS showed the (M+ Na^+) peak at 488.5528 for $C_{27}H_{33}NaFN_3O_3$ which was also in good agreement with elemental analysis results (% C, H, N) that finally confirmed the structure of derivative, **3** (section 3.1.2).

*Derivative, **4***: Ciprofloxacin was heated with 1-cyanoguanidine to give derivative, **4** with 71.80 % yield. The yellow color, melting point, and R_f value give initial information about the formation of the derivative. It was 97.81 % pure as determined by HPLC. The IR spectrum of the derivative exhibited similar bands like ciprofloxacin except for three new strong bands at 3559, 3490.33 and 3402.43 cm^{-1} which can be attributed to νN-H of the primary and secondary amine of cyanoguanidino moiety that confirmed the formation of

the derivative. The ^{1}H-NMR spectrum showed some new peaks, e.g. in the aliphatic region 2H broad singlet δ 5.75 due to H-4' 1° amino group moreover; a broad 2H singlet appeared at δ 9.46 due to H-2', H-4' for (=NH) that confirmed for a second time the formation of the derivative. ^{13}C-NMR decoupled spectrum displayed all peaks of ciprofloxacin besides two extra signals at δ 162.54 and 164.53 due to (C-4') and (C-2') respectively that also evident the derivative. As a final point, the derivative showed m/z peak at 416.1978 for (M+H), $C_{19}H_{23}FN_7O_3$ and the elemental analysis results (% C, H, N) agreed with the molecular formula (section 3.1.3).

*Derivative, **5***: Ciprofloxacin was heated with 1-cyanonaphthalen to give derivative, **5** with 73 % yield. The yellow color, melting point, and R_f value give early information regarding the formation of the derivative. HPLC system had purity 98.64 %. The IR spectrum of **5** showed one new strong band at 3402 cm^{-1} which corresponds to ν =N-H of the secondary amine of cyanonaphthaleno–moiety and absence of the νN-H band at 3350 cm^{-1} of ciprofloxacin; other bands are consistent with the proposed structure, **5**. The ^{1}H-NMR spectrum of **5** showed some new peaks, δ 9.49 (s, 1H, H-2'); δ 8.50 (s, 2H, H-6' H-7'); δ 7.72 (d, 1H, H-10'); δ 7.5- 7.26 (m, 5H, H-4' H-5' H-8' H-9' and H-8) that confirmed the formation the derivative. ^{13}C-NMR decoupled spectrum of **5** displayed all peaks of ciprofloxacin; besides, some extra signals at δ 164.53 for (C-2'); and δ 122-133 (10 C, naphthalene) confirmed the derivative. Finally, the derivative showed m/z peak at 485.1845 for (M+H), $C_{28}H_{25}FN_4O_3$ and the elemental analysis results (% C, H, N) were in agreement with the molecular formula (section 3.1.4).

*Derivative, **6***: Ciprofloxacin was methylated with dimethylsulfate in the presence of sodium hydroxide with 73.72 % yield. The melting point and R_f value give opening information about the formation of the derivative, **6**. HPLC system, the purity was 98.80 %. The IR spectrum revealed similar bands like ciprofloxacin except for a new one at 2856 cm^{-1} for νC-H (N-CH_3) which confirmed the formation of the derivative. The conversion of ciprofloxacin to its N-methyl derivative was confirmed by the ^{1}H and ^{13}C-NMR spectra. The methyl protons of the N-CH_3 group appeared as a 3H singlet at δ 2.57 which are not present in ciprofloxacin. The ^{13}C spectrum N-CH_3 appeared at δ 40.69. This derivative showed m/z peak at 346.3868 for $C_{18}H_{21}FN_3O_4$ as (M+H). There is a good agreement with the elemental analysis report (% C, H, and N) with the formula $C_{18}H_{20}FN_3O_4$ (section 3.1.5).

