Computational Designing, Synthesis & In-vitro antibacterial screening of Ciprofloxacin derivatives for development of new broad spectrum Antibacterial Agents

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Abstract

The present work contains synthesis of five Mannich base analogues of ciprofloxacin NS1-NS5**.**Which has been prepared by the Mannich reaction to investigate their antibacterial activity. The structure of the analogues has been established by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy, and elemental analysis techniques. The derivatives were screened for their antibacterial activityby both in-silico method and disc diffusion method. The antibacterial activityof the five analogues compared with the parent and was evaluated against Gram-positivebacteria*(Staphylococcus aureus)*PDB ID: 3SRS *and*Gram- bacteria strain*(Escherichia coli)*PDB ID: 1T9Y. The synthesized compounds showed enhanced antibacterial activity in contrast to the ciprofloxacin as proved by both the methods.After accessing it can be observed that the proposed molecules NS2 have the highest antibacterial activity against (*S. aureus*)and NS1have highestantibacterial activityagainst *E. coli*)respectively than the parent compound Ciprofloxacin.

Keywords: Ciprofloxacin;Mannich reaction; Docking; Synthesis;Antibacterial Screening

Introduction:

Bacteria that cause bacterial infections and disease are called pathogenic bacteria. Antimicrobial therapeutic agents are a group of materials that fight against pathogenic bacteria by killing or reducing the metabolic activity of bacteria[1,2]. Traditionally, small molecule compounds are the most commonly used agents during the course of antibacterial therapy. Heterocyclic compounds are organic molecules, which include carbazoles, azoles and also mannich base shows good antibacterial effect[3,4,5].Ciprofloxacin, 1[1-cyclopropyl-6-fluoro-l,4-dihydro-4-oxo-7-(l-piperazinyl)-3-quino-line carboxylic acid] is an antibacterial agent. It is a second-generation broad-spectrum synthetic fluoroquinolone antibiotic[6]. Quinolone antibiotics act by targeting DNA gyrase and topoisomerase of the bacterial enzyme[7].Mannich bases result from a three-component condensation between a substrate with acidic hydrogen, an aldehyde and an amine. Heterocycles mannich bases are remarkable compounds with various medicinal properties such as: antimicrobial, anticancer, antiviral, analgesic, anticonvulsant, anti-inflammatory, anti-HIV, antimalarial, anti-Alzheimer, anthelmintic, antioxidant and so forth[8,9,10].

Materials& Methods:

1.**Computational Work** :

a) Molecular Modeling Studies

Molecular modeling studies have been carried out using HEX 5.1 (Grid-based Ligand Docking with Energetics) software workspace was used for all the steps involved in ligand preparation, protein preparation and docking. ACD Chem Sketch is chemical drawing software developed by ACD LAB. The software is user-friendly, provides all details of drawn structures and helped to calculate chemical properties, design professional reports and presentations.

b) Ligand Preparation

The ligands used in this study were prepared using ARGUS LAB (Optimized Potential Liquid Simulations for All Atoms) force fields for energy minimization.

c) Protein Preparation

The X-ray crystal structures retrieved from PDB database as raw could not be suitable for molecular docking studies. A typical PDB structure consists only of heavy atoms, waters, Cofactors, metal ions and can be of multimeric. These structures do not have the information about bond orders, topologies or formal atomic charges. So, the raw PDB structure should be prepared in a suitable manner for docking. ARGUS LAB (Optimized Potential Liquid Simulations for All Atoms) force fields for energy minimization.

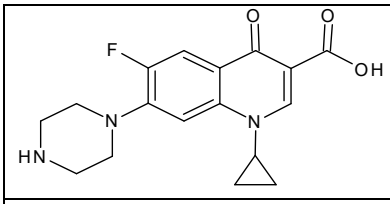
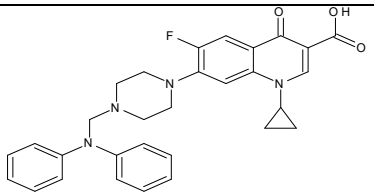
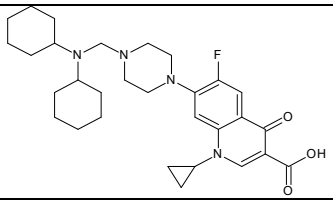
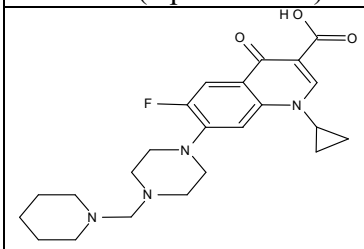
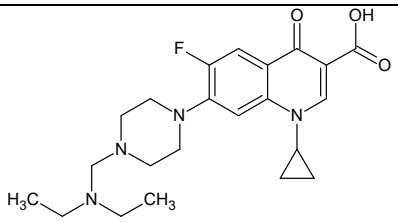
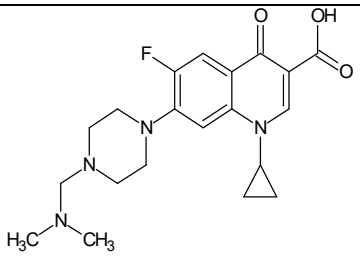
Lipinski Rule of Five Lipinski rule of 5 helps in distinguishing between drug like and non drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 3 or more of the following rules

