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Research Article

SYNTHESIS, BIOLOGICAL EVALUATION, MOLECULAR MODELING, AND DOCKING STUDIES OF CIPROFLOXACIN DERIVATIVES

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ABSTRACT

Ciprofloxacin, a fluoroquinolone analogue has activity against a wide range of Gram-negative and Gram-positive microorganisms by inhibiting the enzymes topoisomerase-II (DNA-gyrase) and topoisomerase-IV which are required for bacterial DNA replication, transcription, repair, and recombination. A series of ciprofloxacin Schiff bases were synthesized (1a-j) via >C=N- linkage by reacting ciprofloxacin with various primary amines through nucleophilic addition reaction in the presence of glacial acetic acid and were characterized on the basis of infrared, nuclear magnetic resonance, mass spectrometry, and elemental analysis techniques. In the present investigation, we screened ciprofloxacin Schiff bases based on a better Docking simulation with QRDR-A. The compound 1g, 1b and 1d resulted in a dock score of −154.82, −145.27 and −144.32 kcal.mol^{-1} ranked first, second, and third, respectively, and the compound 1g along with 1c, 1f, and 1j also interacted with Asp87. It was found that 1a, 1d, and 1e induced marked influence on Gram-negative and Gram-positive antibacterial activity. The compound 1j shows potent antifungal activity against *Aspergillus niger* and *Candida albican.* The compound 1g, shows an excellent anti-tubercular activity. The correlation between experimental data (minimum inhibitory concentration) versus docking score displayed 0.93 r^2, which suggests that parameters for docking simulation are good in reproducing experimental orientation of these compounds. From the observed result, the analogs of ciprofloxacins are suggested to be potent inhibitors with sufficient scope for further exploration.

Keywords: DNA-gyrase, Schiff bases, Docking, Molecular modeling.

INTRODUCTION

Ciprofloxacin is a synthetic chemotherapeutic antibiotic and is a member of the antibiotic class Quinolones (fluoroquinolone drug class) [1]. The presence of a fluorine group at position-6 of the molecule places it into a subclass called the Fluoroquinolones. It is a second-generation fluoroquinolone antibacterial [2]. Its structure and ball-stick 3D model has shown in Figs. 1 and 2 respectively.

Ciprofloxacin is marketed worldwide with over three hundred different brand names. Ciprofloxacin was first patented in 1983 by Bayer A.G. and subsequently approved by the U.S. Food and Drug Administration (FDA) in 1987. Ciprofloxacin has 12 FDA-approved human uses and other veterinary uses [3].

Ciprofloxacin considered a benchmark when comparing new fluoroquinolones, shares with these agents a common mechanism of action, i.e. inhibition of DNA gyrase. While ciprofloxacin demonstrated a fairly good activity against Gram-positive bacteria, it is against Gram-negative organisms that it proved to be more potent than other fluoroquinolones. It is the most active quinolone against *Pseudomonas aeruginosa*, with MIC90s on the order of 0.5 µg/ml. When given orally, ciprofloxacin exhibited 70% bioavailability and attained peak serum levels ranging between 1.5 and 2.9 µg/ml after a single 500-mg dose. Nineteen percent of an oral dose was excreted as metabolites in both urine and feces. In most cases, body fluids and tissue concentrations equaled or exceeded those in concurrent serum samples. In clinical trials, oral and intravenous ciprofloxacin yielded similar clinical and bacteriologic results compared to standard therapy in a wide array of systemic infections, including lower and upper urinary tract infections; gonococcal urethritis, skin, skin structure, bone infections, respiratory tract, and gastrointestinal tract infections. Major benefits with the oral form of this quinolone are expected in chronic pyelonephritis and bone infections and in pulmonary exacerbations in patients with cystic fibrosis [4]. Emergence of ciprofloxacin-resistant micro-organisms has been noted in clinical practice, primarily *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The most frequent side effects are related to the gastrointestinal tract, but attention should be given to adverse central nervous system effects [5].

As of 2011 the FDA has added two black box warnings for this drug in reference to spontaneous tendon ruptures and the fact that ciprofloxacin may cause worsening of myasthenia gravis symptoms, including muscle weakness and breathing problems. Such an adverse reaction is a potentially life-threatening event and may require ventilatory support [6].

Ciprofloxacin has *in vitro* activity against a wide range of Gram-negative and Gram-positive microorganisms. The bactericidal action of

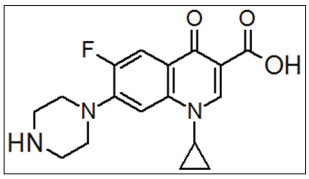


Fig. 1: Ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid)

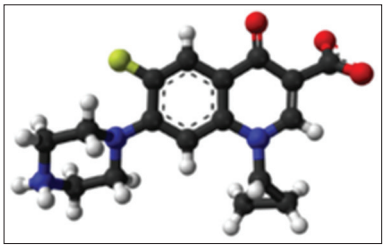


Fig. 2: Ciprofloxacin (Ball and stick 3D model)

ciprofloxacin results from inhibition of the enzymes topoisomerase-II (DNA-gyrase) and topoisomerase-IV, which are required for bacterial DNA replication, transcription, repair, and recombination. The mechanism of action of fluoroquinolones, including ciprofloxacin, is different from that of penicillins, cephalosporins, aminoglycosides, macrolides, and tetracyclines; therefore, microorganisms resistant to these classes of drugs may be susceptible to ciprofloxacin and other quinolones. There is no known cross-resistance between ciprofloxacin and other classes of antimicrobials. *In vitro* resistance to ciprofloxacin develops slowly by multiple step mutations.

Ciprofloxacin has been shown to be active against *Bacillus anthracis* both *in vitro* and by use of serum levels as a surrogate marker. The following *in vitro* data are available, but their clinical significance is unknown. It exhibits *in vitro* minimum inhibitory concentrations (MICs) of 1 µg/mL or less against most (≥ 90%) strains of the following microorganisms; however, the safety and effectiveness of ciprofloxacin in treating clinical infections due to these microorganisms have not been established in adequate and well-controlled clinical trials.

