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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and docking studies of novel ofloxacin analogues as antimicrobial agents

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Abstract A number of novel ofloxacin analogues were synthesized by modifying the carboxylic acid at C-6. To investigate the antimicrobial data on structural basis, *in-silico* docking studies of the tested compounds into the crystal structure of topoisomerase II using Autodock vina 4.0 program was performed in order to predict the affinity and orientation of the synthesized compounds at the activities. R_2 values show good agreement with predicted binding affinities obtained by molecular docking studies. Also, it is verified by *in-vitro* antimicrobial screening, where all the compounds were most active against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*. Among these compounds **3a**, **3b**, **3f** showed good MIC (0.125 μg/ml).

Keywords Ofloxacin · Schiff bases · Antimicrobial · Molecular docking

Introduction

Quinolone antibacterials are known to be very effective therapeutic agents for the treatment of various infectious agents. Introduction of a fluorine atom into the C-6 position of quinolone ring system brought to norfloxacin, the first broad spectrum antibacterial agent, which opened new era of fluoroquinolone antibacterials. The most often used relatively safe and well-tolerated 6-fluoroquinolones as antibacterials include norfloxacin (NFX), ofloxacin (OFX), ciprofloxacin (CPFX), levofloxacin (LVFX), moxifloxacin (MXFX) and gatifloxacin (GTFX) (Chang *et al.*, 1997; Guillaume *et al.*, 2005). Most of the quinolones currently on the market or underdevelopment have only moderate activity against many Gram-positive cocci, including Staphylococci and Streptococci (Foroumadi *et al.*, 2006). Ofloxacin is a second generation fluoroquinolone used to treat various bacterial infections. It is more effective against Gram-negative organisms than Gram-positive ones. This moderate activity against some of the Gram-positive species limited its use in bacterial infections (Dinakaran *et al.*, 2008).

Molecular docking plays an important role in the rational design of drugs. In the field of molecular modelling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Molecular docking can be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest (Hamed *et al.*, 2007; Maria *et al.*, 2005). Schiff bases, heterocyclic compounds such as oxadiazoles and mercaptotriazoles were reported to have a broad spectrum of antibacterial activity (Nagalakshmi and Dhaka 2007; Wu *et al.*, 2007). Therefore, on continuation of our work (Jubie *et al.*, 2010a, b) efforts have been directed toward the synthesis of new quinolone antibacterials that can provide improved Gram-positive antibacterial activity, while retaining good Gram-negative activity. In this study, we introduced various Schiff bases, 1,3,4-oxadiazole-5-thione and 4-amino-1,2,4-triazole-3-thione into the quinolone antibacterial ofloxacin at its C-6 position and

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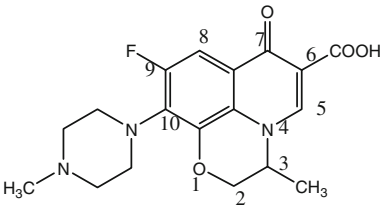
to evaluate the effect of these groups on the antibacterial activity. Molecular docking study was then employed for the analysis with training set composed of 16 novel compounds whose inhibitory activities are unknown, in order to find out the molecular facilities responsible for biological activities. Then antibacterial screening was done to determine their MIC values.