- Molecular mass less than 500 Dalton
- High lipophilicity (expressed as LogP less than 5)
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors
- Molar refractivity should be between 40-130.

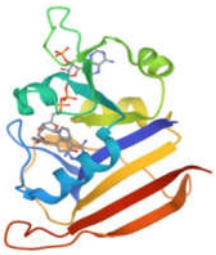
Table 1 :ADME properties& Docking results of Mannich base analogues of ciprofloxacin with 3SRS *and* 1T9Y.

Compound Name	miLogP	TPSA	natoms	MW	nON	nOHNH	nViolations	nrotb	Volume	Rule of 5	E. total 3SRS	E. total 1T9Y
Cipofloxacin.	-0.70	74.57	24	331.35	6	2	0	3	285.46	Followed	-254.21	-287.31
NS1	3.31	69.02	37	512.59	7	1	1	7	458.24	Followed	-334.67	-392.19
NS2	3.72	69.02	38	524.68	7	1	1	7	495.42	Followed	-339.70	-383.75
NS3	0.82	69.02	31	428.51	7	1	0	5	388.59	Followed	-329.60	-333.24
NS4	0.66	69.02	30	416.50	7	1	0	7	382.15	Followed	-320.65	-340.09
NS5	-0.09	69.02	28	388.44	7	1	0	5	348.55	Followed	-330.26	-316.52

Table 2 :Structure of Mannich base analogues of ciprofloxacin

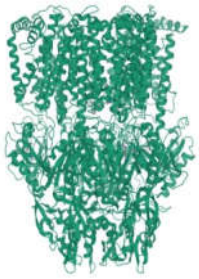
		
NS(ciprofloxacin)	NS1	NS2
		
NS3	NS4	NS5

Receptor :



3SRS(S. aureus)