The most common method of resistance to Quinolones is enzyme mutation that leads to a decrease in susceptibility of the bacteria to the antibiotic. This mechanism of resistance has not been a major problem with the Fluoroquinolones. The dual-enzyme mechanism of action of these antibiotics helps to decrease the incidence of resistance since a bacterial cell would have to possess mutated forms of two different enzymes to be insensitive to the medication. A second method of resistance is through changes in the cell membrane that would decrease nutrient and other uptake into the cell. This is not as common as the first method, but may be a problem that is much more serious and harder to correct. Emergence of ciprofloxacin-resistant microorganisms has been noted in clinical practice, primarily *Pseudomonas aeruginosa* and *S. aureus*. Because of the novel mechanism of action of the Quinolones, bacteria need to make two mutations to become resistant to the antibiotic activity. In addition, doctors have tended to use the Quinolones only in cases where the causative organism has been identified as Quinolone-sensitive. As a result, the Quinolones will probably be clinically important antibiotics for years to come [7].

The present study reports on the syntheses, spectroscopic analysis (including IR and ^{1}HNMR), mass spectrometry and evaluation of biological activities of *N*-substituted piperazinyl Schiff bases of ciprofloxacin (1a-h).

Taking into account, the accuracy aspect of molecular docking and important biological activities of Schiff bases recent efforts have been directed toward modeling of N-piperazinyl Schiff bases ciprofloxacin (1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4- dihydro-quinoline-3- carboxylic acid) with the aim to evaluate the possible relationship between docking score and their contribution to biological activity along with interaction with QRDR-A residues of *Escherichia coli* DNA Gyrase-A (EcGyr-A).

METHODS

Experimental

All the chemicals used were of analytical reagent grade and obtained from Qualigens Ltd. (Fisher Scientific), India. The Melting points of synthesized compounds were determined in an open end capillary tube on Elico melting point apparatus. Reaction progress was monitored by ascending thin layer chromatography on precoated silica gel-G sheets (E. Merck and Co.), visualized by iodine vapors and the purity of compounds was ascertained by a single spot on TLC plates. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in Bruker DRX-300 FT-NMR spectrometer in dimethyl sulfoxide (DMSO)-D_6 and are reported in parts per million (δ) relative to tetramethylsilane (TMS) as an internal standard. Infrared (IR) spectra were recorded on a Bruker FTIR spectrometer (ATR). The MS-ESI spectra were recorded on Micromass Quattro-II. Elemental analysis (CHN) was performed on Elementar Vario EL-III CHNS elemental analyzer. Muller-Hinton and Sabouraud dextrose agars were obtained from Hi-Media Ltd, India. The bacterial and fungal strains were provided by Department of Biotechnology, Saroj Institute of Technology and Management, Lucknow, India. Ciprofloxacin and fluconazole were obtained from S. D. Fine Chemicals and Hi-Media Ltd, India. LogP values for synthesized derivatives were calculated using ChemDraw Ultra 10.0 (http://www.cambridgesoft.com).

Purification and drying of reagents and solvents were carried out according to standard literature procedure (Furniss *et al*., 1980). The general procedure for the preparation of 1 N-piperazinyl Schiff bases ciprofloxacin (1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4- dihydro- quinoline-3- carboxylic acid analogs are described in Scheme 1. ciprofloxacin (0.5 mmol), and various amines (hydrazine, hydroxylamine, semicarbazide, thiosemicarbazide, aniline, phenyl hydrazine, 2,4-dinitrophenyl hydrazine, isonicotinyl hydrazide, and substituted benzoyl hydrazides) (0.5 mmol) was reacted at 85-90°C for 9-14 hrs. respectively, in ethanol with glacial acetic acid for 9-14 hrs. at 110-120°C (Table 1) gave the corresponding 1a-h in 64-98% overall yield. Progress of the reaction was observed by TLC monitoring on silica gel 60 F254 plates until a distinct spot of product was obtained. After total consumption of reactants, the contents were cooled, precipitate was collected, and finally washed with cold ethanol to give the crude Schiff bases. Purification was achieved by passage through a short column, with silica-gel 60 (200-400 mesh, Merck) packing and chloroform: Ethanol (8:2) as solvent system. The product was recrystallized from the mixture of DMF and ethanol (2:8) to give compounds 1a-h. Final product was characterized by melting point and Rf values using solvent system chloroform:Methanol (9:1).

Spectral data

1a.1-Cyclopropyl-6-fluoro-4-hydrazono-7-piperazin-1-yl-1,4-dihydro-quinoline-3-carboxylic acid (CHH)

IR ν_{max} (cm^{-1}, ATR): 3338 (N–H, str.) 3097 (C–H str, Ar.), 2948 (O-H str, carboxylic), 1708 (C=O str, Carboxylic), 1618 (C=N, imine), 1272 (C-F str.), 1020 (C-N str., piperazine), ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.47 – 1.30 (m, 4H, 2H-2'/2H-3'- cyclopropyl), 2.48 (s, 1H, piperazine), 3.10–3.37 (m, 8H, piperazine), 3.65 (m, 1H, H-1'- cyclopropyl), 7.54 (d, 1H, H-8), 7.85 (d, 1H, H-5), 7.90 (s, 2H, NH_2 -Hydrazine), 8.64 (s, 1H, H-2), Elemental analysis (%): Calcd. for $C_{17}H_{20}FN_5O_2$: C, 59.12; H, 5.84; N, 20.28; Found: C, 59.01; H, 5.97; N, 20.38.

