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Synthesis, Characterization, Anti-Microbiological and Methicillin-Resistance *Staphylococeus Aureus,*Evaluation of N-Acyl Ciprofloxacin Derivatives

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Abstract: *We report here a novel amide-piperazine based multiple ligand approach ciprofloxacin derivatives were synthesized and fully characterized by HR-MS, ^{1}H-NMR, ^{13}C-NMR, and FT-IR. All the synthesized compounds were exhibited high antibacterial activity tested against drug-sensitive bacteria Gram positive Staphylococcus Aureus and Bacilus Subtilis and Gram negative bacteria Escherichia Coli and Pseudomonas Aeruginosa. We found that all the compounds are promising candidates as antibacterial agents, along with compound* ***5c****amide-piperazine based ciprofloxacin derivative demonstrated outstanding antibacterial activity against MRSA in the in vitro antibacterial studies. The results of the studies show the synthesized* ***5c*** *derivative can be used for the development of anti-MRSA drugs.*

Keywords: Amide, Piperazine, Ciprofloxacin, Antibacterial, Antifungal and MRSA

I. INTRODUCTION

Quinolones, the group of synthetic antibacterials are generally used in the treatment of many infections. The addition of fluorine atom at position of 7, to the original quinoline compound yielded fluoroquinolines, a new class of antibiotic drug (1).

The second generation antibiotics now called fluoroquinolines are very useful antimicrobial agents that shown activity against a wide range of Gram positive and Gram negative bacteria. They have also proved against the microorganisms which are resistant to other antibacterial agents (2).

Fluoroquinolones have important role in the treatment of many bacterial infections (3). Fluoroquinolones constituted a very important advancement in the management of infectious diseases (3). Ciprofloxacin with advanced systematic activity is the first widely used quinoline marketed in 1987. Ciprofloxacin is found to be an important antibacterial agents. Ciprofloxacin 1-cyclo-propyl-6-fiuoro-1,4-dihydro-4-oxo-7-(1-piperzinyl)-3-quinoline carboxylic acid ($C_{17}H_{15}FN_3O_3$) that belongs to second generation of fluoroquinolines has a broad spectrum antibiotics(3).

Hence ciprofloxacin is highly active against various microorganisms.Extended antimicrobial activity and minimum adverse effects; we have reported here bioactive N-acyl ciprofloxacin derivatives.

II. EXPERIMENTAL WORK

2.1 Materials and Equipments

Ciprofloxacin and all the reagents were of analytical grade. All the glasswares were washed with chromic acid followed by a through washing with deionized water. Melting points were maintained by open capillary method. The IR spectra were obtained on a Perkin Elmer FTIR spectrometer. The absorption peaks were recorded in frequency(cm^{-1}).NMR were recorded on Bruker 400 MHz. NMR spectrometer with the compounds dissolved in DMSO. Chemical shifts are reported in parts per million(s) relative totetramethyl silane as an internal standard.Significant^{1}H NMR data tabulated in the following order: (S,singlet; d,doublet; t,triplet; q,quartet; and m,multiplet and number of protons). The mass spectra were recorded. Reactions were monitored by TLC and compounds visualized under UV lamp.

2.2 General Procedure for Preparation of N-Acyl Ciprofloxacin Derivatives:

Synthesis of various derivatives of ciprofloxacin was attempted with various carboxylic acids i.e. succinic acid, 4-nitro benzene-1,2-dicarboxylic acid and (R)-3-((tert-butoxy carbonyl)amino)-4-(2,4,5-trifluorophenyl) butanoic acid.