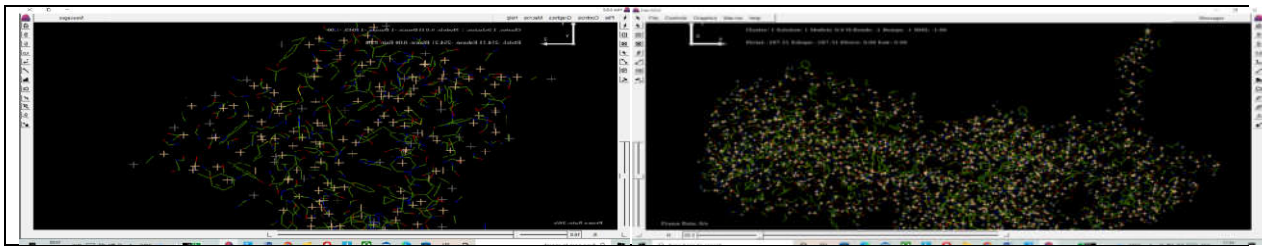
Fig -1

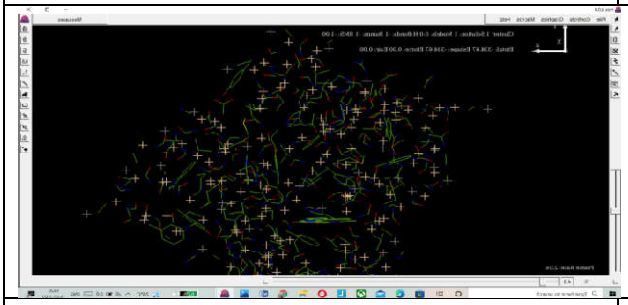
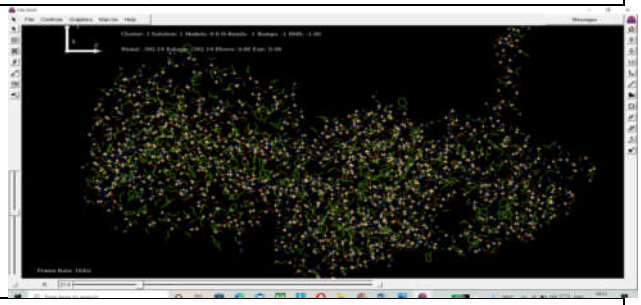
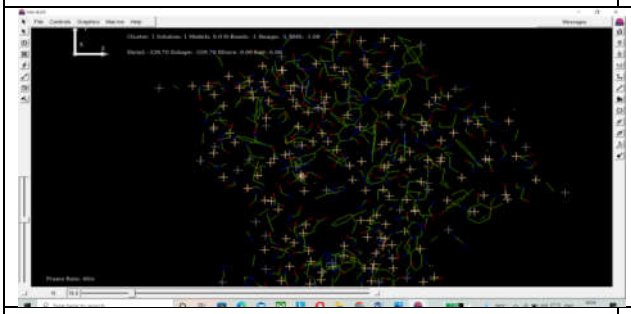
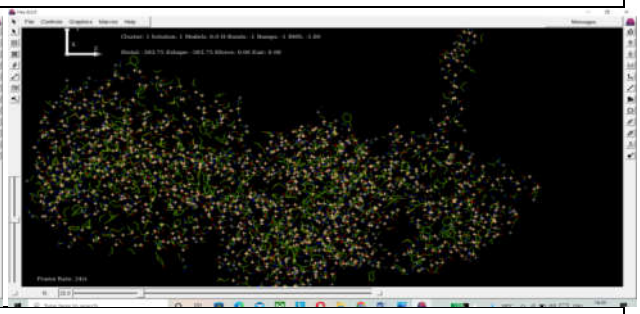
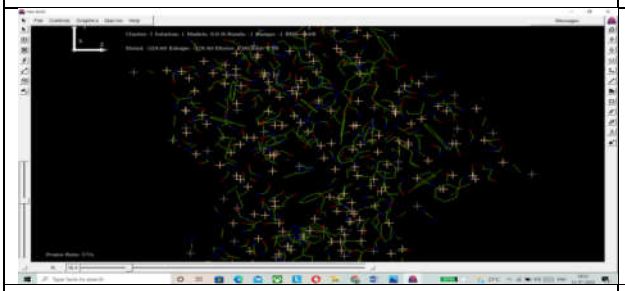
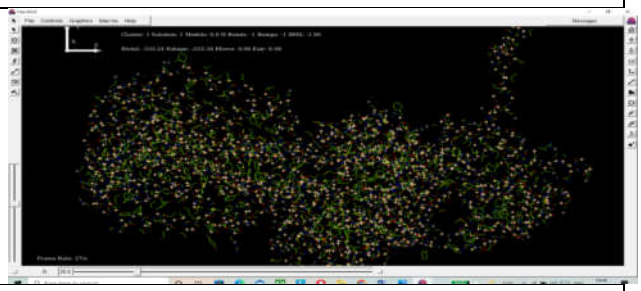
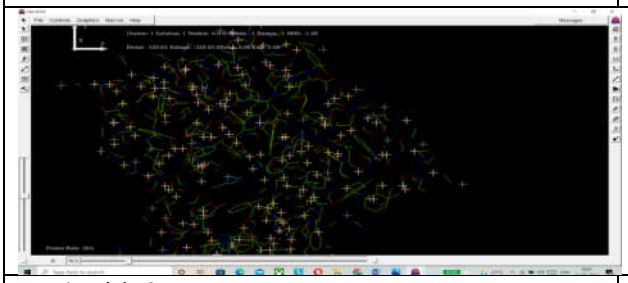
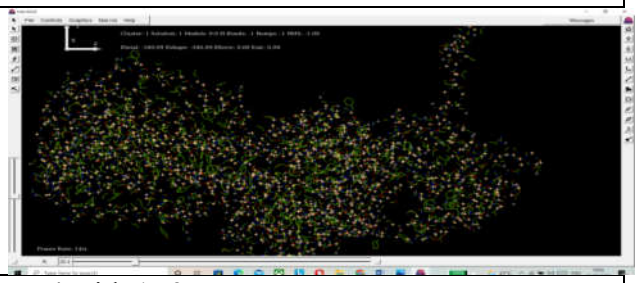
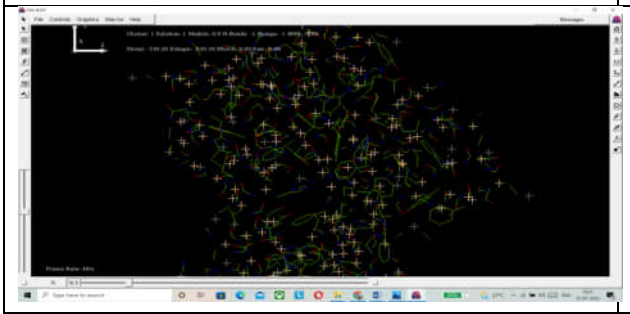
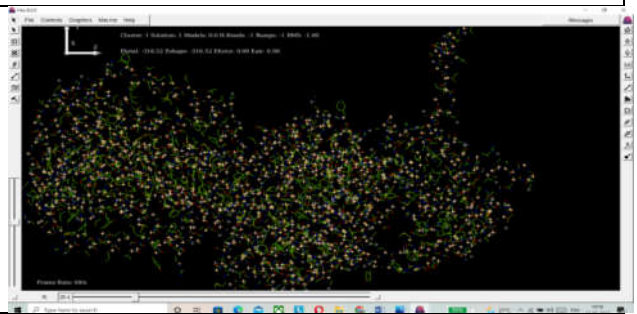