1b. 1-Cyclopropyl-6-fluoro-4-hydroxyimino-7-piperazin-1-yl-1,4-dihydro- quinoline-3-carboxylic acid (3d) CHA

IR ν_{max} (cm^{-1}, ATR): 3372 (N–H, str.) 3085 (C–H str, Ar.), 2956 (O-H str, carboxylic), 1718 (C=O str, carboxylic), 1621 (C=N, imine), 1268 (C-F str.), 1022 (C-N str. piperazine), ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.16-1.31 (m, 4H, 2H-2'/2H-3'- cyclopropyl), 2.48 (s, 1H, piperazine), 3.13- 3.38 (m, 8H, piperazine), 3.68 (m, 1H, 1'- cyclopropyl), 7.58 (d, 1H, H-8), 7.85 (d, 1H, H-5), 8.79 (s, 1H, H-2), 11.09 (s,1H, NOH, D_2O exchangable), MS-ESI: *m/z* 347.15 (M+1), Elemental analysis (%): Calcd. for $C_{17}H_{19}FN_4O_3$: C, 58.95; H, 5.53; N, 16.18; Found: C, 59.08; H, 5.38; N, 16.04.

1c. 4-(2-carbamoylhydrazinylidene)-1-cyclopropyl-6-fluoro-7-(piperazin-1-yl)-1, 4-dihydro quinoline-3-carboxylic acid (3f) CSC

IR ν_{max} (cm^{-1}, ATR): 3334 (N–H, str.) 3045 (C–H str, Ar.), 2962 (O-H str., Carboxylic), 1713 (C=O str, Carboxylic), 1647 (Amide-I), 1621 (C=N, Imine). 1509 (Amide-II), 1265 (C-F str.), 1021 (C-N str. Piperazine), ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.21- 1.36 (m, 4H, 2H-2'/2H-3'- cyclopropyl), 2.51 (s, 1H, piperazine), 3.14 - 3.38 (m, 8H, piperazine), 3.65 (m, 1H, 1'- cyclopropyl), 6.30 (s, 2H, -$CONH_2$), 7.54 (d, 1H, H-8), 7.87 (d, 1H, H-5), 8.13 (s, 1H, -NH), 8.64 (s, 1H, H-2), Elemental analysis (%): Calcd. for $C_{18}H_{21}FN_6O_3$: C, 55.66; H, 5.45; N, 21.64, Found: C, 55.54; H, 5.63; N, 21.45.

1d. 4-(2-carbamothioylhydrazinylidene)-1-cyclopropyl-6-fluoro-7-(piperazin-1-yl)-1,4-dihy-droquinoline-3-carboxylic acid (3h) CTSC

IR ν_{max} (cm^{-1}, ATR): 3336 (N–H, str.), 3022 (C–H str, Ar.), 2968 (O-H str., Carboxylic), 1715 (C=O str, carboxylic), 1626 (C=N, imine), 1272

(C-F str.), 1229 (C=S), 1024 (C-N str., piperazine), ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.18 – 1.29 (m, 4H, 2H-2'/2H-3'- cyclopropyl), 2.53 (s, 1H, piperazine), 3.12-3.37(m, 8H, piperazine), 3.71 (m, 1H, 1'-cyclopropyl), 6.87 (s, 2H, -CSNH_2), 7.54 (d, 1H, H-8), 7.92 (d, 1H, H-5), 8.15 (s, 1H,-NH), 8.73 (s, 1H, H-2), Elemental analysis (%): Calcd. for $C_{18}H_{21}FN_6O_2S$: C, 53.45; H, 5.23; N, 20.78; Found: C, 53.37; H, 5.41; N, 20.89.

1e. 1-Cyclopropyl-6-fluoro-4-(phenyl-hydrazono)-7-piperazin-1-yl-1,4-dihydroquinoline-3-carboxylic acid (3j) CPH

IR ν_{max} (cm^{-1}, ATR): 3352 (N–H, str) 3025 (C–H str, Ar.), 2949 (O-H str, carboxylic), 1730 (C=O str, carboxylic), 1626 (C=N, imine), 1261 (C-F str.), 1037 (C-N str, piperazine), ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.18- 1.31 (m, 4H, 2H-2'/2H-3'- cyclopropyl), 2.48(s, 1H, piperazine), 3.34 - 3.53 (m, 8H, piperazine), 3.84(m, 1H,1'-cyclopropyl), 7.58 (d, 1H, H-8), 7.92 (d, 1H, H-5), 7.96 (m, 5H, Phenyl), 8.29 (s, 1H, -NH), 8.67 (s, 1H, H-2), MS-ESI: *m/z* 422.19 (M+1), Elemental analysis (%): Calcd. for $C_{23}H_{24}FN_5O_2$. C, 65.54; H, 5.74; N, 16.62, Found: C, 65.76; H, 5.53; N, 16.60.

1f. 1-Cyclopropyl-4-[(2,4-dinitro-phenyl)-hydrazono]-6-fluoro-7-piperazin-1-yl-1,4-dihydro -quinoline-3-carboxylic acid(3f) CDNPH

