INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

SYNTHESIS ANDBIOLOGICAL EVALUATION OF NORFLOXACIN DERIVATIVES

Pradip K. Jena[1], Ashish K. Pathak[2], Susanta K. Sahu[2]* and SwayansiddhaTripathy[2]

[1]Department of Chemistry, College of Basic Science and Humanities,

Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India.

[2]University Department of Pharmaceutical Sciences, Utkal University,

Bhubaneswar, Odisha, India.

ABSTRACT

Topoisomerase-II and topoisomerase-IV (DNA-gyrase) enzymes are required for bacterial DNA replication, transcription, repair, and recombination. Fluoroquinolone analogue norfloxacin has activity against a broad range of gram-negative and gram-positive microorganisms by inhibiting the above enzymes. The fluorine atom at the 6 position increases potency against Gm(-ve) organisms and the piperazine moiety at the 7 position is responsible for anti-pseudomonal activity. A series of norfloxacin Schiff bases were synthesized via >C=N- linkage by reacting it with various primary amines through nucleophilic addition reaction in the presence of glacial acetic acid and were characterized by IR, NMR, MS and elemental analysis techniques. All the synthesized compounds were evaluated for antimicrobial activity against both gram positive and gram-negative bacteria and antifungal activity by measuring zone of inhibition. The unsubstituted and substituted phenyl with nitro substituent induced marked influence in Gram+ve activity against *B. subtilis* among all synthesized derivatives. Some synthesized compounds also showed potent antifungal activity and equipotent antitubercular activity against standard drug isoniazid. The docking result of 4-oxo substituted Schiff bases of norfloxacin has been corelated with the experimental data.

Keywords: Norifloxacin, Schiff bases, DNA Gyrase-A, Docking, SAR.

1. INTRODUCTION

Norfloxacin, a nalidixic acid analog, is the first of the fluorinated quinoline carboxylic acids to be marketed in the United States. It demonstrates potent antibacterial activity against aerobic, gram-negative bacteria including the Enterobacteriaceae, gentamicin-resistant*Pseudomonasaeruginosa*, and penicillin-resistant *Neisseria gonorrhoeae*. Norfloxacin exhibits good activity against methicillin-resistant and -sensitive *Staphylococcus aureus*, but less activity against most other aerobic, gram-positive organisms.[1]The mode of action of norfloxacin depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. The bactericidal action of norfloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination. Norfloxacin is a prototype, narrow spectrum (first generation) fluoroquinolones. The interaction of the quinolones with DNA gyrase is greatly influenced by the C-4 oxo group on the standard structure of 4-quinolones-3-carboxylic acid. Yet some of the researcher have synthesized several N-(2-oxo-2-(4-substituted phenyl) ethyl derivatives with different quinolones including norfloxacin and 6,8-difluoro quinolones have been designed for enhanced antibacterial activity against some Gram-positive and Gram-negative

organism as compared to the parent quinolone.[2] The fluorine atom at the 6 position increases potency against Gm(-ve) organisms, and the piperazine moiety at the 7 position is responsible for anti-pseudomonal activity.[3]Norfloxacin shows promise as an antibacterial agent for genitourinary and gastrointestinal infections.[4]Norfloxacin is an oral fluoroquinolone antimicrobial agent recently released for the treatment of uncomplicated and complicated urinary tract infections. The drug antagonizes DNA gyrase, an enzyme essential for bacterial DNA replication. This drug is administered orally twice daily and achieves high concentrations in urine, stool, renal tissue and bile. Norfloxacin was at least as effective as currently used agents in treating urinary tract infections and limited study has been done in bacterial gastroenteritis, gonorrhea, bacterial prostatitis, and prevention of gram-negative bacillary infection in neutropenic patients.[1,5-6]Adverse drug effects were mild and included disturbances of the gastrointestinal tract and the central nervous system.Resistance has been noted as main problem of norfloxacin, which led to its restricted/limited use.[7] Thus there exists continuous need for newer norfloxacinderivatives with better activity profile and tolerability to overcome the problem of resistance. Because of the lack of data in the literature concerning with the analogues of norfloxacin, we are reporting here the same by introducing new functionality as Schiff bases (Hydrazones, oximes and semicarbazones) against microbes. The docking result of 4-oxo substituted Schiff bases of norfloxacinhave been compared with the experimental data.The structure of norfloxacinhas been shown in **figure 1.**