1T9Y(*E. coli*)

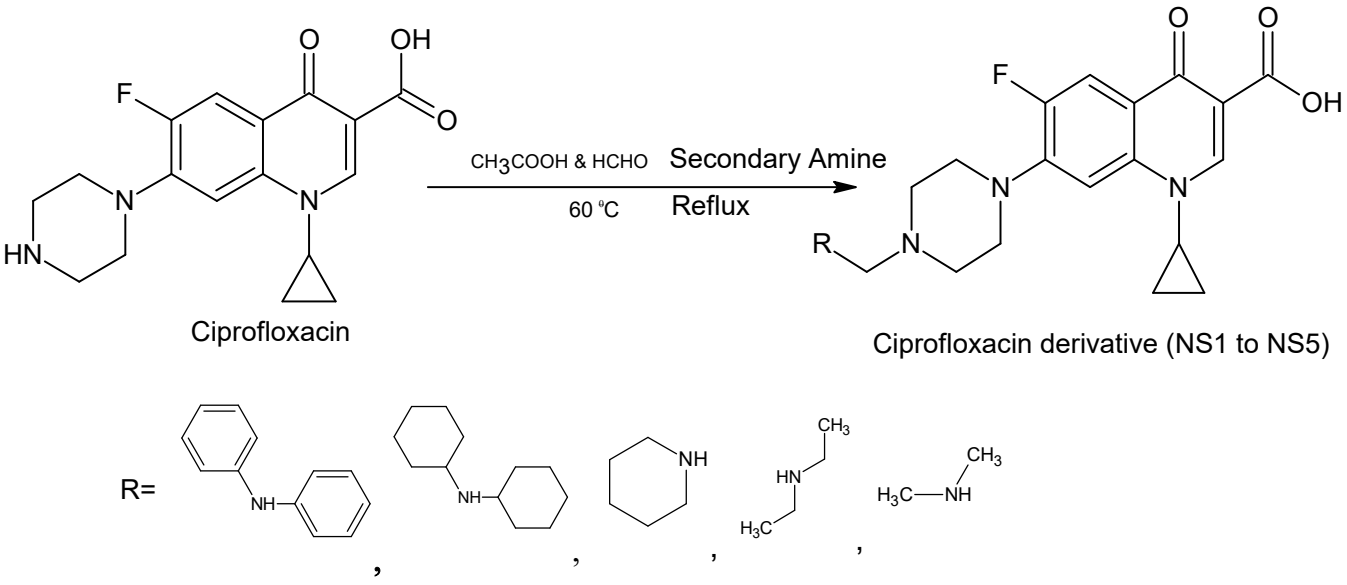
Fig -2

Fig.3 Docking image of compounds with both 3SRS (*S. aureus*) and 1T9Y (*E. coli*)



NS(Ciprofloxacin) with3SRS (*S. aureus*)	NS (Ciprofloxacin) with 1T9Y (*E. coli*)
	