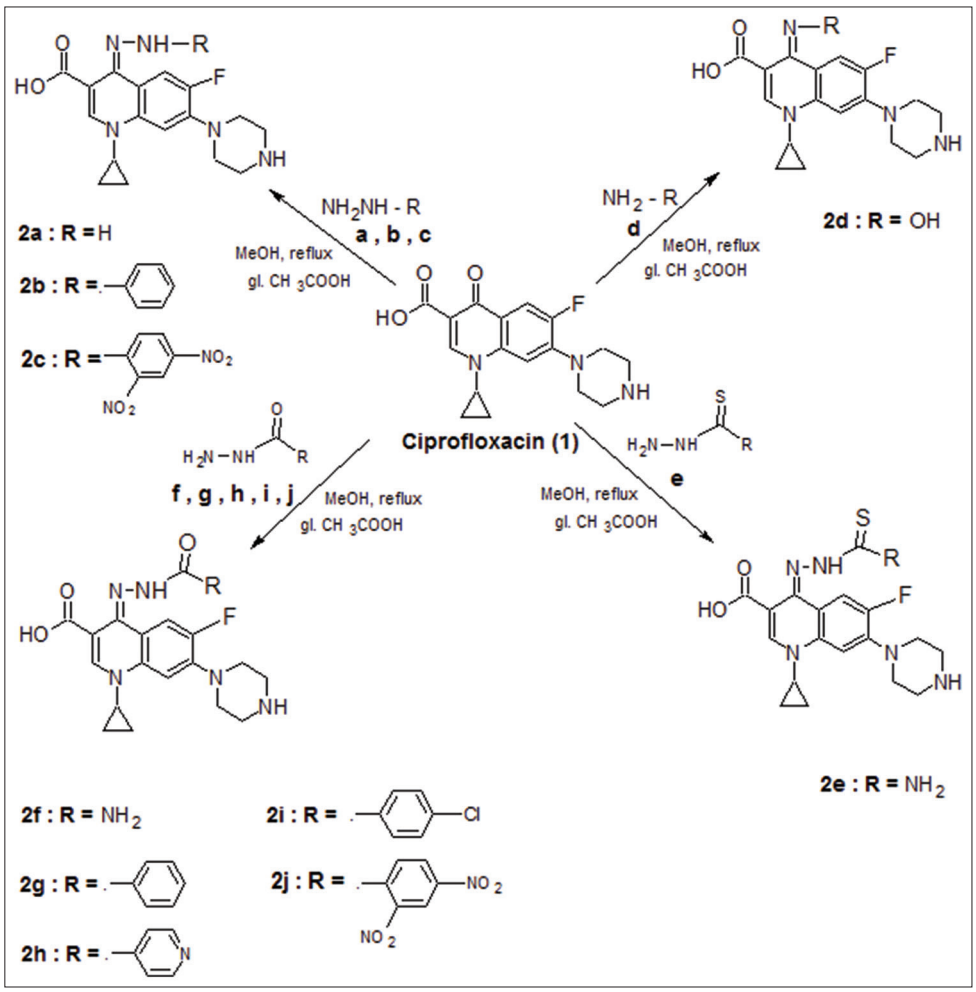
IR ν_{max} (cm^{-1}, ATR): 3280 (N–H, str.) 3039 (C–H stre, Ar.), 2970 (O-H str., Carboxylic), 1722 (C=O str, Carboxylic), 1620 (C=N, Imine). 1533 ($ArNO_2$, str., Assym.) 1347 ($ArNO_2$, str., Symm.), 1269 (C-F str.), 1033 (C-N str. Piperazine), ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.14- 1.28 (m, 4H, 2H-2'/2H-3'- cyclopropyl), 2.48 (s, 1H, piperazine), 3.32- 3.54(m, 8H, piperazine),3.87(m, 1H, 1'- cyclopropyl), 7.54 (d, 1H, H-8), 7.92 (d, 1H, H-5), 7.95- 8.21 (m, 3H, Phenyl), 8.29 (s, 1H, -NH), 8.67 (s, 1H, H-2), MASS $[M+H]^+$: m/e: 511.16 (100.0%), 512.16 (28.2%), 513.17 (4.5%), Elemental analysis (%): Calcd. for $C_{23}H_{22}FN_7O_6$: C, 54.01; H, 4.34; N, 19.17, Found: C, 53.87; H, 4.42; N, 19.29.

1g.1-Cyclopropyl-6-fluoro-7-piperazin-1-yl-4-[(pyridine-4-carbonyl)-hydrazono]-1,4-dihydro-quinoline-3-carboxylic acid (3g) CINH

IR ν_{max} (cm^{-1}, ATR): 3287 (N–H, str.) 3035 (C–H stre, Ar.), 2982 (O-H str., Carboxylic), 1720 (C=O str, Carboxylic), 1689 (Amide-I), 1617 (C=N, Imine), 1522 (Amide-II), 1447 (C-N ring str., Pyridine), 1262 (C-F str.), 1026 (C-N str. Piperazine), ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.17- 1.32 (m, 4H, 2H-2'/2H-3'- cyclopropyl), 8.64 (s, 1H, H-2), 7.87 (d, 1H, H-5), 2.48 (s, 1H, piperazine), 3.30- 3.39 (m, 8H, piperazine), 3.68 (m, 1H, 1'- cyclopropyl), 7.54 (d, 1H, H-8), 8.35-8.72 (4H, Pyridine), 8.25 (s, 1H, -NH), MASS $[M+H]^+$: m/e: 450.18 (100.0%), 451.18 (27.8%), 452.19 (3.9%), Elemental analysis (%): Calc for: $C_{23}H_{23}FN_6O_3$: C, 61.32; H, 5.15; N, 18.66, Found: C, 61.48; H, 5.23; N, 18.52.

1h.4-(Benzoyl-hydrazono)-1-cyclopropyl-6-fluoro-7-piperazin-1-yl-1,4-dihydro-qui-noline-3-carboxylic acid (3h) CBHZ

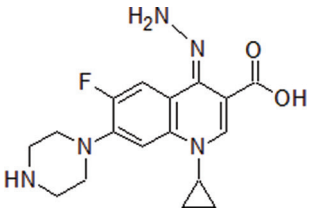
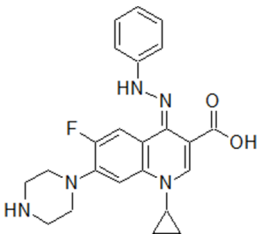
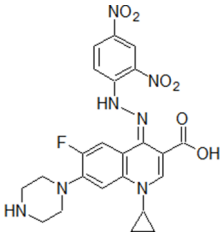
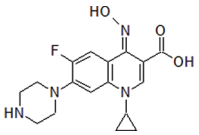
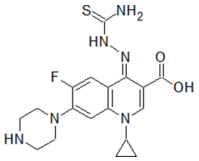
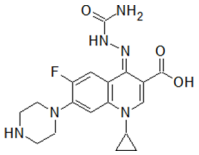
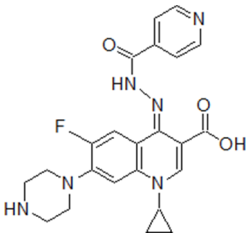
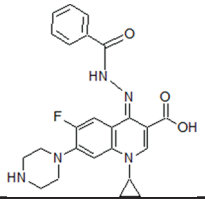
IR ν_{max} (cm^{-1}, ATR): 3358 (N–H, str.) 3042 (C–H stre, Ar.), 2965