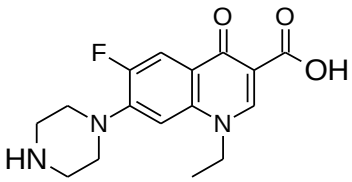
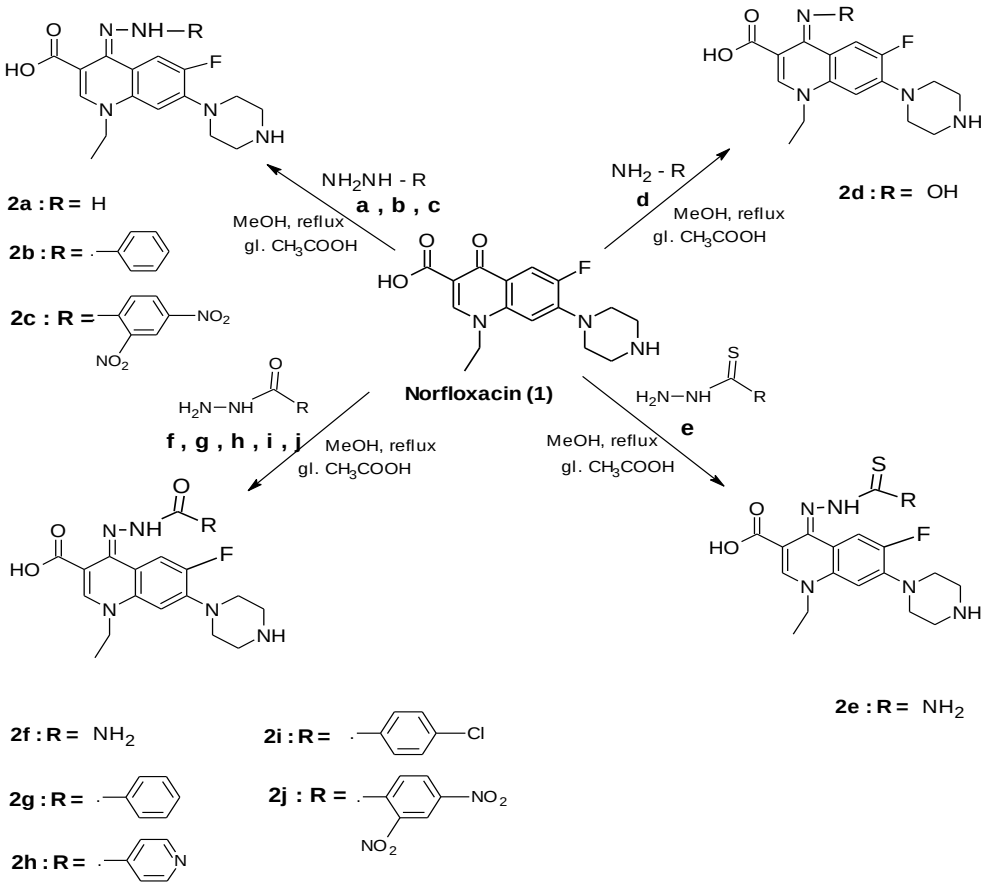


Fig. 1:Structure of Norfloxacin
(1-Ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1,4-dihydro-quinoline-3-carboxylic acid)

2.MATERIALS AND METHODS

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 single spot on TLC plates. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in Bruker DRX-300 FT-NMR spectrometer in 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 ElementarVario 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. norfloxacin and fluconazole were obtained from S. D. Fine Chemicals and Hi-Media Ltd, India. LogPvalues for synthesized derivatives were calculated using ChemDraw Ultra 10.0 (http://www.cambridgesoft.com).

Purification and drying of reagents and solvents was carried out according to standard literature procedure (Furniss et al., 1980). The general procedure for the preparation of 1N-piperazinylSchiff bases norfloxacin(1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4- dihydro-quinoline-3- carboxylic acid analogues are described inScheme1.norfloxacin(0.5 mmol), andvarious amines (hydrazine, hydroxylamine, semicarbazide,thiosemicarbazide, aniline, phenyl hydrazine, 2,4-dinitrophenyl hydrazine, isonicotinylhydrazideand substituted benzoyl hydrazides) (0.5 mmol) was reacted at 85-90^{o}C for 9-14 hrs. respectively, in ethanol with glacial acetic acid for 9-14 hrs. at 110-120^{o}C. Progress of the reaction was observed by TLC monitoring on silica-gel 60 F254 platesuntil 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 2**a-h.** Final product was characterized by measuring melting point and Rfvalues using solvent system chloroform: methanol (9:1) has been shown in Table 1.



Scheme 1- Synthesis of Schiff bases of norfloxacin.
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** = $NH_2NHC(=O)Ph(NO_2)_2$

Spectral data

2.1.1-Ethyl-6-fluoro-4-hydrazono-7-piperazin-1-yl-1,4-dihydro-quinoline-3-carboxylic acid (2a) NHH

IR ν_{max}(cm^{-1}, ATR): 3362 (N–H, str.) 3046 (C–H str, Ar.), 2972 (O-H str., Carboxylic), 1714 (C=O str, carboxylic), 1625 (C = N, imine),1257 (C-F str.) 1037 (C-N str. piperazine), **^{1}H NMR (300 MHz, DMSO) δ:** 1.32- 1.45 (t, 3H, ethyl), 2.47 (s, 1H, piperazine), 3.24 -3.48 (m, 8H, piperazine), 4.45 (q, 2H, ethyl), 7.20 - 7.27 (d, 1H, H-8), 8.10 (s, 2H, NH_2 -hydrazine), 8.11 (d, 1H, H-5), 8.64 (s, 1H, H-2), 14.79 (s, 1H, -COOH, quinolone), **MS-ESI:** ***m/z*** 334.16 (M+1),**Elemental analysis (%):** Calcd.for $C_{16}H_{20}FN_5O_2$ (333.36) Cal%: C, 57.65; H, 6.05; N, 21.01 Found: C, 57.72; H, 6.01; N, 20.92.