NS1 with 3SRS	NS1 with 1T9Y
	
NS2 with 3SRS	NS2 with 1T9Y
	
NS3 with 3SRS	NS3 with 1T9Y
	
NS4 with 3SRS	NS4 with 1T9Y
	
NS5 with 3SRS	NS5 with 1T9Y

Scheme



2.Synthesis:

General procedure for preparation of derivatives:

In general, derivatives (NS1-NS5) were obtained by the reaction of ciprofloxacin with varioussecondary amine i.e., Diphenylamine, Di cyclohexylamine, Piperidine, Diethyl amine andDimethyl amine respectively in acetic acid and formalin (Scheme). The solution of ciprofloxacin (0.5 g, 1.508 mmol) in water (10 ml) and acetic acid (2 ml) was added in equal mmol of formalin and a secondary amine (R) mixture with vigorous stirring for each reaction. Each of the reaction mixtures was warmed at 60 °C for 80 min. and kept at room temperature overnight. The crystalline products were thus deposited. The reaction masses were filtered off, washed with 60 % aqueous ethanol, and dried under vacuum in a desiccator[11].

Table 3 :Physiochemical properties& TLC solvent with Rf value of Mannich base analogues of ciprofloxacin

Compound Name	Nomenclature and Chemical formula	Composition	Yield	Color	Rf Value	M.P.
NS1	1-cyclopropyl-6-fluoro-4-oxo-7-diphenyl-amino methyl-piperazin-1-ylquinoline-3-carboxylic acid M.F.- $C_{30}H_{29}FN_4O_3$	C=70%, H=5.5%, F=3.5%, N=10%, O=9%	65%	Pale Yellow	Rf = 0.64 (Ethyl acetate: N-Hexane:	143-145 °C

	M.W.- 512.57				Chloroform),	
NS2	1-cyclopropyl-6-fluoro-4-oxo-7-dicyclohexyl-aminomethyl-piperazin- 1-ylquinoline-3-carboxylic acid M.F.- $C_{30}H_{41}FN_4O_3$ M.W.- 524.66	C=68.5%, H=7.5%, F=3.5%, N=10%, O=9%	72%	Floral White	Rf = 0.68 (Ethyl acetate: N-Hexane: Chloroform)	250-252 °C
NS3	1-cyclopropyl-6-fluoro-4-oxo-7-piperidine-1-yl-methyl-piperazin-1-ylquinoline-3-carboxylic acid M.F.- $C_{23}H_{29}FN_4O_3$ M.W.- 428.50	C=64.2%, H=7%, F=4%, N=13%, O=11%	62%	Beige Brown	Rf = 0.73 (Methanol : Chloroform)	158-160 °C
NS4	1-cyclopropyl-6-fluoro-4-oxo-7-diethylamino-methyl-piperazin-1-ylquinoline-3-carboxylic acid M.F.- $C_{22}H_{29}FN_4O_3$ M.W.- 416.48	C=63%, H=7%, F=4.5%, N=13.5%, O=11.5%	68%	Sand Brown	Rf = 0.71 (Ethyl acetate: Methanol: Chloroform)	173-175°C
NS5	1-cyclopropyl-6-fluoro-4-oxo-7-dimethylamino-methyl-piperazin-1-ylquinoline-3-carboxylic acid M.F.- $C_{20}H_{25}FN_4O_3$ M.W.- 388.43	C=62%, H=6.5%, F=5%, N=14.3%, O=12%	70%	Warm Ivory Brown	Rf = 0.62 (Ethyl acetate: Methanol: Chloroform)	189-190°C

3.Spectral Characterizations of Synthesized compounds (NS1-NS5)