Scheme 1: Synthesis of Schiff bases of ciprofloxacin, Reagents: a = NH_2NH_2, b = NH_2NHPh, c = $NH_2NHPh(NO_2)_2$, d = NH_2OH, e = $NH_2NHC(=S)NH_2$, f = $NH_2NHC(=O)NH_2$, g = isonicotinylhydrazide, h = $NH_2NHC(=O)Ph$, i = $NH_2NHC(=O)PhCl$, j = $NH_2NHC(=O)Ph(NO_2)_2$

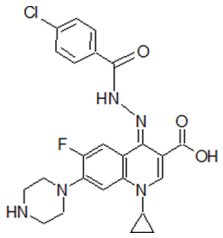
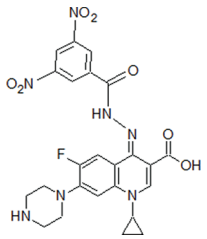
(O-H str., Carboxylic), 1718 (C=O str, Carboxylic), 1643 (Amide-I), 1621 (C=N, Imine). 1532 (Amide-II), 1252 (C-F str.), 1022 (C-N str. Piperazine), ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.18- 1.32 (m, 4H, 2H-2'/2H-3'- cyclopropyl), 2.51 (s, 1H, piperazine), 3.30- 3.54 (m, 8H, piperazine), 3.85 (m, 1H, 1'- cyclopropyl), 7.58 (d, 1H, H-8), 7.82- 8.17 (m, 5H, Phenyl), 7.92 (d, 1H, H-5), 8.34 (s, 1H, -NH), 8.67 (s, 1H, H-2), MASS $[M+H]^{+:}$ m/e: 449.19 (100.0%), 450.19 (27.2%), 451.19 (4.6%), 450.18 (1.9%), Elemental analysis (%): Calc. for $C_{24}H_{24}FN_5O_3$: C, 64.13; H, 5.38; N, 15.58, Found: C, 64.02; H, 5.24; N, 15.71.

Table 1: Physicochemical parameter of the synthesized compounds

Compound Code	Compound	Structure	Mol. Formula	Mol wt.	Yield (%)	Melting point (°C)	[a]Rf value	[b]Log P	[c]R.t (hr.)
CHH	1a		$C_{17}H_{20}FN_5O_2$	345.37	79.59	259-261	0.69	1.06	6
CPH	1b		$C_{23}H_{24}FN_5O_2$	421.47	85.37	240-242	0.85	2.96	6
CDNPH	1c		$C_{23}H_{22}FN_7O_6$	511.46	80.28	197-199	0.81	3.25	12
CHA	1d		$C_{17}H_{19}FN_4O_3$	346.36	87.84	252-254	0.66	1.71	8
CTSC	1e		$C_{18}H_{21}FN_6O_2S$	404.46	69.15	170-172	0.75	1.24	8
CSC	1f		$C_{18}H_{21}FN_6O_3$	388.40	72.71	220-223	0.76	0.68	8
CINH	1g		$C_{23}H_{23}FN_6O_3$	450.47	67.43	248-250	0.59	1.57	31
CBHZ	1h		$C_{24}H_{24}FN_5O_3$	449.48	73.46	211-213	0.47	2.91	11

(Cont)

Table 1: (Continued...)

CPCBHZ	1i		$C_{24}H_{23}ClFN_5O_3$	483.92	96.22	188-190	0.52	3.46	11
CDNBHZ	1j		$C_{24}H_{22}FN_7O_7$	539.47	74.37	233-235	0.63	1.98	11

[a]Solvent system: Chloroform: methanol (9:1), [b]Calculated by ChemDraw Ultra 10.0 (http://www.cambridgesoft.com), [c]Rt: Reaction time (hrs)

1i. 4-[(4-Chloro-benzoyl)-hydrazono]-1-cyclopropyl-6-fluoro-7-piperazin-1-yl-1,4-dihydroquinoline-3-carboxylic acid (3i) CPCBHZ

IR ν_{max} (cm^{-1}, ATR): 3372 (N–H, str.) 3052 (C–H stre, Ar.), 2985 (O-H str., Carboxylic), 1709 (C=O str, Carboxylic), 1647 (Amide-I), 1622 (C=N, Imine), 1537 (Amide-II), 1270 (C-F str.)1032 (C-N str. Piperazine), 724 (C-Cl str.), ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.19- 1.29 (m, 4H, 2H-2'/2H-3'- cyclopropyl), 2.53 (s, 1H, piperazine), 3.32- 3.53 (m, 8H, piperazine), 3.71 (m, 1H, 1'- cyclopropyl), 7.59 (d, 1H, H-8), 7.65- 7.83 (m, 4H, Phenyl), 7.87 (d, 1H, H-5), 8.71 (s, 1H, 4-NH), 8.73 (s, 1H, H-2), MASS $[M+H]^+$: m/e: 483.15 (100.0%), 485.14 (32.0%), 484.15 (27.2%), 486.15 (8.9%), 485.15 (4.6%), 484.14 (1.9%), 487.15 (1.3%), Elemental analysis (%): Calc. for $C_{24}H_{23}ClFN_5O_3$: C, 59.57; H, 4.79; N, 14.47, Found: C, 59.70; H, 4.86; N, 14.32.