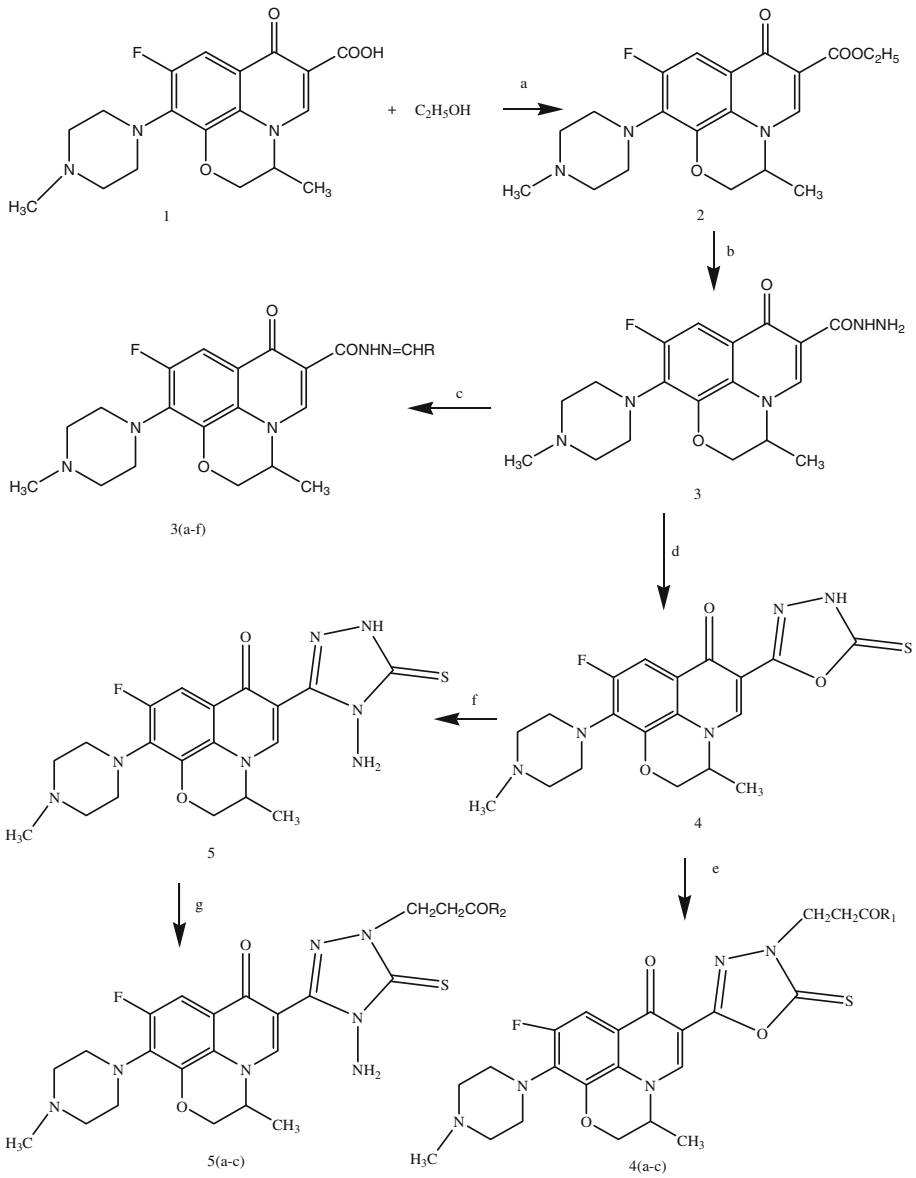


Ofloxacin

Results and discussion

Chemistry

The compounds described in this article were synthesized by the multi-step reaction protocol described in the Scheme 1. All the new compounds were characterized by spectroscopic data (^{1}HNMR, MS and IR). The spectral data of the new compounds reported in this study correlate with the proposed structures. Ofloxacin ester (**1**) was prepared from a reaction between ofloxacin and ethyl alcohol by Fischer esterification reaction. It was converted to Ofloxacin hydrazide (**2**) by the reaction with hydrazine hydrate. Three series of Ofloxacin derivatives were prepared from Ofloxacin hydrazide. Schiff bases (**3a–f**) were prepared from a reaction of primary amine of hydrazide with different



Scheme 1 Synthesis of the compounds. Reagents and conditions. (*a*) H_2SO_4, reflux 48 h; (*b*) NH_2NH_2, reflux 12 h; (*c*) RCHO, C_2H_5OH, MWI 240 W, 15 min; (*d*) Aq. KOH/C_2H_5OH, CS_2/reflux 18 h; (*e*) 40% HCHO, R_1COCH_3; (*f*) NH_2NH_2, reflux 18 h; (*g*) 40% HCHO, R_2COCH_3

substituted aromatic aldehydes. Ofloxacin hydrazide was undergone ring closure reaction to form 1,3,4-oxadiazole-5-thione (**4**) by the reaction with a mixture of carbon disulphide and aqueous potassium hydroxide. It was converted into 4-amino-1,2,4-triazole-3-thione (**5**) by the reaction with hydrazine hydrate. A series of Mannich bases (**4a–c** and **5a–c**) were prepared by condensing active hydrogen atom of substituted ketones with formaldehyde and the secondary amino function (–NH of oxadiazole and triazole).