2.2. 1-Ethyl-6-fluoro-4-(phenyl-hydrazono)-7-piperazin-1-yl-1,4-dihydro-quinoline-3-carb-oxylic acid (2b) NPH

IR ν_{max} (cm^{-1}, ATR): 3364 (N–H, str.) 3045 (C–H str, Ar.), 2969 (O-H str., Carboxylic), 1715 (C=O str, Carboxylic), 1625 (C=N, Imine), 1265 (C-F str.), 1041 (C-N str. Piperazine), **^{1}H NMR (300 MHz, DMSO) δ:** 1.37 – 1.42 (t, 3H, CH_3, ethyl), 2.48 (s, 1H, piperazine), 3.28 – 3.50 (m, 8H, piperazine), 4.59 (q, 2H, CH_2, ethyl), 7.23 – 7.25 (d, 1H, H-8), 7.94 (d, 1H, H-5), 7.98 (m , 5H, Phenyl), 8.47 (s, 1H, -NH), 8.96 (s, 1H, H-2), 14.80 (s, 1H, -COOH, quinolone), **Elemental analysis (%):** Calcd.for $C_{22}H_{24}FN_5O_2$(409.45) Cal%: C, 64.53; H, 5.91; N, 17.10; Found: C, 64.68; H, 6.03; N, 16.98.

2.3. 4-[(2,4-Dinitro-phenyl)-hydrazono]-1-ethyl-6-fluoro-7-piperazin-1-yl-1,4-dihydro-quinoline- 3-carboxylic acid (2c) NDNP*H*

IR ν_{max} (cm^{-1}, ATR): 3361 (N–H, str.) 3045 (C–H str., Ar.), 2973 (O-H str., Carboxylic), 1714 (C=O str, Carboxylic), 1626 (C=N, Imine), 1529 ($ArNO_2$, str., Assym.), 1352 ($ArNO_2$, str., Symm.), 1262 (C-F str.), 1039 (C-N str. Piperazine), **^{1}H NMR (300 MHz, DMSO-d_6) δ:** 1.38 – 1.46 (t, 3H, CH_3, ethyl), 2.52 (s, 1H, piperazine), 3.31-3.52 (m, 8H, piperazine), 4.88 (q, 2H, CH_2, ethyl), 6.46-7.10 (m , 3H, Phenyl), 7.27-7.32 (d, 1H, H-8), 8.32 (d, 1H, H-5), 8.80 (s, 1H, -NH)8.96 (s, 1H, H-2), 14.79 (s, 1H, -COOH, quinolone), **Elemental analysis** (%)**:** Calcd.for $C_{22}H_{22}FN_7O_6$:C,

52.91; H, 4.44; N, 19.63; Found:C, 53.07; H, 4.31; N, 19.78.

2.4.1-Ethyl-6-fluoro-4-hydroxyimino-7-piperazin-1-yl-1,4-dihydro-quinoline-3-carboxylic acid (2d)NHA

IR v_{max} (cm^{-1}, ATR): 3394 (N–H, str.) 3045 (C–H str, Ar.), 2965 (O-H str., Carboxylic), 1715 (C=O str, Carboxylic), 1626 (C=N, Imine), 1260 (C-F str.), 1035 (C-N str. Piperazine), **^{1}H NMR (300 MHz, DMSO) δ:** 1.35 – 1.44 (t, 3H, CH_3, ethyl), 2.46 (s, 1H, piperazine), 3.31 – 3.50 (m, 8H, piperazine), 4.52 (q, 2H, CH_2, ethyl), 7.54 – 7.61 (d, 1H, H-8), 8.07 (d, 1H, H-5), 8.72 (s, 1H, H-2), 11.65 (s,1H, NOH, D_2O exchangable), 14.83 (s, 1H, -COOH, quinolone), **Elemental analysis (%):** Calcd.for $C_{16}H_{19}FN_4O_3$ (334.34) Cal%: C, 57.48; H, 5.73; N, 16.76; Found: C, 57.34; H, 5.85; N, 16.79.

2.5. 4-(2-carbamoylhydrazinylidene)-1-ethyl-6-fluoro-7-(piperazin-1-yl)-1,4-dihydro-quin-oline -3- carboxylic acid (2e) NSC

IR v_{max} (cm^{-1}, ATR): 3365 (N–H, str) 3042 (C–H str, Ar.), 2962 (O-H str, Carboxylic), 1714 (C=O str, Carboxylic), 1667 (Amide-I), 1626 (C=N, Imine), 1529 (Amide-II), 1259 (C-F str.), 1035 (C-N str, Piperazine), **^{1}H NMR (300 MHz, DMSO) δ:** 1.38 – 1.42 (t, 3H, CH_3, ethyl), 2.51 (s, 1H, piperazine), 3.26 – 3.54 (m, 8H, piperazine), 4.52 (q, 2H, CH_2, ethyl), 6.10 (s, 2H, -$CONH_2$), 7.96 – 8.07 (d, 1H, H-8), 8.10 (s, 1H, -NH), 8.14 (d, 1H, H-5), 8.76 (s, 1H, H-2), 14.83 (s, 1H, -COOH, quinolone), **Elemental analysis (%):** Calcd.for $C_{17}H_{21}FN_6O_3$(376.38) Cal%: C, 54.25; H, 5.62; N, 22.33; Found: C, 54.39; H, 5.71; N, 22.40.