Compound 1 : (NS1) 1-cyclopropyl-6-fluoro-4-oxo-7-diphenyl-amino methyl-piperazin-1-ylquinoline-3-carboxylic acid: Paleyellowsolid, m.p: 143-145˚C, R*f*=0.64, Yield -65%, FT-IR(cm^{-1}):3152.45, 2923.52, 2855.42, 1716.58, 1615.79, 1486.43, 1461.51, 1394.77, 1325.79, 1264.69and 942.79.^{1}H NMR: δ 0.75-0.93 (4H, 0.84 (dddd, *J* = 8.1, 7.8, 7.5, 7.1 Hz), 0.84 (dddd, *J* = 8.1, 7.8, 7.5, 7.1 Hz)), 2.61 (4H, ddd, *J* = 14.7, 6.7, 2.5 Hz), 3.05 (4H, ddd, *J* = 13.6, 6.7, 2.5 Hz), 3.89 (1H, tt, *J* = 8.1, 7.5 Hz), 4.33 (2H, s), 6.68 (1H, d, *J* = 0.5 Hz), 6.93 (2H, tt, *J* = 8.1, 1.2 Hz), 7.04-7.29 (8H, 7.10 (dtd, *J* = 8.2, 1.2, 0.5 Hz), 7.22 (dddd, *J* = 8.2, 8.1, 1.3, 0.5 Hz)), 7.92 (1H, d, *J* = 0.5 Hz), 8.53 (1H, s).^{13}C NMR: δ 7.2 (2C, s), 34.4 (1C, s), 49.1 (2C, s), 52.6 (2C, s), 67.2 (1C, s), 106.2 (1C, s), 107.6 (1C, s), 115.0 (1C, s), 124.0 (1C, s), 124.5 (4C, s), 127.8 (2C, s), 128.2 (4C, s), 132.7 (1C, s), 139.1 (1C, s), 141.5 (1C, s), 145.1 (2C, s), 152.3 (1C, s), 165.9 (1C, s), 174.0 (1C, s).EI-MS: m/z512, 492, 484, 343, 267, 203, 169, 76, 44.

Compound 2 : (NS2) 1-cyclopropyl-6-fluoro-4-oxo-7-dicyclohexyl-aminomethyl-piperazin-1-ylquinoline-3-carboxylic acid: Floral white solid,m.p: 254-255˚C, R*f*=0.68, Yield- 72%, FT-IR(cm^{-1}):: 2925.74, 2851.99, 1740.08, 1660.12, 1612.63, 1456.87, 1380.30, 1306.08 and 956.50.^{1}H NMR: δ 0.75-0.93 (4H, 0.84 (dddd, *J* = 8.1, 7.8, 7.5, 7.1 Hz), 0.84 (dddd, *J* = 8.1, 7.8, 7.5, 7.1 Hz)), 1.29-1.74 (20H, 1.36 (dquint, *J* = 12.3, 2.8 Hz), 1.38 (dtt, *J* = 12.3, 10.3, 2.8 Hz), 1.52 (dtt, *J* = 12.9, 6.5, 2.8 Hz), 1.52 (dtt, *J* = 12.9, 6.5, 2.8 Hz), 1.66 (dtd, *J* = 12.9, 6.5, 2.8 Hz), 1.66 (dtd, *J* = 12.9, 6.5, 2.8 Hz)), 2.42-2.61 (6H, 2.49 (tt, *J* = 10.3, 2.8 Hz), 2.53 (ddd, *J* = 12.7, 6.7, 2.5 Hz)), 3.04 (4H, ddd, *J* = 13.6, 6.7, 2.5 Hz), 3.29 (2H, s), 3.89 (1H, tt, *J* = 8.1, 7.5 Hz), 6.68 (1H, d, *J* = 0.5 Hz), 7.92 (1H, d, *J* = 0.5 Hz), 8.53 (1H, s).^{13}C NMR: δ 7.2 (2C, s), 24.8 (4C, s), 25.6 (2C, s), 30.3 (4C, s), 34.4 (1C, s), 49.1 (2C, s), 52.6 (2C, s), 56.6 (2C, s), 67.2 (1C, s), 106.2 (1C, s), 107.6 (1C, s), 115.0 (1C, s), 124.0 (1C, s), 132.7 (1C, s), 139.1 (1C, s), 141.5 (1C, s), 152.3 (1C, s), 165.9 (1C, s), 174.0 (1C, s).EI-MS: m/z 524, 504, 484, 442, 343, 331, 279, 203, 181, 82, 44.