3.1.1. *Finding of derivative, **2***:

The product was obtained as white crystals; yield 1.28 g, 69 %; m.p. 222-223 °C; TLC R_f 0.64; HPLC system had purity 98.64 %; IR (KBr, ν cm^{-1}): 3420 (O-H str.); 3055 (C-H str., aromatic); 2902 (C-H str., CH_2); 2856 (C-H str., CH_3); 1717 (C=O, conjugated COOH); 1631 (C=O str., conjugated quinolone); 1290 (C-N str.); 1238 (C-O str.); 1227 (C-F str.); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 10.92 (s, 1H, H-14, COOH); 8.67 (s, 1H, H-2, aryl H);

7.92 (d, 1H, J_{HF}= 13.2 Hz, H-5, aryl H); 7.58 (d, 1H, J_{HF}= 4.2 Hz, H-8, aryl H); 3.84 (m, 1H, H-11, cyclopropane); 3.43 (s, 4H, H-16, H-20 piperazinyl H); 3.33 (s, 1H, H-6'); 3.17 (s, 4H, H-17, H-19, piperazinyl H); 2.93 (s, 3H, H-3', N-CH_3); 2.69 (s, 1H, H-4'); 2.23 (s, 1H, H-5'); 1.31(m, 2H, H-12, cyclopropane); 1.18 (m, 2H, H-13, cyclopropane); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 182.11 (C-4, C=0 quinolone); 161.63 (C-2'); 166.64 (C-14, COOH); 154.32 (C-6); 148.82 (C-2); 146.10 (C-7); 139.9 (C-10); 111.73 (C-9); 107.53 (C-5); 107.3 (C-3); 99.15 (C-8); 73.1 (C-6'); 50-52 (2C, C-4' and N-methyl); 36-39 (4C, C piperazin); 27.6 (C-5'); 8-14 (3C, C cyclopropane); *Anal*. calcd. for $C_{22}H_{25}FN_4O_3$: C, 64.06; H, 6.11; N, 13.58 %; found: C, 63.56; H, 6.21; N, 12.66 %; ESI-MS m/z calcd. for $C_{22}H_{25}FN_4O_3$+ (H^+) : 412.1946; found: 416.1848.

3.1.2. *Finding of derivative,* ***3***:

The product was obtained as white crystals; yield 1.44 g, 68.60 %; m.p. 260-261 °C; TLC R_f 0.65; HPLC system purity 98.80 %; IR(cm^{-1}): 3454 (O-H str.); 3101, 3057(C-H str., aromatic, C-H str.,); 2954(C-H str., CH_2); 2859(C-H str., CH_3); 1718(C=O str., COOH); 1632(C=O str., qunilone); 1301(C-N str.); 1258(C-O str.); 1231(C-F str.); ^{1}H-NMR (DMSO-d_6,400 MHz): δ 10.80 (s, 1H, H-14, COOH); 8.64 (s, 1H, H-2); 7.85(d, 1H, H-5); 7.52(d, 1H, H-8); 5.02 (d, 1H, H-3'); 3.82 (m, 1H, H-11); 3.22 (t, 4H, H-16, H-20); 2.88 (t, 4H, H-17, H-19); 1.19 -1.54 (br m, 14H, H-4', H-12', H-5', H-6', H-9' H-10' & H-11'); 1.14 (m, 2H, H-12, H-13); 1.09 (m, 2H, H-12, H-13); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 182.81 (C-4, C=0 quinolone); 166.04 (C-14, COOH); 158.62 (C-2'); 152.82 (C-6); 148.22 (C-2); 146.55 (C-7); 137.92 (C-10); 113.26 (C-9); 113.53 (C-5); 107.34 (C-3); 104.15 (C-3'); 100.15 (C-8); 50-56 (7C, C-16, C-17, C-19, C-20, C-4' C-7' and C-8'); 36-25 (3C, C-11 piperazin, C-5', C-6'); 8-14 (5C, C-12, C-13 cyclopropane, C-9', C-10' and C-11'); *Anal.* calcd. for $C_{27}H_{32}FN_3O_3$: C, 69.66; H, 6.93; N, 9.03 %; found: C, 69.54; H, 6.80; N, 9.22 %; ESI-MS m/z calcd. for $C_{27}H_{32}FN_3O_3$+ (Na+): 488.2325; found: 488.3528.