2.6.4-(2-carbamothioylhydrazinylidene)-1-ethyl-6-fluoro-7-(piperazin-1-yl)- 1,4-dihydro quinoline-3-carboxylic acid (2f) NTSC

IR v_{max} (cm^{-1}, ATR): 3345 (N–H, str.) 3044 (C–H str, Ar.), 2971 (O-H str, Carboxylic), 1713 (C=O str, Carboxylic), 1624 (C=N, Imine), 1261 (C-F str.), 1036 (C-N str. Piperazine), 1235 (C=S), **^{1}H NMR (300 MHz, DMSO) δ:** 1.38- 1.48 (t, 3H, CH_3, ethyl) 2.51 (s, 1H, piperazine), 3.31-3.61 (m, 8H, piperazine), 4.57 (q, 2H, CH_2, ethyl), 6.73 - 6.90 (s, 2H, -$CSNH_2$), 7.96 - 8.09 (d, 1H, H-8), 8.20 (s, 1H, -NH), 8.32 (d, 1H, H-5), 8.68 (s, 1H, H-2), 14.76 (s, 1H, -COOH, quinolone), **Elemental analysis (%):** Calcd.for $C_{17}H_{21}FN_6O_2S$ (392.45) Cal%: C, 52.03; H, 5.39; N, 21.41; Found; C, 51.92; H, 5.54; N, 21.32.

2.7.1-Ethyl-6-fluoro-7-piperazin-1-yl-4-[(pyridine-4-carbonyl)-hydrazono]-1,4-dihydro-quinoline-3-carboxylic acid (2g) NINH

IR v_{max} (cm^{-1}, ATR): 3395 (N–H, str.) 3046 (C–H str, Ar.), 2978 (O-H str., Carboxylic), 1716 (C=O str, Carboxylic), 1665 (Amide-I), 1623 (C=N, Imine), 1527 (Amide-II) 1434 (C-N ring str., Pyridine) 1240 (C-F str.), 1042 (C-N str. Piperazine), **^{1}H NMR (300 MHz, DMSO-d_6) δ:** 1.37 - 1.44 (t, 3H, CH_3, ethyl), 2.48 (s, 1H, piperazine), 3.30- 3.61 (m, 8H, piperazine), 4.52 (q, 2H, CH_2, ethyl), 7.54- 7.64 (d, 1H, H-8), 8.36 (d, 1H, H-5), 8.40 (s, 1H, -NH), 8.73 – 9.70 (q, 4H, Pyridine), 8.82 (s, 1H, H-2), 14.82 (s, 1H, -COOH, quinolone), **Elemental analysis** (%)**:** Calcd.for $C_{22}H_{23}FN_6O_3$:C, 60.27; H, 5.29; N, 19.17; Found:C, 60.12; H, 5.42; N, 18.98.

2.8. 4-(Benzoyl-hydrazono)-1-ethyl-6-fluoro-7-piperazin-1-yl-1,4-dihydro-quinoline-3-car-boxylic acid (2h) NBHZ

IR v_{max} (cm^{-1}, ATR): 3378 (N–H, str.) 3042 (C–H str., Ar.), 2965 (O-H str., Carboxylic), 1714 (C=O str, Carboxylic), 1667 (Amide-I), 1624 (C=N, Imine), 1534 (Amide-II), 1265 (C-F str.)1049 (C-N str. Piperazine), **^{1}H NMR (300 MHz, DMSO-d_6) δ:** 1.31 – 1.41 (t, 3H, CH_3, ethyl), 2.52 (s, 1H, piperazine), 3.26 – 3.48 (m, 8H, piperazine), 4.81 (q, 2H, CH_2, ethyl), 8.68 (s, 1H, H-2), 6.92 – 7.26 (m, 5H, Phenyl), 7.27 – 7.33 (d, 1H, H-8), 7.98 (d, 1H, H-5), 8.95 (s , 1H, -NH),14.78 (s, 1H, -COOH, quinolone),**Elemental analysis** (%)**:** Calcd.for $C_{23}H_{24}FN_5O_3$:C, 63.15; H, 5.53; N, 16.01; Found:C, 63.31; H, 5.37; N, 15.92.

2.9.4-[(4-Chloro-benzoyl)-hydrazono]-1-ethyl-6-fluoro-7-piperazin-1-yl-1,4-dihydroquino-line-3-carboxylic acid (2i) NPCBHZ