Compound 3 : (NS3) 1-cyclopropyl-6-fluoro-4-oxo-7-piperidine-1-yl-methyl-piperazin-1-ylquinoline-3-carboxylic acid: Beige brown solid, m.p: 158-160˚C, R*f*=0.73, Yield -62%, FT-IR(cm^{-1}):: 2929.46, 2855.03, 1715.79, 1663.86, 1616.94, 1455.18, 1400.60, 1330.06, 1255.79 and 931.91.^{1}H NMR: δ 0.75-0.93 (4H, 0.84 (dddd, *J* = 8.1, 7.8, 7.5, 7.1 Hz), 0.84 (dddd, *J* = 8.1, 7.8, 7.5, 7.1 Hz)), 1.48 (2H, dtt, *J* = 10.9, 6.7, 2.8 Hz), 1.72 (4H, ddddd, *J* = 10.9, 6.7, 6.6, 2.8, 2.7 Hz), 2.48-2.68 (8H, 2.55 (ddd, *J* = 11.8, 6.7, 2.5 Hz), 2.61 (ddd, *J* = 7.5, 6.6, 2.7 Hz)), 2.93-3.12 (6H, 2.98 (s), 3.04 (ddd, *J* = 13.6, 6.7, 2.5 Hz)), 3.89 (1H, tt, *J* = 8.1, 7.5 Hz), 6.68 (1H, d, *J* = 0.5 Hz), 7.92 (1H, d, *J* = 0.5 Hz), 8.53 (1H, s).^{13}C NMR: δ 7.2 (2C, s), 24.3 (1C, s), 25.6 (2C, s), 34.4 (1C, s), 49.1 (2C, s), 52.6 (2C, s), 53.1 (2C, s), 82.3 (1C, s), 106.2 (1C, s), 107.6 (1C, s), 115.0 (1C, s), 124.0 (1C, s), 132.7 (1C, s), 139.1 (1C, s), 141.5 (1C, s), 152.3 (1C, s), 165.9 (1C, s), 174.0 (1C, s).EI-MS: m/z428, 408, 388, 358, 331, 203, 183, 181, 97, 75, 44.

Compound 4 : (NS4) 1-cyclopropyl-6-fluoro-4-oxo-7-diethylamino-methyl-piperazin-1-ylquinoline-3-carboxylic acid: Sand brown solid, m.p: 170-172˚C, R*f*=0.71, Yield- 68%, FT-IR(cm^{-1}):3361.75, 3000.26, 2803.64, 1719.92, 1661.79, 1620.91, 1551.73, 1501.92, 1457.85, 1395.93, 1253.22 and 932.74.^{1}H NMR: δ 0.75-1.05 (10H, 0.84 (dddd, *J* = 8.1, 7.8, 7.5, 7.1 Hz), 0.84 (dddd, *J* = 8.1, 7.8, 7.5, 7.1 Hz), 0.99 (t, *J* = 7.2 Hz)), 2.48-2.68 (8H, 2.55 (ddd, *J* = 11.8, 6.7, 2.5 Hz), 2.62 (q, *J* = 7.2 Hz)), 3.04 (4H, ddd, *J* = 13.6, 6.7, 2.5 Hz), 3.20 (2H, s), 3.89 (1H, tt, *J* = 8.1, 7.5 Hz), 6.68 (1H, d, *J* = 0.5 Hz), 7.92 (1H, d, *J* = 0.5 Hz), 8.53 (1H, s).^{13}C NMR: δ 7.2 (2C, s), 12.9 (2C, s), 34.4 (1C, s), 49.1 (2C, s), 49.9 (2C, s), 52.6 (2C, s), 82.3 (1C, s), 106.2 (1C, s), 107.6 (1C, s), 115.0 (1C, s), 124.0 (1C, s), 132.7 (1C, s), 139.1 (1C, s), 141.5 (1C, s), 152.3 (1C, s), 165.9 (1C, s), 174.0 (1C, s).EI-MS: m/z416, 396,388, 343,331,203,171,169, 85,73, 44.

Compound 5 : (NS5) 1-cyclopropyl-6-fluoro-4-oxo-7-dimethylamino-methyl-piperazin-1-ylquinoline-3-carboxylic acid: Warm ivory brownsolid,m.p: 189-190˚C, R*f*=0.62, Yield -70%, FT-IR(cm^{-1}):3081.30, 2882.08, 2828.89, 2738.21, 1716.41, 1663.05, 1549.09, 1387.19, 1333.73, and 1308.58.^{1}H NMR: δ 0.75-0.93 (4H, 0.84 (dddd, *J* = 8.1, 7.8, 7.5, 7.1 Hz), 0.84 (dddd, *J* = 8.1, 7.8, 7.5, 7.1 Hz)), 2.33 (6H, s), 2.58 (4H, ddd, *J* = 11.4, 6.7, 2.5 Hz), 2.96-3.19 (6H, 3.04 (ddd, *J* = 13.6, 6.7, 2.5 Hz), 3.14 (s)), 3.89 (1H, tt, *J* = 8.1, 7.5 Hz), 6.68 (1H, d, *J* = 0.5 Hz), 7.92 (1H, d, *J* = 0.5 Hz), 8.53 (1H, s).^{13}C NMR: δ 7.2 (2C, s), 34.4 (1C, s), 38.2 (2C, s), 49.1 (2C, s), 52.6 (2C, s), 67.2 (1C, s), 106.2 (1C, s), 107.6 (1C, s), 115.0 (1C, s), 124.0 (1C, s), 132.7 (1C, s), 139.1 (1C, s), 141.5 (1C, s), 152.3 (1C, s), 165.9 (1C, s), 174.0 (1C, s).EI-MS: m/z388, 368, 360, 343, 331, 318, 203, 143, 141, 57, 44.