3.1.3. *Finding of derivative,* ***4***:

The product was obtained as yellow crystals; yield 0.90 g, 71.80 %; m.p. 235-236 °C; TLC R_f 0.53; and HPLC system purity 97.81 %; IR (cm^{-1}): 3559, 3490 and 3402 (N-H str., 1^0 and 2^0 amine); 3093 (C-H str., aromatic); 2917 (C-H str. CH_2); 1720 (C=O str., COOH, 1627 (C=O str., conjugated quinolone); 1285 (C-N str.); 1241(C-O str.); 1228 (C-F str.); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 10.78 (s, 1H, H-14, COOH); 9.46 (br s, 2H, H-2', H-4'); 8.65 (s, 1H, H-2, aryl H); 7.92 (d, 1H, J_{HF}= 13.2 Hz, H-5, aryl H); 7.58 (d, 1H, J_{HF}= 4.2 Hz, H-8, aryl H); 5.75 (br s, 2H, H-4' 1° amine); 3.84 (m, 1H, H-11, cyclopropane); 3.43 (s, 4H, H-16, H-20 piperazinyl H); 3.17 (s, 4H, H-17, H-19, piperazinyl H); 1.31(m, 2H, H-12, cyclopropane); 1.10 (m, 2H, H-13, cyclopropane); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 188.11 (C-4, C=0 quinolone); 167.10 (C-14, COOH); 164.53 (C-4'); 162.54 (C-2'); 154.32 (C-6); 148.82 (C-2); 146.10 (C-7); 139.9 (C-10); 111.73 (C-9); 107.53 (C-5); 107.3 (C-3); 99.15 (C-8); 36-39 (4C, C piperazin); 8-14 (3C, C cyclopropane); *Anal*.

calcd. for $C_{19}H_{22}FN_7O_3$: C, 54.93; H, 5.34; N, 23.60 %; found: C, 54.03; H, 5.84; N, 23.31 %; ESI-MS m/z calcd. for $C_{19}H_{22}FN_7O_3$+ (H^+) : 416.1846; found: 416.1978.

3.1.4. *Finding of derivative,* ***5***:

The product was obtained as yellow crystals; yield 1.06 g, 73 %; m.p. 285-286 °C; TLC R_f 0.57; HPLC system purity 98.84 %; IR (cm^{-1}): 3402 (=N-H str.); 3093 (C-H str., aromatic); 2917 (C-H str. CH_2); 1628 (C=O str., conjugated quinolone); 1287 (C-N str.); 1244 (C-O str.); 1233 (C-F str.); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 10.92 (s, 1H, H-14, COOH); 9.49 (s, 1H, H-2'); 8.50 (s, 2H, H-6' H-7'); 8.68 (s, 1H, H-2, aryl H); 7.98 (d, 1H, J_{HF}= 13.2 Hz, H-5, aryl H); 7.72 (d, 1H, H-10'); 7.5-7.26 (m, 5H, H-4' H-5' H-8' H-9' and H-8); 3.84 (m, 1H, H-11, cyclopropane); 3.43 (s, 4H, H-16, H-20 piperazinyl H); 3.17 (s, 4H, H-17, H-19, piperazinyl H); 1.31 (m, 2H, H-12, cyclopropane); 1.10 (m, 2H, H-13, cyclopropane); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 186.11 (C-4, C=0 quinolone); 167.10 (C-14, COOH); 164.53 (C-2'); 154.32 (C-6); 148.82 (C-2); 146.10 (C-7); 139.9 (C-10);122-133 (10C, naphthalene); 111.73 (C-9); 107.53 (C-5); 107.3 (C-3); 99.15 (C-8); 36-39 (4C, C piperazin); 8-14 (3C, C cyclopropane); Anal. calcd. for $C_{28}H_{25}FN_4O_3$: C, 69.41; H, 5.20; N, 11.56 %: found: C, 69.40; H, 5.80; N, 11.22 %; ESI-MS m/z calcd. for $C_{28}H_{25}FN_4O_3$+ (H+): 485.1845; found: 485.1908.

3.1.5. *Finding of derivative,* ***6***:

The derivative, **6** was obtained as white crystals; yield 1.15 g, 73.72 %; m.p. 288 °C; TLC R_f 0.64; HPLC purity 98.15 %; IR (cm^{-1}): 3412 (O-H str., H-bonded); 3055(C-H str., aromatic); 2926 (C-H str., aliphatic); 2856 (C-H str., CH_3); 1720 (C=O, COOH); 1629 (C=O str., conjugated quinolinone); 1429 (C-N str.); 1367 (C-O str); 1306 (C-F str.); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 10.96 (s, 1H, H-14, COOH); 8.64 (s, 1H, H-2); 7.86 (d, 1H, H-5); 7.52 (d, 1H, H-8); 3.82 (s, 1H, H-11); 3.32 (s, 4H, H-16, H-20 piperazinyl H); 2.59 (s, 4H, H-17, H-19, piperazinyl H); 2.57 (s, 3H, H-2', N- methyl); 1.29 (m, 2H, H-12, cyclopropane); 1.17 (m, 2H, H-13, cyclopropane); ^{13}C-NMR (DMSO-d_6,100 MHz): δ 187.11 (C-4, quinolinone C=O); 167.64 (C-9, COOH); 154.32 (C-2); 148.82 (C-6); 146.10 (C-10); 139.90 (C-7); 131.04 (C-5); 111.73 (C-9); 107.53 (C-3); 96.34 (C-8); 57.84 (2C, C-17, C-19); 54.56 (2C, C-16, C-20); 40.69 (C-2'); 36.58 (C-11); 8.41(C-12, C-13); *Anal.* calcd. for $C_{18}H_{20}FN_3O_3$: C, 62.60; H, 5.84; N, 12.17 %; found, C, 62.74; H, 6.04; N, 12.24 %; ESI-MS m/z calcd. for $C_{18}H_{20}FN_3O_3$+ (H^+): 346.1556; found: 346.1668.

3.2. *Antibacterial activity*

Zones of inhibition for Gram-positive bacteria (Table 1) indicate that the derivatives, **2-6** showed various degrees of activities compared to ciprofloxacin against the Gram-positive bacterial strains. The derivatives **2** (23.12±0.01 mm), **3** (24.80±0.04 mm), and **4** (23.12±0.01 mm) showed significantly enhanced activity compared to ciprofloxacin (18.02±0.02 mm) against *Staphylococcus aureus* whereas derivative **6 (**18.10±0.02 mm)

exhibited similar activity but **5** (14.52±0.02 mm) and **6** (13.10±0.05 mm) exhibited less activity compared to ciprofloxacin. The derivative, **2** (17.30±0.03 mm) and **3** (15.94±0.02 mm) showed significantly enhanced activities compared to ciprofloxacin (14.10±0.01 mm) but compounds **4** (14.06±0.02 mm), **5** (9.02±0.02 mm**),** and **6** (13.94±0.11 mm**)** exhibited less activity than parent against *Streptococci.* The compounds **2** (16.84±0.02 mm), and **3** (18.02±0.01 mm) exhibited enhanced activity but derivatives **4, 5** and **6** were found to be similar or poor in activity against *Bacillus* spp compared to ciprofloxacin (16.30±0.02 mm).

Zones of inhibition (Table 2) signify that the derivatives exhibited different activities compared to ciprofloxacin against the Gram-negative bacterial strains. The compounds **2** (14.20 ±0.05 mm) and **3** (18.24±0.08 mm) were found to show enhanced activity but the derivative **6** (8.82±0.04 mm) exhibited poor activity and rest of derivatives **4** and **5** were found to possess no activity compared to ciprofloxacin (12.22±0.04 mm) against *E. coli*. Among the derivatives, only compound **3** (26.28±0.04 mm) exhibited enhanced activity compared to ciprofloxacin (24.24±0.08 mm) but derivatives **2** (15.44±0.04 mm), **4** (13.32±0.06 mm), **5** (12.52±0.04 mm) and **6** (13.28±0.04 mm) showed poor activities compared to ciprofloxacin against *Klebsiella pneumoniae*.

The derivatives **2** (24.20±0.04 mm) and **3** (27.82±0.03 mm) showed enhanced activities compared to ciprofloxacin (21.68 ±0.01 mm) but compounds **4** (14.20±0.04 mm)**, 5** (12.42±0.12 mm) and **6** (21.60±0.08 mm) are found to be similar or less active compared to ciprofloxacin against *V. cholerae*. The derivatives, **2** (26.48±0.04 mm) and **3** (27.38±0.04 mm) showed enhanced activities compared to ciprofloxacin (26.20±0.01 mm) but the compounds **4** (19.82±0.03 mm), **5** (20.24±0.02 mm)**,** and **6** (21.32±0.01 mm) are found to be less active compared to ciprofloxacin against *Salmonella* spp. Only the compound **3** (28.36±0.06 mm) possessed enhanced activity compared to ciprofloxacin (26.24±0.08 mm) but derivatives **2**, **4**, **5,** and **6** showed less activity compared to ciprofloxacin against *Shigella dysenteriae.*

3.3. *Antifungal activity*

Zones of inhibition for the fungi (Table 3) indicate that the derivatives, **2** (14.20±0.04 mm), **3** (15.04 ±0.02 mm), **4** (12.64 ±0.04 mm), **5** (12.04±0.02 mm), and **6** (9.40±0.01 mm) exhibited effective activities compared to ciprofloxacin (9.92 ±0.02 mm) against *Candida albicans* but less than that of miconazole nitrate (34.00±0.02 mm). Ciprofloxacin and its derivatives **2-6** exhibited poor activity against *Fusarium solani* and *Aspergillus fumigatus* compared to miconazole nitrate; however, among the derivatives compound, **3** is found to be most potent.