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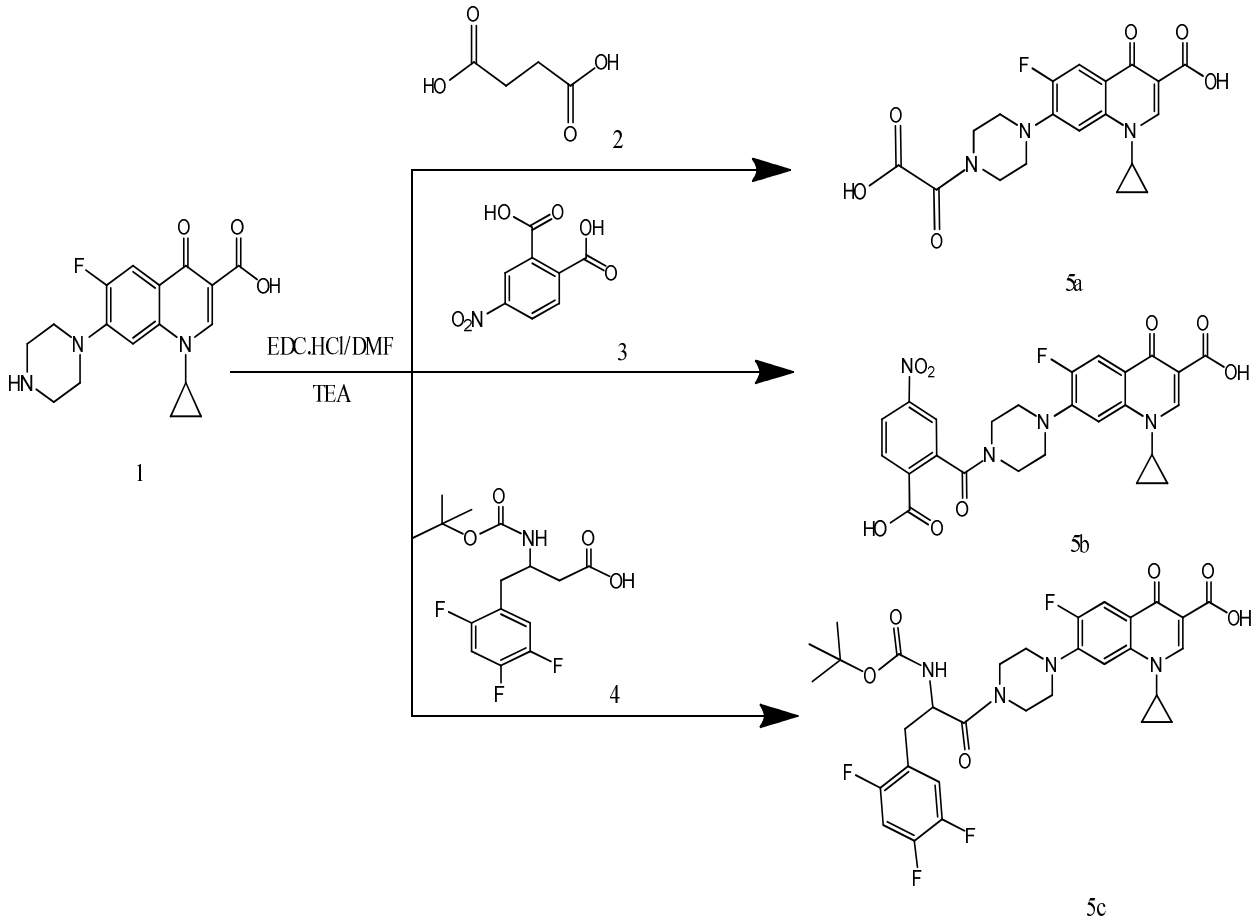
Ciprofloxacin (2.0 g, 0.006 mol), triethyl amine (0.84 mL, 0.006 mol) and EDC.HCl (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.15 g, 0.006 mol) and corresponding acid (0.006 mol) was refluxed at 10-15^{0}C for 3-4 hours. Thereafter temperature was raised to 45-60^{0}C. The progress of reaction was monitored using TLC and completion of reaction, in reaction mixture 40 ml ice cold water added. The reaction mixture was kept for 1 hour with continuous stirring. Then it was washed thoroughly with water and filtered. Obtain the crude product was recrystallized with suitable solvents and purified it.



Reaction Scheme: Synthesis of N-Acyl Ciprofloxacin

Spectral Data:

5a.7-(4-(3-carboxypropanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquuinoline-3-carboxylic acid

Yield: 76.85%,

M.P.: 179^{0}C.

IR (KBr): 3224 cm^{-1}(O-H), 3340 cm^{-1}(O-H), 3417 cm^{-1}(N-H),1608cm^{-1} (C=O)

^{1}H NMR (400 M Hz): δ 1.33 (m,4H, (-CH_2cyclopropane) 2.43, 2.6 (2H,t, -CH_2), 3.57 (m, 8H, piperazinyl H), 7.57, 7.93(dd, 2H, benzene) 8.66,(s, 1H, methylene), 11 (s,1H, carboxylic acid)

^{13}C NMR: δ 7.2 (2C, s), 32.2 (1C, s), 32.3 (1C, s), 32.9 (1C, s), 34.4 (1C, s), 46.5 (2C, s), 49.1 (2C, s), 94.4 (1C, s), 115.0-115.1 (2C, 115.0 (s), 115.1 (s)), 127.3 (1C, s), 132.7 (1C, s), 139.1 (1C, s), 141.5 (1C, s), 152.3 (1C, s), 168.1 (1C, s), 169.1 (1C, s), 177.8 (1C, s).

m/z: (obs) 431.80 and (cal) 431.41

5b.7-(4-(2-carboxy-5-nitrobenzoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

Yield: 78.8%

M.P.:118^{0}C.