In-silico drug docking

Binding affinities of the synthesized compounds into topoisomerase-II

The molecular docking studies showed a good correlation between their MIC and auto dock binding free energy. Almost all the compounds used for docking showed best fit Root Mean Square Difference (RMSD) value of 0.000 with topoisomerase II (3ILW). Among the compounds tested for docking study, **3a** showed high affinity with low energy of −7.4 kcal/mol with employed protein. Binding between 3ILW and compound **3a** indicates very good inhibition with calculated rmsd. Compounds **3**, **3a**, **3b**, **3c**, **3d**, **3e**, **3f**, **4**, **4a**, **4b**, **4c**, **5**, **5a**, **5b**, **5c** showed good inhibition with affinity range between −7.4 to −6.4 kcal/mol. Docking between **1** and **2** with 3ILW protein indicated weak inhibition with low energy value of −6.3 kcal/mol with calculated rmsd. The statistical analysis revealed that most of the compounds showed a significant linear regression coefficient ($R^2 = 0.8–0.9$).

Antimicrobial studies

Two factors influence the Minimal Inhibitory Concentration (MIC) of fluoroquinolones; the rate of penetration into the bacterial cell and its inhibitory activity of DNA gyrase. Although, most of the studies proposed that a substituent at tenth position of the ofloxacin ring is related to the binding site with enzyme through electrostatic interactions, our aim here was to study the influence the structural change of the carboxylic group of the quinolone ring. The results of antibacterial activity of ofloxacin derivatives against a panel of Gram positive and Gram negative bacteria are represented in Table 1 in comparison with that of the reference drug ofloxacin. The compounds were screened for antimicrobial activity against three Gram-positive bacterial strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*) and three Gram-negative bacterial strains (*Escherichia coli*, *Shigella* and *Pseudomonas aeruginosa*) and one fungal strain (*Candida albicans*) by twofold serial dilution method. DMSO was used as control, showed no zone of inhibition. The compounds exhibited good antibacterial effect towards gram positive species when compared to the standard ofloxacin. At the same time, the analogues were retaining antibacterial activity towards gram negative species when compared to standard ofloxacin. Among the synthesized compounds tested for antimicrobial activity compounds **3a–3f**, **4a–4c**, **5a–5c** have good antibacterial activity against *S. aureus* and **3a**, **3b**, **3f** showed good MIC value of 0.125 μg/ml. The modification of carboxylic acid group into Schiff bases leads to increase the antibacterial activity against gram positive species. The presence of N=C moiety may be the reason for this in compounds **3a–f**. Introduction of heterocyclic compounds into the carboxylic acid group also increase the antibacterial activity particularly against the gram positive species. The other titled compounds also had antimicrobial activity at a concentration of 0.25 μg/ml and showed good activity against *S. aureus*. The synthesized compounds **3b**, **3f**, and **4c** showed good antimicrobial activity at a concentration of 0.25 μg/ml against *S. epidermidis* and *B. subtilis* when compared to the standard. The other titled compounds showed antimicrobial activity at a concentration of 0.5 μg/ml and had good activity against *S. epidermidis* when compared to standard. The synthesized compounds showed moderate antibacterial activity against gram negative organisms (Tables 2, 3).

Table 1 Antibacterial activity of tested compounds and ofloxacin (μg/ml)

Comp.	S.a	S.e	B.s	E.c	K.p	P.a
3a	0.125	0.5	0.5	0.5	0.12	0.25
3b	0.125	0.25	0.5	0.5	0.5	0.5
3c	0.25	0.25	0.5	1	1	0.12
3d	0.25	0.25	0.5	1	1	1
3e	0.25	0.25	0.5	0.5	0.5	0.5
3f	0.125	0.25	0.5	1	0.25	1
4a	0.25	0.5	0.5	1	0.25	2
4b	0.25	0.5	0.5	0.25	0.5	0.5
4c	0.25	0.25	0.5	0.25	0.12	0.25
5a	3.125	0.5	0.5	0.50	0.12	0.5
5b	0.25	0.5	0.5	1	0.12	0.5
5c	0.25	0.5	0.5	1	0.12	0.25
Ofl	0.25	0.5	1	1	0.06	0.12

S.a, *Staphylococcus aureus*; S.e, *Staphylococcus epidermis*; B.s, *Bacillus epidermis*; E.c, *Escherichia coli*; K.p, *Klebsiella pneumonia*; P.a, *Pseudomonas aeruginosa*; Ofl, ofloxacin