3.4. *Cytotoxicity*

Ciprofloxacin and its analogues **2-6** demonstrated a varying degree of cytotoxic activities (Table 4). Most of the derivatives are found to have slightly more cytotoxic activities compared to ciprofloxacin. Among the compounds the lowest LC_{50} is shown by

derivatives **3** (20.72 µg/mL), **4** (18.20 µg/mL) and **5** (8.46 µg/mL), confirmed the most potent cytotoxic agent compared to ciprofloxacin (36.42 µg/mL) but less than vincristine sulfate (0.78 µg/mL). However, amongst the derivatives analogue, **5** is found to be the most potent.

5. Conclusion

In this paper, five analogues of ciprofloxacin have been successfully synthesized. The structure of the analogues was confirmed by different techniques i.e. IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry together with elemental analysis. The structural analogues of ciprofloxacin, **1** showed varying degree of antibacterial activity against the tested bacterial strains. Zones of inhibition of bacterial strains implied that derivative, **2** exhibited enhanced activities against *Staphylococcus aureus, Streptococci, Bacillus* spp, *E. coli, V. cholerae,* and *Salmonella* spp compared to ciprofloxacin. Derivative **3** exhibited enhanced activity against all of the Gram-positive and Gram-negative tested bacterial strains; Compound, **4** showed enhanced activities against *Staphylococcus aureus, Streptococci*, *Bacillus spp*; **5** showed poor activity against all of the Gram-positive and Gram-negative tested bacterial strains; **6** exhibited more or less similar activity against tested bacterial strains compared to the parent. On the other hand, most of the derivatives possessed valuable antifungal properties against *Candida albicans* but poor activity against *Fusarium solani* and *Aspergillus fumigatus* whereas parent, **1** did not demonstrate any activity. Most of the derivatives showed cytotoxic activity where derivatives **4** and **5** were found to be the most potent cytotoxic agent compared to ciprofloxacin. The comparison of the activities of different analogues of ciprofloxacin indicated that the amidic linkage of an alkyl group at piperazine moiety may be responsible for the change in the biological properties of the parent.

References

1. G. C. Crumplin and J. T. Smith, Nature **260**, 643 (1976). https://doi.org/10.1038/260643a0
2. J. C. Wang, Annu. Rev. Biochem. **54**, 665 (1985). https://doi.org/10.1146/annurev.bi.54.070185.003313
3. M. Gellert, K. Mizuuchi, and M. H. O'Dea, Proc. Natl. Acad. Sci. USA **74**, 4772 (1977). https://dx.doi.org/10.1073%2Fpnas.74.11.4772
4. S. H. Choi, Y. E. Kim, and Y. J. Kim, Kor. J. Pediatr. **56**, 196 (2013). https://doi.org/10.3345/kjp.2013.56.5.196
5. K. J. Aldred, R. J. Kerns, and N. Osheroff, Biochemistry **53**, 1565 (2014). https://doi.org/10.1021/bi5000564
6. M. M. Masadeh, K. H. Alzoubi, S. I. Al-Azzam, O. F. Khabour, and A. M. Al-Buhairan, Pathogens **5**, 28 (2016). https://doi.org/10.3390/pathogens5010028
7. S. Correia, P. Poeta, M. Hebraud, J. L. Capelo, and G. Igrejas, J. Med. Microbiol. **66**, 551 (2017). https://doi.org/10.1099/jmm.0.000475
8. D. C. Hooper and J. S. Wolfson, N. Engl. J. Med. **324**, 384 (1991). https://doi.org/10.1056/NEJM199102073240606
9. M. L. Bennish, M. A. Salam, W. A. Khan, and A. M. Khan, Ann. Intern. Med. **117**, 727 (1992). https://doi.org/10.7326/0003-4819-117-9-727