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IR (KBr): 1539 cm^{-1}(NO_2), broad 3377 cm^{-1}(O-H), 1653 cm^{-1}(C=O)
^{1}H NMR: δ1.33 (m, 4H, CH_2, cyclopropane), 3.05 (m, 8H, piperazinyl H), 7.85, 7.95 (t, 2H benzene), 7.57 (d, 1H benzene), 8.63 (s, 1H, ethylene)
^{13}C NMR: δ 7.2 (2C, s), 34.4 (1C, s), 46.5 (2C, s), 49.1 (2C, s), 106.2 (1C, s), 107.6 (1C, s), 115.0 (1C, s), 124.0 (1C, s), 126.9 (1C, s), 127.7 (1C, s), 128.3-128.5 (2C, 128.4 (s), 128.4 (s)), 129.3 (1C, s), 130.2 (1C, s), 132.7 (1C, s), 139.1 (1C, s), 141.5 (1C, s), 152.3 (1C, s), 164.9 (1C, s), 165.9 (1C, s), 167.5 (1C, s), 174.0 (1C, s)..
m/z: (obs) 524.5and (cal) 524.45
5c.7-(4-(2-((tert-butoxycarbonyl)amino)-3-(2,4,5-trifluorophenyl)propanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
Yield: 81.55 %,
M.P.:134^{0}C.
IR (KBr): 3429 cm^{-1}(N-H), 2976 cm^{-1}(-CH), 1716 cm^{-1}(C=O), 1626cm^{-1}1442 cm^{-1}(-CH_3)
^{1}H NMR: δ 1.1-1.17 (m, 4H, CH_2cyclopropane), 1.4 (s, 9H, - CH_3) 3.63 – 3.8 (m, 8H, piperazinyl H), 4.1 (t, 1H, - CH), 7.26- 7.54 (m, 4H, benzene), 8.6 (s, 1H, - NH)
^{13}C NMR: δ 7.2-7.3 (2C, 7.2 (s), 7.2 (s)), 28.2 (3C, s), 34.4 (1C, s), 37.2 (1C, s), 43.2 (1C, s), 49.0 (1C, s), 49.1 (2C, s), 49.2 (1C, s), 53.5 (2C, s), 80.4 (1C, s), 106.2 (1C, s), 107.4 (1C, s), 107.6 (1C, s), 115.0-115.1 (2C, 115.0 (s), 115.1 (s)), 121.3 (1C, s), 124.0 (1C, s), 132.7 (1C, s), 139.1 (1C, s), 141.5 (1C, s), 149.3 (1C, s), 152.3 (1C, s), 153.9 (1C, s), 155.4 (1C, s), 160.4 (1C, s), 165.9 (1C, s), 174.0 (1C, s), 204.0 (1C,
m/z: (obs) 646.35 and (cal) 645.65