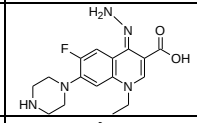
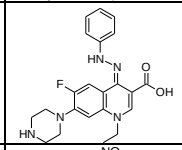
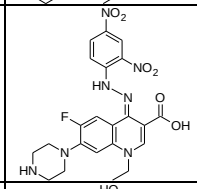
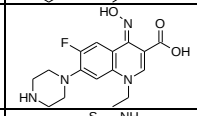
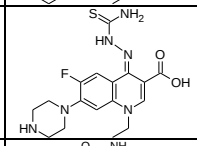
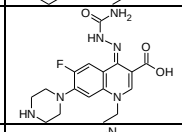
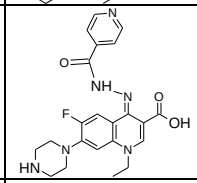
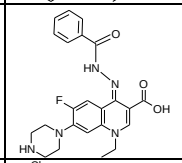
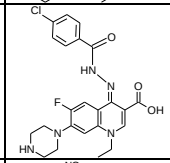
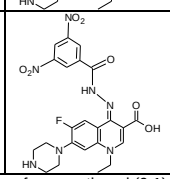
IR v_{max} (cm^{-1}, ATR): 3384 (N–H, str.) 3049 (C–H stre, Ar.), 2972 (O-H str., Carboxylic), 1715 (C=O str, Carboxylic), 1532 (Amide-II), 1622 (C=N, Imine), 1668 (Amide-I), 1267 (C-F str.), 1052 (C-N str. Piperazine), 750 (C-Cl str.), **^{1}H NMR (300 MHz, DMSO-d_6) δ:** 1.35 – 1.47 (t, 3H, CH_3, ethyl), 2.47 (s, 1H, piperazine), 3.28 – 3.50 (m, 8H, piperazine), 4.57 (q, 2H, CH_2, ethyl), 6.71 – 7.05 (m , 4H, Phenyl), 7.23 – 7.27 (d, 1H, H-8), 8.14 (d, 1H, H-5), 8.73 (s, 1H, -NH),8.76 (s, 1H, H-2), 14.69 (s, 1H, -COOH, quinolone), **Elemental analysis** (%)**:** Calcd.for $C_{23}H_{23}ClFN_5O_3$:C, 58.54; H, 4.91; N, 14.84 ; Found:C, 58.70; H, 4.83; N, 15.03.

2.10. 4-[(3,5-dinitro-benzoyl)-hydrazono]-1-ethyl-6-fluoro-7-piperazin-1-yl-1,4-dihydro-quinoline-3-carboxylic acid (2j) NDNBHZ

IR v_{max} (cm^{-1}, ATR): 3385 (N–H, str.) 3046 (C–H str., Ar.) 2976 (O-H str., Carboxylic), 1716 (C=O str, Carboxylic), 1662 (Amide-I), 1625 (C=N, Imine), 1529 (Amide-II), 1521 ($ArNO_2$,

str., Assym.), 1343 ($ArNO_2$, str., Symm.), 1267 (C-F str.), 1053 (C-N str. Piperazine), **^{1}H NMR (300 MHz, DMSO-d_6) δ:** 1.38- 1.46 (t, 3H, CH_3, ethyl), 2.51 (s, 1H, piperazine), 3.31 – 3.51 (m, 8H, piperazine), 4.47 (q, 2H, CH_2, ethyl), 7.77 – 8.13 (m, 3H, Phenyl), 7.94 – 8.02 (d, 1H, H-8), 8.32 (d, 1H, H-5), 8.35 (s , 1H, -NH),8.96 (s, 1H, H-2), 14.75 (s, 1H, -COOH, quinolone), **Elemental analysis** (%)**:** Calcd.for $C_{23}H_{22}FN_7O_7$:C, 52.37; H, 4.20; N, 18.59; Found:C, 52.51; H, 4.27; N, 18.43.

Table 1:Physicochemical parameter of the synthesized compounds

Compd.	Synthesized	Structures	Mol. Formula	Mol wt.	Yield (%)	Melting point (^{0}C)	[a]Rf Value	[b]LogP	[c] R.t (hr.)
1a HH	**2a**		$C_{16}H_{20}FN_5O_2$	333.36	65.57	196-198	0.54	1.10	11
1b PH	**2b**		$C_{22}H_{24}FN_5O_2$	409.46	78.80	144-145	0.77	3.00	6
1c DNPH	**2c**		$C_{22}H_{22}FN_7O_6$	499.45	67.85	137-139	0.87	3.41	11
1d HA	**2d**		$C_{16}H_{19}FN_4O_3$	334.35	76.85	248-250	0.48	1.75	8
1e TSC	**2e**		$C_{17}H_{21}FN_6O_2S$	392.45	88.82	242-244	0.68	1.28	8
1f SC	**2f**		$C_{17}H_{21}FN_6O_3$	376.39	91.39	257-258	0.75	0.72	8
1g INH	**2g**		$C_{22}H_{23}FN_6O_3$	438.45	95.37	186-188	0.58	1.61	11
1h BHZ	**2h**		$C_{23}H_{24}FN_5O_3$	437.47	64.36	181-183	0.43	2.95	11
1i PCBHZ	**2i**		$C_{23}H_{23}ClFN_5O_3$	471.91	97.47	171-173	0.64	3.51	31
1j DNBHZ	**2j**		$C_{23}H_{22}FN_7O_7$	527.46	61.58	220-222	0.83	2.14	10

[a]Solvent system: chloroform: methanol (9:1), [b]Calculated by ChemDraw Ultra 10.0 (http://www.cambridgesoft.com), [c]R. t = Reaction time (hours) at 110-1