1j. 1-Cyclopropyl-4-[(3, 5-dinitro-benzoyl)-hydrazono]-6-fluoro-7-piperazin-1- yl- 1,4-dihydro-quinoline-3-carboxylic acid (3j) CDNBHZ

IR ν_{max} (cm^{-1}, ATR): 3364 (N–H, str.) 3048 (C–H stre, Ar.), 2972 (O-H str., Carboxylic), 1711 (C=O str, Carboxylic), 1649 (Amide-I), 1625 (C=N, Imine), 1537 ($ArNO_2$, str., Assym.) 1535 (Amide-II), 1353 ($ArNO_2$, str., Symm.), 1268 (C-F str.)1036 (C-N str. Piperazine), ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.19- 1.31 (m, 4H, 2H-2'/2H-3'- cyclopropyl), 2.53 (s, 1H, piperazine), 3.34- 3.54 (m, 8H, piperazine), 3.85 (m, 1H, 1'- cyclopropyl), 7.58 (d, 1H, H-8), 7.96 (d, 1H, H-5), 7.75- 8.10 (m, 3H, Phenyl), 8.79 (s, 1H, H-2), 8.96 (s, 1H, -NH), MASS $[M+H]^+$: m/e: 539.16 (100.0%), 540.16 (27.3%), 541.16 (5.6%), 540.15 (2.6%), Elemental analysis (%): Calc. for: $C_{24}H_{22}FN_7O_7$ C, 53.43; H, 4.11; N, 18.17, Found: C, 53.31; H, 4.20; N, 18.28.

Biological evaluations

Determination of in vitro antimicrobial and antifungal activity

Compounds 1a-j was screened for antibacterial activity against Gram-negative and Gram-positive bacterial strains by the agar dilution method [8]. Two-fold serial dilutions of the compounds and reference drugs (ciprofloxacin and fluconazole) were prepared in Mueller-Hinton agar for bacteria and in Sabouraud dextrose agar for fungi. Drugs (10.0 mg) were dissolved in DMSO (1 ml), and the solution was diluted with water (9 ml). Further progressive double dilution with melted Mueller-Hinton and Sabouraud dextrose agars were performed to obtain the required concentrations of 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.19, 0.098, 0.049, 0.025, 0.013, 0.006, and 0.003 µg/mL. The bacterial and fungal inocula were prepared by suspending overnight colonies from Mueller-Hinton and Sabouraud dextrose agars media in 0.85% saline. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to approximately 0.5 McFarland standards (1.5×10^8 CFU/ml). The suspensions were then diluted in 0.85% saline to give 10^7 CFU/ml for bacteria and 10^5 CFU/ml for fungi. Petridishes were spot inoculated with 1 µl of each prepared bacterial and fungal suspensions. Finally, the petridishes were incubated at 35-37°C for 18-20 hrs for bacteria and 28-30°C for 48-72 hrs for fungi and the MIC was determined. The MIC was the lowest concentration of the test compound which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial and fungal growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment [9]. The amount of DMSO never exceeded 1% v/v. The physicochemical parameter of the synthesized compounds is mentioned in Table 1.

MOLECULAR DOCKING STUDIES OF CIPROFLOXACIN ANALOGUES

The molecular docking study of Ciprofloxacin analogs with well-established structure of EcGyr-A was done using MolDock docking engine of Molegro Virtual Docker, version 5.5.0 (MVD) software from CLC Bio (http://www.clcbio.com/products/molegro, Aarhus, Denmark) [10]. All calculations were conducted on IntellCore2 Duo T6400, 1.20 GHz dual processing machine. Docking of Ciprofloxacin and it's analogs with EcGyr-A proceeds in three steps; the first is ligand preparation; second is retrieval, preparation, and validation of 3D X-ray crystal structure of EcGyr-A and third is identification of QRDR-A along with molecular docking of reference ligand and designed analogs to QRDR-A. The ducking result is shown in Table 2.

RESULTS AND DISCUSSION

Chemistry

The synthetic route to obtain the necessary derivatives from commercially available reagents is briefly outlined in scheme 1. The title Schiff base of ciprofloxacin formed via >C=N- linkage were accomplished by reaction of ciprofloxacin and various primary amines (1a-j) through nucleophilic addition reaction in the presence of glacial acetic acid. The structures of all synthesized compounds were confirmed by IR, ^{1}H NMR, mass spectral and elemental analysis techniques. Herewith, this procedure acclaims an efficient and promising synthetic strategy with good to excellent yields for the production of titled derivatives. IR spectrums were recorded in the range of 4000-650 cm^{-1} to ensure the presence of various functional groups. In this context, the characteristic group stretching frequencies of carbonyl (C=O) of parent compound (ciprofloxacin) tends to appears at 1630 cm^{-1} whereas the imines (>C=N) at 1617-1626 cm^{-1}, indicates the disappearance of carbonyl peak and thus confirms the synthesis of desired compounds. Moreover, our investigations in the ^{1}H NMR

spectrum showed multiple signals corresponding to the resonance of quinolone protons from δ 3.65-3.87 ppm as multiplet for 1-cyclopropyl and δ 1.14-1.36 ppm for 2/3-cyclopropyl at N-1 position was observed. A singlet δ 8.64-8.79 ppm for 1H, C-2 and δ 14.78-15.08 ppm for 1H, C-6 has been observed in the spectrum. A doublet for δ 7.85-7.96 ppm (1H, C-5) and δ 7.54-7.59 ppm 1H, C-8 were attributed for C-5 and C-8 in synthesized derivatives. The NMR spectra showed δ 2.48-2.53 ppm corresponding to 1H, N-H and δ 3.10-3.54 ppm, as multiplet (8H, piperazine) were attributed for piperazine ring at C-7 position were observed in the spectrum clearly confirms the synthesized analogues. The mass spectrum of compound is characterized by their M+1 peak. Elemental analysis was within ±/ 0.4% of the theoretical composition in agreement with the proposed structures.