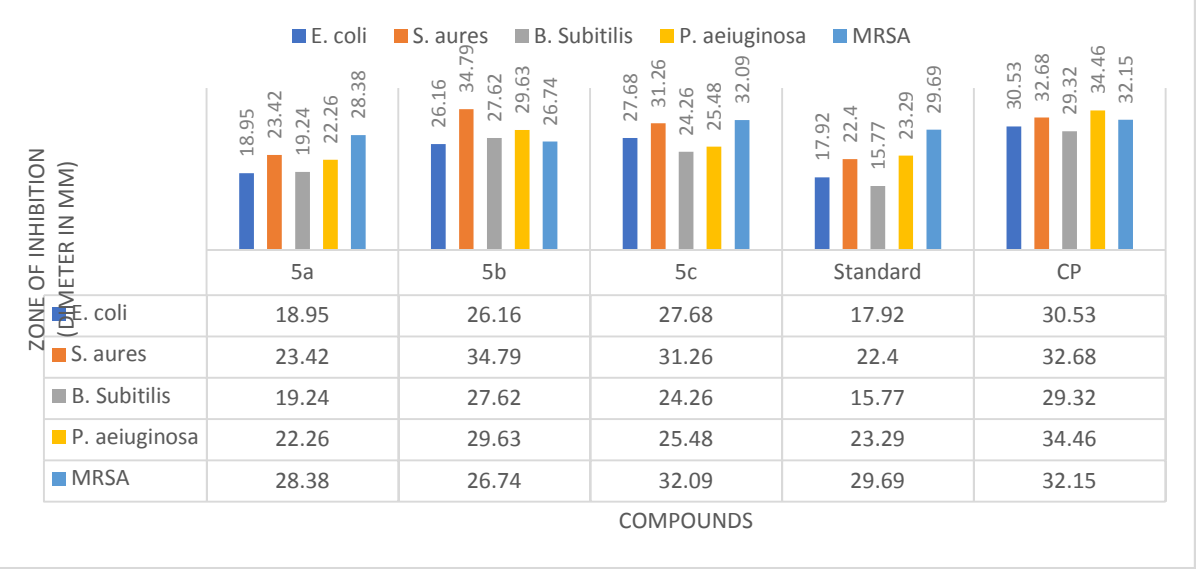
2.3 Anti Microbial Assay

The antimicrobial susceptibility of all the derivatives was tested by the disc diffusion technique developed by Bauer et. al.(4). For this purpose 1000 μgmL^{-1} stock solution of ciprofloxacin and its derivatives were prepared.

Table 1: Zone of Inhibition of Ciprofloxacin and its Derivatives

Sr. No.	Compound	*E. Coli*	*S. Aureus*	*B. Subtillis*	*P. Aeruginosa*	*A. Niger*	*C. Albicans*	*A. Flavus*	MRSA
1	**5a**	**18.95**	**23.42**	**19.24**	22.26	-	-	-	28.38
2	**5b**	**26.16**	**34.79**	**27.62**	**29.63**	-	-	-	26.74
3	**5c**	**27.68**	**31.26**	**24.26**	**25.48**	-	-	-	**32.09**
Standard	Chloramphenicol	17.92	22.40	15.77	23.29	-	-	-	29.69
Standard	Amphotericin –B	NA	NA	NA	NA	13.65	17.76	19.24	NA
Cp	Ciprofloxacin	**30.53**	**32.68**	**29.32**	**34.46**	6.31	-	6.39	32.15

Figure 1: Antimicrobial Assay of N-Acyl Ciprofloxacin Derivatives



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Antibacterial Activity(11)

For the antibacterial assay, 1000 μgmL^{-1} stock solution of ciprofloxacin and its derivatives were prepared in distilled water. The assay was carried out by taking concentration 100 microgram per disk. Commercially available filter paper discs were soaked in the prepared drug and derivatives 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 ^{0}C for 24 hours. This method is based on the determination of an inhibited zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The results were compared with the parent against 04 different strains of Gram positive and Gram negative organism (*Staphylococcus Aureus, Bacillus Subtilis, Escherichia Coli* and *Pseudomonas Aeruginosa*).

Antifungal Assay(12)

For the antifungal assay, 1000 μgmL^{-1} stock solution of ciprofloxacin and its derivatives were prepared in distilled water. The assay was carried out by taking concentration 100 microgram per disk. Commercially available filter paper discs were impregnated with the prepared solutions of the drugs and its analogues, dried and applied on the surface of the agar (Potato Dextrose Agar) plate over which a culture of micro organism was already streaked. After 24 hours at 37^{0}C of incubation the clear zone of inhibition around the disc was determined, this is proportional to the fungal susceptibility for the antimicrobial agent present in the disk. Ciprofloxacin and its derivatives were tested for antifungal activity against the fungi; *Aspergillus Niger, Aspergillus Flavus* and *Candida Albicans*.

Anti-Methicillin Resistant Staphylococcus Aureus (MRSA) activity(13)

The synthesized compounds having target specific activity against multi drug resistance gram positive bacteria Methicillin Resistant Staphylococcus Aureus (MRSA) ATCC43300. Method used for activity is Agar diffusion assay (Disc diffusion method, Disc size 6 mm).

Concentration of Compounds:

Stock solution [1000 microgram per ml] of each compound was prepared in DMSO. Assay carried out by taking concentration 100 microgram per disk. Hi-media antibiotics disk: Methicillin (5 microgram/disk) moistened with water are used as standard.