10. H. A. Ludlam, I. Barton, L. White, C. Mc Mullin, A. King, and I. Phillips, J. Antimicrob. Chemother. **25**, 843 (1990). https://doi.org/10.1093/jac/25.5.843
11. L. R. Peterson, L. M. Lissack, K. Canter, C. E. Fasching, C. Clabots, and D. N. Gerding, Am. J. Med. **86**, 801 (1989). https://doi.org/10.1016/0002-9343(89)90476-2
12. C. Jean-Didier, R. Francoise, and G. Monique, J. Antimicrob. Chemother. **46**, 2306 (2002).
13. Y. Yamashita, T. Ashizawa, M. Morimoto, J. Hosomi, and H. Nakano, Cancer Res. **52**, 2818 (1992).
14. T. D. Goots and K. E. Brighty, Med. Res. Rev. **16**, 433 (1996). https://doi.org/10.1002/(SICI)1098-1128(199609)16:5<433::AID-MED3>3.0.CO;2-W
15. N. J. de Souza, S. V. Gupte, P. K. Deshpande, V. N. Desai, S. B. Bhawsar, R. D. Yeole, M. C. Shukla, J. Strahilevitz, D. C. Hooper, B. Bozdogan, P. C. Appelbaum, M. R. Jacobs, N. Shetty, M. V. Patel, R. Jha, and H. F. Khorakiwala, J. Med. Chem. **48**, 5232 (2005). https://doi.org/10.1021/jm050035f
16. M. G. Rabbani, M. R. Islam, and M. Ahmad, J. Bangl. Chem. Soc. **26**, 30 (2013).
17. R. Siddiqui, N. Sultana, K. M. Khan, N. Akbar, M. Ali, and S. Arayne, J. Chin. Clin. Med. **2**, 188 (2007).
18. S. Jubie, P. Sikdar, R. Kalirajan, B. Gowramma, S. Gomathy, S. Sankar, and K. Elango, J. Pharma. Res. **3**, 511 (2010).
19. M. G. Rabbani, M. R. Islam, M. Ahmad, and A. M. L. Hossion, Bangl. J. Pharmacol. **6**, 8 (2011). https://doi.org/10.3329/bjp.v6i1.7720
20. H. Ahmad, T. K. Pal, M. A. Alam, J. Hossen, S. Paul, and M. C. Sheikh, J. Sci. Res. **10**, 291 (2018). https://doi.org/10.3329/jsr.v10i3.36379
21. A. Cipurković, E. Horozić, N. Ljubijankić, A. Odobašić, S. Galijašević, and M. Saletović, Rasayan J. Chem. **10**, 1381 (2017).
22. N. Ljubijankić, M. Stanković, V. Tešević, S. Grgurić-Šipka, M. Jadranin, S. Begić, and E. Šabanović, Rasayan J. Chem. **11**, 647 (2018). https://doi.org/10.31788/RJC.2018.1123004
23. A. Scozzafava, L. Menabuoni, and F. Mincione, Bioorg. Medic. Chem. Lett. **11**, 575 (2001). https://doi.org/10.1016/S0960-894X(00)00722-8
24. A. Scozzafana and C. T. Supuran, J. Med. Chem. **43**, 3677 (2000). https://doi.org/10.1021/jm000027t
25. D. J. Austin, K. G. Kristinsson, and R. M. Anderson, Proc. Natl. Acad. Sci. USA **96**, 1152 (1999). https://doi.org/10.1073/pnas.96.3.1152
26. M. G. Rabbani and M. R. Islam, J. Sci. Res. **11**, 351 (2019). http://dx.doi.org/10.3329/jsr.v11i3.38843
27. A. W. Bauer, W. M. M. Kirby, J. C. Sheriss, and M. Turck, Am. J. Clin. Path. **45**, 493 (1966). https://doi.org/10.1093/ajcp/45.4_ts.493
28 M. H. Islam, M. C. Sheikh, and M. A. A. A. A. Islam, J. Sci. Res. **11**, 121 (2019). http://dx.doi.org/10.3329/jsr.v11i1.37863
29. B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols, and J. L. McLaughin, Planta Medica. **45**, 31 (1982). https://doi.org/10.1055/s-2007-971236
30. A. K. Azad, M. A. Jainul and Z. K. Labu, J. Sci. Res. **10**, 175 (2018). https://doi.org/10.3329/jsr.v10i2.34820