Experimental

Chemistry-general aspects

Melting points were taken in glass capillary tubes on a Veego VMP-1 Apparatus and are uncorrected. The

Table 2 Antifungal activity of the synthesized compounds against *Candida albicans*

Comp.	Zone of inhibition		
	25 μg/ml	50 μg/ml	100 μg/ml
3a	16	18	22
3b	18	20	23
3c	15	18	21
3d	18	21	23
3e	15	17	20
3f	15	18	21
4a	16	19	22
4b	17	18	21
4c	15	18	22
5a	16	18	22
5b	15	17	21
5c	16	18	21
Ofl	21	26	28

Table 3 Best affinity mode of docked compounds

Docking (kcal/mol)	Mode	Affinity	Dist from best mode	
			rmsd l.b	rmsd u.b
3ILW vs. **1**	1	−6.3	0.000	0.000
3ILW vs. **2**	1	−6.3	0.000	0.000
3ILW vs. **3**	1	−6.6	0.000	0.000
3ILW vs. **3a**	1	−7.4	0.000	0.000
3ILW vs. **3b**	1	−7.2	0.000	0.000
3ILW vs. **3c**	1	−7.0	0.000	0.000
3ILW vs. **3d**	1	−6.7	0.000	0.000
3ILW vs. **3e**	1	−7.0	0.000	0.000
3ILW vs. **3f**	1	−7.3	0.000	0.000
3ILW vs. **4**	1	−6.8	0.000	0.000
3ILW vs. **4a**	1	−6.8	0.000	0.000
3ILW vs. **4b**	1	−6.9	0.000	0.000
3ILW vs. **4c**	1	−6.4	0.000	0.000
3ILW vs. **5**	1	−7.0	0.000	0.000
3ILW vs. **5a**	1	−6.8	0.000	0.000
3ILW vs. **5b**	1	−7.1	0.000	0.000
3ILW vs. **5c**	1	−6.8	0.000	0.000

3ILW Topoisomerase II

^{1}H-NMR were recorded on Bruker DRX-300 (300 MHz FT-NMR) using DMSO as solvent and TMS as an internal standard. The IR spectra of compounds were recorded on Shimadzu FT-IR spectrometer using KBr pellet technique and are expressed in cm^{-1}. ESI-MS spectra were recorded on a Mariner System 5304 mass spectrometer.

*Synthesis of ethyl 9-fluoro-3,7-dihydro-3-methyl-10-(4-methylpiperazin-yl)-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinolone-6-carboxylate (**2**)*

Ofloxacin (3.61 g, 1 mmol) was dissolved in 100 ml of ethanol, a few drops of concentrated sulphuric acid were added to this and the reaction mixture was refluxed for 48 h. The excess ethanol was distilled off under reduced pressure and this solution was poured into ice cold water. The solid obtained was filtered and recrystallized from absolute ethanol.

Yield 75%. m.p. 212–216°C. ^{1}H NMR (DMSO-d_6) δ; 7.7 (s, 1H, Ar–H), 7.9 (s, 8H, CH_2-piperazine),7 (s, 1H, N–CH), 4.9 (q, 2H, CH_2 ester), 4.4–4.6 (d, 2H, O–CH_2 oxazine), 3.2–3.4 (m, 1H, CH oxazine), 1.38 (t, 2H, CH_3 ester). IR ν_{max} cm^{-1} (KBr): 3041.84, 1737, 1116.17, 1028.24. MS: 390.13 (M^+, 100%).

*Synthesis of 9-fluoro-3,7-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinolone carbohydrazide (**3**)*

Compound **2** (3.89 g, 1 mmol) in 50 mL of ethanol was taken in a round bottom flask. To that hydrazine hydrate (0.75 g, 1.5 mmol) was added and refluxed for 18 h. The total volume of the solution was reduced to half and was cooled in ice water. The solid was precipitated out and recrystallized from ethanol.