Media used:

Microbiological media used for bacteria [Staphylococcus aureus (MRSA)] is Nutrient agar (Hi-media) Composition (gL-1); Sodium chloride, 5.0, Beef extract 10.0; Peptone 10.0 (pH 7.2)

For this, pure culture of Staphylococcus aureus (ATCC 4330) was picked with a bop, and the growth was transferred into a tube containing 5 ml of a nutrient broth medium having composition (gl-1)- sodium chloride, 5.0; beef extract 10.0; peptone 10.0 (pH 7.2). The broth culture was incubated at 37°C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (usually to 6 hours). The turbidity of the actively growing broth culture is adjusted with sterile saline or both to obtain turbidity optically comparable to that of the 0.5 McFarland standards. This result in a suspension contains 2 x 108 CFU/ml of bacterial cells. Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The suspension was spread on the surface of a nutrient agar plate by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking several times, rotating the plate approximately 60°C each time to ensure an even distribution of inoculum. Stock solutions [1000 microgram per ml] of each newly synthesized compound were prepared in DMF. The sterile discs of 6 mm diameter were used in this assay. The disc diffusion assay was carried out by taking concentration 100microorganism per disc. The discs immersed with compounds were dispensed onto the surface of the inoculated agar plate. Also, Methicillin (5 microgram/disk) [Hi-media, Mumbai, disc diameter 6 mm] moistened with DMSO/DMF were placed on agar plate as standard. Each disc was pressed down to ensure complete contact with the agar surface. The plates were placed in a refrigerator at to 8°C for 30 minutes after the discs are applied. Then the plates were incubated in incubator at 37°C for 24 hours. After 24 hours of incubation, each plate was examined. The diameters of the zones of as shown in Figure, were measured using Vernier calliper, the results are summarized in Table 1.

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III. RESULT AND DISCUSSION

The antibacterial activity of Ciprofloxacin derivatives against drug-sensitive bacteria, Gram positive *Staphylococeus Aureus, Bacilus Subtillis* and Gram negative bacteria *Escherichia Coli* and *Pseudomonas Aeruginosa* are summarised in Table 1.

All analogues, we aimed to synthesize showed comparable activity towards ciprofloxacinand Standard Chloramphenicol against all the tested strains. Result indicates that compound **5b** and **5c** were active against *S. Aureus*, *B. Subtillis, E. Coli* and *P. Aeruginosa*. Compound **5a** active against *S. Aureus, B. Subtillis,* and *E. Coli* show stronger activity. All synthesized compounds possess stronger potency against *S. Aureus* among that compound found more potency than their parent compound Ciprofloxacin.Compound **5d** is active against *S. Aureus, B. Subtillis, E. Coli* and *P. Aeruginosa* and show stronger activity.

The ciprofloxacin is antimicrobial drug and inactive against fungi.But in order to evaluate the result of addition of different functional groups to the structure, the antifungal activity of its derivatives was carried out against *A. Niger, C. Albicans* and *A. Flavus*. All the compounds show no activity against any fungi.

Methicillin Resistant Staphylococcus Aureus (MRSA) is the most common resistant microbe that is primarily associated with healthcare related infection; infection and antibiotic resistance of MRSA are increasing worldwide (5,6). The severity of the anti microbial drug resistant of MRSA requires the development of new antibiotics against it and other pathogens. To overcome this resistance problem, the identification of anti microbial compounds that have a novel mechanism of action towards new target enzymes, such as those involved in bacterial fatty acid biosynthesis, is critical.

We found here all the synthesize compounds **5a, 5b** and **5c** has target specific activity against multi drug resistance gram positive bacteria Methicillin Resistant Staphylococcus Aureus (MRSA) ATCC 43300. Method used for activity is Agar Diffusion Assay (Disc diffusion method, Disc size 6 mm).

IV. CONCLUSION

Conclusively, we have described a convenient synthesis of N-acyl derivatives of ciprofloxacin. Development of bacterial resistance has led to the synthesis ofnewer and more potent quinolones. As detailed above, three analogues have been synthesized, characterized and evaluated for their biological activities in vitro in order to discover potent agents against Gram-positive bacteria and Gram negative bacteria. It was observed that, significant enhancements of potency against organisms were achieved from the ciprofloxacin nucleus. All the synthesize compounds posses potent activity but compound **5c** having more potency target specific activity against multi drug resistance gram positive bacteria Methicillin Resistant Staphylococcus Aureus (MRSA) ATCC 43300.

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