Biological evaluation

Synthesized norfloxacin analogues were screened for their antibacterial activity against Gram-negative; *Helicobacter pylori* (ATCC 26695), *Klebsiellapneumoniae*(ATCC 15380), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa*(ATCC 27893), *Salmonella typhi*(MTCC 3216), Gram-positive; *Bacillus subtilis*(ATCC 6633), *Bacillus thuringiensis*(MTCC 4714), *Staphylococcus aureus*(ATCC 25323), methicillin resistant*Staphylococcus aureus* (ATCC 33591) (MRSA) bacterial strains and on *Aspergillusniger*(ATCC 9029) and *Candida albicans* (ATCC 90028) fungal strains using conventional agar dilution method.[8]Two-fold serial dilutions of the compounds and reference drugs (nalidixic acid and fluconazole) were prepared in Mueller-Hinton agar for bacteria and in Sabouraud dextrose agar for fungi. Drugs (10.0 mg) were dissolved in dimethyl-sulfoxide (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 $\mu g.mL^{-1}$. 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 35-37ºC for 18-20 hrs for bacteria and 28-30ºC for 48-72 hrs for fungi and the minimum inhibitory concentration (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.Compounds**2a-h** was screened for their antibacterial activity against Gram-negativeand Gram-positive bacterial strains by the agar dilution method.[10]The anti-mycobacterial activity of the synthesized compounds **2a-j** were assessed against M. tuberculosis H37Rv (ATCC 2729411) using the MicroplateAlamar Blue Assay (MABA). [11-12]This methodology is non-toxic, uses thermally stable reagent, and shows good correlation with proportional and BACTEC radiometric methods.[13] The activity was expressed as minimum inhibitory concentration (MIC) in µg/mL. The drug concentration tested was in the range 0.1-100.0 µg/mL. A blue color in the well was interpreted as absence of bacterial growth, and pink color was scored as growth. MIC (minimal inhibition concentration) was defined as the lowest drug concentration, which prevented a color change from blue to pink. Isoniazid was used as reference standard. The result has been shown in **Table 2**.

Molecular docking studies of norfloxacin analogues

The molecular docking study of norfloxacin 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).14 All calculations were conducted on IntellCore2 Duo T6400, 1.20 GHz dual processing machine. Docking of norfloxacin 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.15-19 The ducking result has been shown in Table 3.

Table 2:*In vitro* antimicrobial and antitubercular activities of compounds 2a-j, expressed as [a] MIC (µg.mL^{-1})

Comp.		Antimicrobial study (MIC, µg.mL^{-1})												
		Antibacterial activity									Antifungal activity		Antitub-ercular activity	
		Gram -ve					Gram +ve							
		H. p	*K. p*	*E. c*	*P. a*	*S. t*	*B. s*	*B. t*	*S. a*	*MRSA*	*A. n*	*C. a*	*M. tcip*	Actual
2a NHH		6.25	0.78	6.25	3.12	3.12	3.12	6.25	3.12	3.12	12.5	6.25	1.56	3.12
2e NPH		6.25	3.12	3.12	3.12	1.56	0.78	1.56	1.56	3.12	NS	12.5	0.78	6.25
2f NDNPH		12.5	3.12	1.56	0.39	6.25	0.78	12.5	0.78	6.25	50	6.25	6.25	12.5
2b NHA		1.56	6.25	12.5	6.25	1.56	6.25	3.12	1.56	1.56	12.5	6.25	3.12	3.12
2d NTSC		6.25	6.25	1.56	0.78	1.56	1.56	3.12	0.78	3.12	3.12	0.78	6.25	6.25
2c NSC		3.12	0.78	12.5	1.56	3.12	3.12	1.56	1.56	1.56	12.5	1.56	0.78	6.25
2g NINH		12.5	1.56	0.39	3.12	0.78	3.12	6.25	0.39	3.12	NS	NS	0.39	1.56
2h NBHZ		6.25	1.56	0.19	1.56	1.56	1.56	6.25	1.56	6.25	3.12	12.5	3.12	3.12
2i NPCBHZ		12.5	6.25	3.12	3.12	3.12	3.12	3.12	3.12	12.5	50	12.5	1.56	0.78
2j NDNBHZ		6.25	3.12	6.25	6.25	0.39	6.25	3.12	1.56	25	6.25	6.25	6.25	6.25
[c] NFX		3.12	1.56	0.19	1.56	0.78	1.56	1.56	0.78	1.56	NA	NA	NA	NA
[d] FCZ		NA	NA	NA	NA	NA	NA	NA	NA	NA	6.25	3.12	NA	NA
INH		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.78	0.78
[e] Control		---	---	---	---	---	---	---	---	---	---	---	---	---

Key: Mean values (n = 3), Gram-negative bacteria:*H.p: *Helicobacter pylori* (ATCC 26695), ***K.p:****Klebsiellapneumoniae*(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*Staphylococcusaureus* (ATCC 33591), **Fungal strains:*A.n:****Aspergillusniger*(ATCC 9029), ***C.a:****Candidaalbicans* (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]**NFX:** Norfloxacin (antibacterial standard).

[c]**FCZ:** Fluconazole (antifungal standard)

[d]**INH =** Isoniazid(antitubercular standard)

[e]**Control:** DMSO (1%).