Yield: 61%. m.p. 238–242°C. ^{1}H NMR (DMSO-d_6) δ; 8.7 (s, 1H, NH), 7.9 (s, 8H, CH_2 piperazine), 7.7 (m, 1H, Ar–H quinolone), 4.4–4.6 (d, 2H, OCH_2 oxazine), 3.2–3.4 (m, 1H, CH oxazine), 2.3 (s, 3H, N–CH_3), 1.2 (d, 3H, CH_3 oxazine). IR ν_{max} cm^{-1} (KBr): 3455.39, 3495.30, 3491, 3045.70, 1716.70, 1599.04, 1473.66, 1047.38.MS: 385.43 (M^+, 100%).

*General method of synthesis of Schiff bases (**3a–f**)*

A mixture of compound 3 (3.75 g, 0.01 mol), substituted benzaldehyde (0.001 mol) and 4–5 drops of conc. H_2SO_4 in ethanol medium was mixed and then were irradiated for 15 min at 240 W. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol, and recrystallized with hot ethanol.

*9-Fluoro-3,7-dihydro-3-methyl-6-(2-chloro-N′-methylenebenzohydrazide) 10-(4-methylpiperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**3a**)*

Yield: 39%. m.p. 158–161°C. ^{1}H NMR (DMSO-d_6) δ: 8.9 (s, 1H, NH(CONH)), 8.1 (d, 1H, N=CH), 7.5 (m, 4H, Ar–H), 7.9 (s, 8H, CH_2 piperazine), 7.7 (m, 1H, Ar–H

quinolone), 4.4–4.6 (d, 2H, OCH_2 oxazine), 3.2–3.4 (m, 1H, CH oxazine), 2.3 (s, 3H, N–CH_3), 1.2 (d, 3H, CH_3 oxazine). IR ν_{max} cm^{-1} (KBr): 3068.85, 3045.27, 1047.38, 1616.40, 1587.47, 750.33. MS: 498.60 (M^+, 100%).

*9-Fluoro-3,7-dihydro-3-methyl-6-(4-methoxy-N-methylenebenzohydrazide)10-(4-methylpiperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**3b**)*

Yield: 42%. m.p. 176–179°C. ^{1}H NMR (DMSO-d_6) δ: 8.9 (s, 1H, NH–CONH), 8.1 (d, 1H, N=CH), 7.5 (m, 4H, Ar–H), 7.9 (s, 8H, CH_2 piperazine), 7.7 (m, 1H, Ar–H), 6.8 (d, 1H, Ar–H), 4.4–4.6 (d, 2H, OCH_2), 3.73 (s, 3H, OCH_3), 3.2–3.4 (m, 1H, CH oxazine), 2.3 (s, 3H, N–CH3), 1.2 (d, 3H, CH_3 oxazine). IR ν_{max} cm^{-1} (KBr): 3068.85, 3045.27, 1047.38, 1616.40, 1587.47. MS: 494.51 (M^+, 100%).

*9-Fluoro-3,7-dihydro-3-methyl l-6-(2-hydroxy-N′-methylenebenzohydrazide)10-(4-methylpiperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**3c**)*

Yield: 55%. m.p. 196–199°C. ^{1}H NMR (DMSO-d_6) δ: 12 (s, 1H, OH), 8.9 (s, 1H, NH (CONH)), 8.1 (d, 1H, N=CH), 7.5 (m, 4H, ArH), 7.9 (s, 8H, CH_2 piperazine), 7.7 (m, 1H, Ar–H), 5.1 (s, 1H, OH), 4.4–4.6 (d, 2H, OCH_2 oxazine), 3.2–3.4 (m, 1H, CH oxazine), 2.3 (s, 3H, N–CH_3), 1.2 (d, 3H, CH_3 oxazine). IR ν_{max} cm^{-1} (KBr): 3435.78, 3068.85, 3045.2, 3007.12, 1047.38, 1616.40, 1587.47.MS: 480.18 (M^+, 100%).

*9-Fluoro-3,7-dihydro-3-methyl l-6-(N′-methylene-hydroxy-2-carbohydrazide)10-(4-methylpiperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**3d**)*

Yield: 45%. m.p. 172–175°C. ^{1}H NMR (DMSO-d_6) δ: 8.9 (s, 1H, NH(CONH)), 8.1 (d, 1H, N=CH), 7.5 (m, 4H, Ar–H),7.9 (s, 8H, CH_2 piperazine),7.7 (m, 1H, Ar–H), 4.4–4.6 (d, 2H, OCH_2 oxazine), 3.2–3.4 (m, 1H, CHoxazine), 2.3(s, 3H, N–CH_3), 1.2 (d, 3H, CH_3 oxazine). IR ν_{max} cm^{-1} (KBr):3068.85, 3045.27, 1047.38, 1616.40, 1587.47. MS: 442.38 (M^+, 100%).