4. In-vitro Antibacterial studies :

The antimicrobial activity of the derivatives was determined by the disc diffusion method[12] against Gram-positive and Gram-negative bacteria. 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 gram-positive strains, i.e., *Staphylococcus aureus* and a gram-negative strain, i.e., *Escherichia coli.*The results are presented in Table No.4

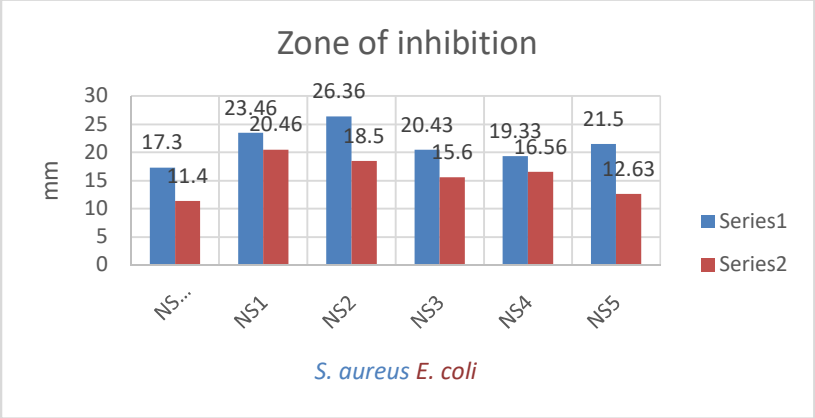
Results and Discussion:

Table No.4: Zone of inhibition of the compounds (100 µg/ml) against bacteria.

Serial No.	Compound Name	Gram-positive bacteria *(S. aureus)* in mm	Gram-negative bacteria *(E. coli)*in mm
1	NS (ciprofloxacin)	17.30±0.20	11.40±0.26
2	NS1	23.46±0.30	20.46±0.25
3	NS2	26.36±0.15	18.50±0.36
4	NS3	20.43±0.15	15.60±0.36
5	NS4	19.33±0.32	16.56±0.35
6	NS5	21.50±0.36	12.63±0.30

*Results are expressed as mean ± SD, n=3 no of observations.

Fig.4



The antimicrobial activities of derivatives Gram-positive and Gram-negative bacteria are presented in Table 4. Zones of inhibition indicate that the derivatives, (NS1-NS5)showed various degrees of activity compared to ciprofloxacin (NS) against both the Gram-positive bacterial strain*(Staphylococcus aureus) and* Gram- bacteria strain ***(****Escherichia coli****)***. The derivatives NS1(23.46±0.30mm)**,** NS2(26.36±0.15mm)**,** NS3(20.43±0.15mm)**,** NS4(19.33±0.32mm)andNS5(21.50±0.36mm)showed significantly enhanced activity compared to ciprofloxacin(NS) (17.30±0.20) against Gram-positive bacteria*(Staphylococcus aureus).*Zones of inhibition signify that the derivatives exhibited antimicrobial activity NS1(20.46±0.25mm)**,** NS2(18.50±0.36mm)**,** NS3(15.60±0.36 mm)**,** NS4(16.56±0.35mm)andNS5(12.63±0.30mm)showed significantly enhanced activity compared to ciprofloxacin(NS) (11.40±0.26) against Gram-negative bacteria*(Escherichia coli).*

Conclusion:A novel series of Mannich base analogues of ciprofloxacin (NS1-NS5) were successfully designed through computational method and synthesized, characterized by IR, NMR& Mass spectral data. The final compounds were screened for in vitro antibacterial activity against both Gram-positive and Gram-negative strains of bacteria by Disc Diffusion method. all the compounds showed significant activity against *Escherichia coli* and *Staphylococcus aureus* as compared to standard drug ciprofloxacin which also support the docking result obtained by Computational drug designing study.

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