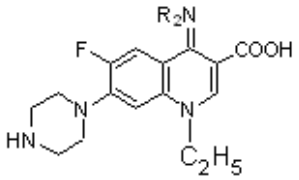
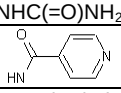


Table 3:Docking result of 4-oxo substituted Schiff bases of norfloxacin

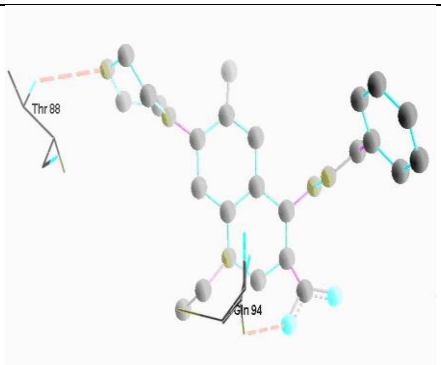
Compd.	R	MIC values (µg.mL^{-1})	Docking Score [a] (kcal.mol^{-1})	Interacting residues
2a	NH_2	6.25	-98.37	Arg91, Phe96, Ser97
2b	NHPh	3.12	-118.45	Arg91
2c	NHPh $(NO_2)_2$	1.56	-120.35	Ser97
2d	OH	12.5	-90.35	Arg91
2e	$NHC(=S)NH_2$	1.56	-117.14	Ser97, Arg91
2f	$NHC(=O)NH_2$	12.5	-88.88	Arg91
2g		0.39	-141.23	Arg91,Thr219 andGly114
2h	NHC(=O)Ph	0.19	-138.64	Thr88 and Gln94
2i	NHC(=O)PhCl	3.12	-114.84	Arg91
2j	$NHC(=O)Ph(NO_2)_2$	6.25	-108.55	Gln94
[b]NOR	= O	0.19	-99.79	Arg91

[a]Based on MolDock score [b] NOR = Norfloxacin (Standard drug)

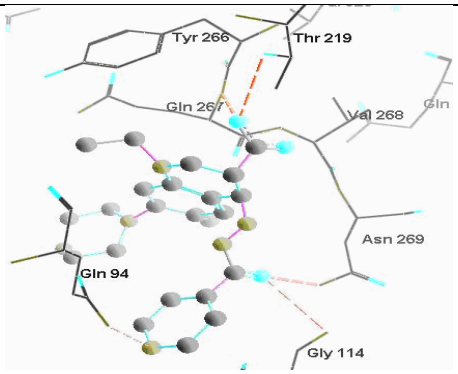
3.RESULTS AND DISCUSSION

The synthetic route to obtain the necessary derivatives from commercially available reagents is briefly outlined in **scheme1.** The title Schiff base of norfloxacin formed via >C=N- linkage were accomplished by reaction of norfloxacin and various primary amines **(a-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 production of titled derivatives. IR spectrums were recorded in the range of 4000-650 cm^{-1} to ensure presence of various functional groups. In this context, the characteristic group stretching frequencies of carbonyl (C=O) of parent compound (norfloxacin) tend to appears at 1631 cm^{-1}, whereas the imines (>C=N) at 1622-1626 cm^{-1}, indicated 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 resonance of quinolone protons, from δ 8.64-8.96 ppm as a singlet for 1H, C-2 position, δ 7.94-8.58 ppm as a doublet for 1H, C-5 position, δ 3.08-3.66 ppm as a multiplet for 8H and δ 2.46-2.52 ppm as a singlet for 1H, -NH, C-7 piperazine. A singlet/triplet from δ 1.31-1.93 ppm and a quartet from δ 4.47-4.88 ppm were attributed to methyl and methylene protons of ethyl chain at N-1 position. Other protons corresponded to doublet 1H, C-8 and singlet, 1H carboxylic, C-3 was observed in the spectrum at δ 7.20-8.09 ppm and δ 14.72-15.13 ppm respectively. The mass spectrum of compound is characterized by their M+1 peak. Elemental analysis was within ± 0.4% of the theoretical values in agreement with the proposed structures.

The title Schiff bases of norfloxacin2 **(a-j)** showed excellent to significant susceptibilities towards Gram-ve, Gram+ve bacterial and fungal strainsas shown in **Table 2**.All the synthesized compounds were evaluated for antimicrobial activity against both gram positive and gram-negative and antifungal activity by measuring zone of inhibition.Theunsubstitutedand substituted phenyl with nitro substituent induced marked influence in Gram+ve activity against *B. subtilis* among all synthesized derivatives.On result analysis of antifungal activitythe compound **2b**, **2c**, **2h** showed potent antifungal action.The compound **2i** showed an equipotent antitubercular activityagainst standard drug isoniazid.A series of designed norfloxacin Schiff bases were docked within the "Quinolone Resistance Determining Region" (QRDR) of E. coli DNA Gyrase-A (EcGyr-A) chain (QRDR-A), to evaluate the possible relationship between docking scores and their contribution to biological activity along with the interaction with target residues. The compound **2b**, **2h** and **2g** resulted in a dock score of -120.35, -138.64 and -141.23 $kcal.mol^{-1}$ respectively. Evaluation of the docking results was based on protein-ligand complementarities considering steric and electrostatic properties as well as calculated potential interaction energy in the complex. The Compound **2g**showed highest docking score interacting with QRDR residue Arg91, Thr219 and Gly114 followed by compound **2h**which interacts with Thr88 and Gln94 have been shown in **Fig.2**.