Antibacterial activity

The title Schiff bases of ciprofloxacin 1(a-j) showed excellent to significant susceptibilities towards Gram-negative, Gram-positive bacterial and fungal strains as well as for *M. tuberculosis* H37Rv as shown in Table 3. Result indicates that compound 1a, with hydrazinylidene substitution an excellent activity was observed against *S. typhi* (0.04 µg/mL), *Bacillus thuringiensis* (0.19 µg/mL) and *S. aureus* (0.09 µg/mL). At the same time, the same compound showed equipotent activity against *H. pylori* (0.78 µg/mL) and methicillin-resistant *S. aureus* (MRSA) (1.56 µg/mL). In the case of next compound 1b, twofold increase in antibacterial activity was reported against *B. thuringiensis* (0.19 µg/mL) and equipotent activity against *S. aureus* (0.39 µg/mL) with 2-phenyl hydrazinylidene substitution. Introduction of hydroxyimino substituents in compound 1d, an excellent inhibition pattern was observed in case of *E. coli* (0.02 µg/mL), *B. thuringiensis* (0.19 µg/mL), *S. aureus* (0.19 µg/mL), MRSA (0.78 µg/mL), whereas equipotent activity against *K. pneumoniae* (0.19 µg/mL), *P. aeruginosa* (0.78 µg/mL) and *S. typhi* (0.09 µg/mL). Notably, twofold amplified antibacterial activity was observed in the case of derivative 1e, against *H. pylori* (0.39 µg/mL) and potent activity against *S. aureus* (0.09 µg/mL), whereas equipotent activity against *P. aeruginosa* (0.78 µg/mL), *B. subtilis* (0.78 µg/mL), *B. thuringieusis* (0.39 µg/mL), and MRSA (1.56 µg/mL) with structural variation of 2-carbamothioyl-hydrazinylidene, at C-4 position in ciprofloxacin. No considerable change in antibacterial activity was observed in the case of compound 1c (2-[2,4-dinitrophenyl] hydrazinylidene), 1f (2-carbamoyl-hydrazinylidene), 1g (2-[pyridine-4-ylcarbonyl] hydrazinylidene), 1h (2-[phenylcarbonyl]hydraziylidene), 1i (2-[(4-chlorophenyl) carbonyl] hydrazinylidene), and 1j (2-[(3, 5-dinitrophenyl) carbonyl] hydrazinylidene). However, drastic decline decrease in activity was reported against rest of the strains except 1g, showed equipotent activity against *E. coli* (0.04 µg/mL).

It was surprising to see that minor structural variation of hydrazinylidene, hydraoxyimino, and 2-carbamothioyl-hydrazinylidene

Table 2: Docking result of 4-oxo substituted (R) Schiff bases of ciprofloxacin

Compounds	R	MIC values (µg/mL)	Docking Score[a] (kcal/mol)	Interacting EcGyr-A QRDR residue with ciprofloxacin analogues
1a	CHH	0.09	−139.52	Ser111, Gly114, Gln267, Asn269
1b	CPH	0.09	−145.27	Arg91, Ser97, Ser111, Ser116, Gln267, Tyr266, Gln267, Asn269
1c	CDNPH	1.56	−102.46	Asp87, Gln94, Phe96, Ser111, Ala117, Thr219, Asn269, Arg272
1d	CHA	0.02	−144.32	Arg91, Ser97, Asp115, Tyr266, Gln267
1e	CTSC	0.78	−119.69	Arg91, Ser97, Phe96, Asp115, Thr219, Tyr266, Gln267
1f	CSC	0.09	−135.56	Asp87, Arg91, Ser171
1g	CINH	0.04	−154.82	Asp87, Arg91, Ser97, Gln267,
1h	CBHZ	0.39	−136.59	Arg91, Gly114, Thr219, Gln267, Asn269
1i	CPCBHZ	1.56	−96.77	Arg91, Ser97, Gly114, Thr219, Tyr266, Gln267
1j	CDNBHZ	0.78	−124.53	Asp87, Arg91, Ser97, Thr219, Gln267, Pro265, Val268, Asn269, Arg272
[b]CFX	=O	0.04	−112.51	Arg91, Ser97, Ser111, Ser116, Gln267

[a]Based on MolDock score, [b]CFX: Ciprofloxacin (standard drug)

Table 3: *In vitro* antimicrobial and antitubercular activities of compounds 1a-j, expressed as MIC (µg/mL)

Compound	Antimicrobial study (MIC, µg/mL)											
	Antibacterial activity									Antifungal activity		Antitub ercular activity
	Gram-negative					Gram positive						
	H. p	*K. p*	*E. c*	*P. a*	*S. t*	*B. s*	*B. t*	*S. a*	*MRSA*	*A. n*	*C. a*	*M. t*
1a	0.78	0.39	0.09	1.56	0.04	3.12	0.19	0.09	1.56	6.25	12.5	1.56
1b	1.56	0.39	0.09	1.56	0.19	6.25	0.19	0.39	3.12	12.5	6.25	0.78
1c	3.12	0.78	1.56	3.12	0.78	1.56	3.12	0.78	6.25	12.5	6.25	6.25
1d	1.56	0.19	0.02	0.78	0.09	1.56	0.19	0.19	0.78	12.5	6.25	3.12
1e	0.39	0.78	0.78	0.78	0.39	0.78	0.39	0.09	1.56	12.5	25	6.25
1f	3.12	0.78	0.09	6.25	0.19	3.12	0.78	0.78	3.12	25	100	0.78
1g	1.56	1.56	0.04	6.25	0.19	3.12	1.56	1.56	3.12	NA	25	0.39
1h	1.56	0.78	0.39	1.56	1.56	1.56	0.78	1.56	6.25	12.5	6.25	3.12
1i	6.25	0.39	1.56	3.12	3.12	6.25	0.78	3.12	12.5	12.5	12.5	1.56
1j	3.12	0.39	0.78	1.56	0.39	1.56	1.56	0.78	12.5	1.56	1.56	6.25
[b]CFX	0.78	0.19	0.04	0.78	0.09	0.78	0.39	0.39	1.56	NA	NA	NA
[c]FCZ	NA	NA	NA	NA	NA	NA	NA	NA	NA	6.25	3.12	NA
[d]INH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.78
[e]Control	-	-	-	-	-	-	-	-	-	-	-	-

Key: Mean values (n=3), Gram-negative bacteria: *H.p: Helicobacter pylori* (ATCC 26695), *K.p: Klebsiella pneumoniae* (ATCC 15380), *E.c: Escherichia coli* (ATCC 25922), *P.a: Pseudomonas aeruginosa* (ATCC 27893), *S.t: Salmonella typhi* (MTCC 3216), Gram-positive bacteria: *B.s: Bacillus subtilis* (ATCC 6633), *B.t: Bacillus thuringiensis* (MTCC 4714), *S.a: Staphylococcus aureus* (ATCC 25323), MRSA: Methicillin resistant *Staphylococcus aureus* (ATCC 33591), Fungal strains: *A.n: Aspergillus niger* (ATCC 9029), *C.a: Candida albicans* (ATCC 90028), Tuberculosis strain: *M. t: Mycobacterium tuberculosis*, NA=Not applicable

[a]MIC: Lowest concentration of an antimicrobial agent that significantly inhibits the visible growth of microorganism after a period of incubation,

[b]CFX: Ciprofloxacin (antibacterial standard), [c]FCZ: Fluconazole (antifungal standard), [d]INH=Isoniazid (antitubercular standard), [e]Control: DMSO (1%)

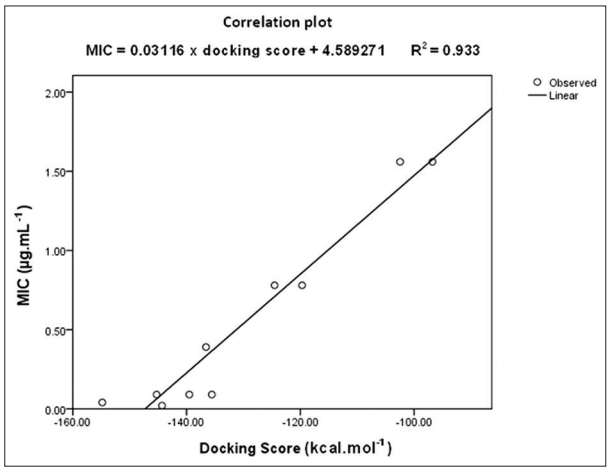


Fig. 3: Correlation graph between minimum inhibitory concentration (µg/mL) values (*Escherichia coli*) of ciprofloxacin derivatives and their docking scores (kcal/mol)

in compound 1a, 1d, and 1e, respectively, induced marked influence on Gram-negative and Gram-positive antibacterial activity. Thus, it is summarized that substitutions of 4-oxo position in ciprofloxacin with above substituents were the main determinant for generation and escalation of bioactivity. In terms of structure-activity relationship, results suggest that the antibacterial activity profile against all bacteria was altered by the formation of hydrazones, oximes, and semicarbazones with ciprofloxacin molecule. It seems that the expansion of activity may be due to better interaction of the molecule with target enzyme or for penetration into these bacteria.

It was surprising to see that minor structural variation of hydrazinylidene and hydraoxyimino in compound 1a, 1d respectively, induced marked influence on Gram-negative and Gram-positive antibacterial activity. Thus, it is summarized that substitution of 4-oxo position in ciprofloxacin with $-NH_2$ and –OH was the main determinant for generation and escalation of bioactivity with regard to structure activity relationships.

Antifungal activity

On result analysis of antifungal activity, the compound 1a showed equipotent activity against *A. niger* (6.25 µg/mL). An excellent antifungal activity was also reported for compound 1j, against *A. niger* (1.56 µg/mL) and *C. albicans* (1.56 µg/mL).

Antitubercular activity

Result indicates that compound 1g (0.39 µg/mL) showed an excellent (twofold) antitubercular activity, whereas equipotent activity for compounds 1b,1f (0.78 µg/mL), and 1a (1.56 µg/mL) against standard drug isoniazid (0.78 µg/mL).

After docking simulation from obtained poses, the binding mode(s) of derivatives with QRDR-A was observed. Evaluation of docking results was based on protein-ligand complementarities considering steric and electrostatic properties, as well as calculated potential interaction energy in the complex. All the compounds interacted with eQRDR-A residue through hydrogen bonds, except compound 1a. The main residues Asp87, Arg91, Gln94, and Ser97 were found interacted with ciprofloxacin derivatives. The docking of all ciprofloxacin derivatives with an active site of QRDR-A showed improved docking score, when compared with the reference ligand ciprofloxacin (−122.51 kcal/mol) except compound 1c, 1e, and 1i. Docking simulation with QRDR-A bound ligand 1g, 1b, and 1d resulted in a dock score of −154.82, −145.27, and −144.32 kcal.mol^{-1} ranked first, second, and third, respectively. In all three compounds, only 1g interacts with Asp87. Along with compound 1g, the compounds 1c, 1f and 1j also interacted with Asp87. The correlation between experimental data (MIC) versus docking score [11,12] displayed 0.93 r^2 (Fig. 3) which suggests that, parameters for docking simulation are good in reproducing experimental orientation of these compounds. On structural analysis of compounds, it was observed that compounds with =N-N-C(=O)-R substituents showed hydrogen bonding with Asp87; the residue which play a major role in *E. coli* resistance.

Parent drug ciprofloxacin interact with Arg91 and Ser97 of eQRDR-A residue, so it may be speculated that the presented ciprofloxacin derivatives 1g, 1b, 1d, 1f, 1h, and 1j may be a successful drug candidates and can play major role to combat bacterial resistance.

CONCLUSION

Finally, it may be concluded that 4-oxo substitution on ciprofloxacin induced marked influence equally on Gram-negative and Gram-positive except compound 1c, 1f, 1h, 1i, and 1j. In comparison with other compounds synthesized, the benzohydrazide analogs 1h and 1j showed potent antifungal activity. The antitubercular activity results up to some extent correlated well with those of antimicrobial activity. Thus, it is summarized that derivatization of 4-oxo position as Schiff bases is optimum and a determinant for generation of bio-activity with regard to structure-activity relationships. The findings of this work should be helpful to medicinal chemists involved in further drug development in this field.

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