NBHZ (2h) -1AB4-02



NINH (2g) -1AB4 – 00

Fig.2:Interaction of compounds 2g and 2h shown by dotted lines (blue) with QRDR along with other residues of *E.coli* DNA Gyrase-A

4.CONCLUSION

In summing up, a series of 4-oxo substituted Schiff bases of norfloxacin have been modelled successfully and docking analysis was carried out with QRDR of EcGyr-A to investigate the role of these derivatives, which indicated the importance of oximes, hydrazones and semicarbazones moieties. The docking score showed significance in prediction of antimicrobial activity. It can be concluded from the presented research that this work shows substantial promise to understand the basis of the mechanism of inhibition of QRDR of EcGyr-A and also in the prediction of the antimicrobial activity of other novel fluoroquinolone derivatives. Again the findings of this work would be helpful to medicinal chemists involved in further drug development in this field.

REFERENCES

1. Randall CRPD, Deniel JMPD and Judith CTPD.Norfloxacin: Clinicalpharmacology and clinical use.Pharmacotherapy.1987;9(4):92-106.
2. Friedman SM, Lu T and Drlica K. Mutation in the DNA Gyrase A gene of Escherichia coli that expands the Quinolone Resistance-Determining Region.Antimicrob. Agents Chemother.2001;45(8):2378-2380.
3. Newsom SWB.The antimicrobial spectrum of Norfloxacin, Journal of Antimicrobial Chemotherapy. 1984;13(B):25-31.
4. JohnsWolfson and David C. Hooper.Norfloxacin: A New Targeted Fluoroquinolone Antimicrobial Agent.ANN INTERN MED.1988;108(2):238-251.
5. Dupont HL, Corrado ML and Sabbaj J. Use of norfloxacin in the treatment of acute diarrheal disease. The American J. of Medicine.1987;82(6):79-83.
6. Kamili MA. Shah MY, Ali G, Kowl RL, Aziz A, Allaqaband GQ and Hussain A. Norfloxacinin multiple Drug Resistant Typhoid fever.Journal of the Indian Medical Association. 1991;23(1):29-31.
7. Colovos C and Yeates TO.Protein Science. 1993;2:1511-1519.
8. Oliphant GMand Green GM.American Family Physician. 2002;65:455-464.
9. Andres CJ, Bronson JJ, D'Andrea SV, Deshpande MS, Falk PJ, Grant-Young KA, Harte W, Ho HT, Misco PF, Robertson JG, Stock D, Sun Y and Walsh WA.Bioorg MedChemlett. 2000;10:715-722.
10. Letafat B, Emami S, Mohammadhosseini N, Faramarzi MA, Samadi N, ShafieeA andForoumadi A. Synthesis and antibacterial activity of new N-[2-(thiophen-3-yl)ethyl]piperazinyl quinolones, Chemical and Pharmaceutical Bulletin.2007;55(6):894-898.
11. Baker CN, BanerjeeSN and TenoverFC. Evaluation of Alamar colorimetric MIC method for antimicrobial susceptibility testing of gramnegative bacteria. J ClinMicrobiol. 1994;32:1261–1267.
12. Chung GAC, Aktar Z, Jackson S and Duncan K. High-throughput screen for detecting antimycobacterial agents. Antimicrob Agents Chemother. 1995;39:2235–2238.
13. Cummings DM, Ristroph D, Camargo EE, Larson SM and Wagner HN.Jr Radiometric detection of the metabolic activity of Mycobacterium tuberculosis. J Nucl Med. 1975;6(12):1189–1191.
14. Bambeke FV, Michot JM,Eldere JV and Tulkens PM.ClinMicrobiolInfection. 2005;11:256-280.
15. SchuttelkopfAW and Alten DMFV.PRODRG-a tool for high-throughput crystallography of protein-ligand complexes.Acta Crystallographica.2004 ;D(60) :1355-1363.
16. Raghuvir RS, Pissur L, Mushtaque SS, Radhakrishnan PI and Evans CC. Molecular mechanics force fields and their applications in drug design.Anti-infective Agents in Medicinal Chemistry.2009;8(2):128-150.
17. Yoshida H, Bogaki M, Nakamura M and Nakamura S. Quinolone resistance-determining region in the DNA gyrasegyrA gene of *Escherichia coli*. Antimicrobial Agents Chemotherapy.1990;34(6):1271-1272.
18. Conrad S, Oethinger M, Kaifel TK, Klotz G, Marre R and Kern MV. gyrA Mutations in high-level fluoroquinolone-resistant clinical isolates of Escherichia coli.Journal of Antimicrobial Chemotherapy.1996;38(3):443-455.
19. Friedman SM, Lu T and Drlica K. Mutation in the DNA Gyrase A gene of *Escherichia coli* that expands the Quinolone Resistance-Determining

Region.Antimicrobial Agents Chemotherapy.2001;45(8):2378-2380.
20. ChemDraw Ultra 10.0 (http://www.cambridgesoft.com).
21. 21.http://www.druglib.com/druginfo/noroxin/description_pharmacology/