*9-Fluoro-3,7-dihydro-3-methyl-6-(N′-methylene-3-nitrobenzohydrazide) 10-(4-methyl piperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**3e**)*

Yield: 56%. m.p. 208–211°C. ^{1}H NMR (DMSO-d_6) δ: 8.6(m, 1H, Ar–H), 8.9(s, 1H, NH(CONH)), 8.1 (d, 1H, N=CH), 7.5(m, 4H, Ar–H benzene), 7.9(s, 8H, CH_2 piperazine), 7.7(m,1H, Ar–H quinolone), 4.4–4.6 (d, 2H, OCH_2 of oxazine), 3.2–3.4 (m, 1H, CH of oxazine), 2.3 (s, 3H, NCH_3), 1.2 (d, 3H, CH_3 of oxazine). IR ν_{max} cm^{-1} (KBr): 3068.85, 3045.27, 1047.38, 1616.40 (C=O), 1587.47. MS: 489.37 (M^+, 100%).

*9-Fluoro-3,7-dihydro-3-methyl-6-(N′-methylenebenzohydrazide) 10-(4-methyl piperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**3f**)*

Yield: 59%. m.p. 200–203°C. ^{1}H NMR (DMSO-d_6) δ :8.9 (s, 1H, NH (CONH)), 8.1 (d, 1H, N=CH),7.5 (m, 4H, Ar–H benzene), 7.9 (s, 8H, CH_2 piperazine), 7.7 (m, 1H, Ar–H quinolone), 4.4–4.6 (d, 2H, OCH_2 oxazine), 3.2–3.4 (m, 1H, CH oxazine), 2.3 (s, 3H, N–CH3) 1.2 (d, 3H, CH_3 oxazine). IR ν_{max} cm^{-1} (KBr): 3068.85, 3045.27, 1047.38, 1616.40, 1587.47. MS: 464.13 (M^+, 100%).

*Synthesis of 9-fluoro-2,3-dihydro-6-(4,5-dihydro-5-thioxo-1,3,4-oxadiazol-2-yl)-3-methyl-1,10-(4-methylpiperazin-1-yl)-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**4**)*

To a solution containing ethanol (20 ml) and potassium hydroxide (0.28 g, 5 mmol), the hydrazide (1.87 g, 5 mmol) was added. After solution has occurred, carbon disulphide (0.42 g, 5.51 mmol) was added and the mixture was stirred at room temperature for 14 h. After concentration of the solution to a small volume, the residue was dissolved in water (10 ml).A precipitate was obtained by adding the solution to ice containing concentrated hydrochloric acid. The solid was filtered under suction and recrystallized with ethanol.

Yield: 62%. m.p. 228–231°C. ^{1}H NMR (DMSO-d_6) δ: 7.7 (m, 1H, Ar–H quinolone),7.9 (s, 8H, CH_2 piperazine), 7 (s, 1H, NH oxadiazole), 2.1 (s, 3H, N–CH_3), 4.4–4.6 (d, 2H, OCH_2 oxazine), 3.2–3.4(m, 1H, CH oxazine), 1.3 (d, 3H, CH_3 oxazine). IR ν_{max} cm^{-1} (KBr): 3406.40, 3041.84, 1624.12, 1207.48, 1055.10, 979.87. MS: 416.44 (M^+, 100%).

*Synthesis of 9-fluoro-2, 3-dihydro-6-[3-(4-Amino -5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)]-3-methyl-10-(4-methylpiperazin-1-yl)-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**5**)*

A solution of compound 4 (1.42 g, 3.42 mmol), water (3 ml) and 95% hydrazine hydrate (0.12 g, 2.42 mmol) was heated under reflux for 14 h. After cooling, the solution was diluted with coldwater (20 ml), acidified by drop wise addition of concentrated HCl and filtered. The solid was washed with a minimum of cold water and recrystallized from 1:1 ethanol:water.

Yield: 41%. m.p. 256–259°C. ^{1}H NMR (DMSO-d_6) δ:7.7 (m, 1H, Ar–H quinolone), 7 (s, 1H, NH),6.9 (s, 2H, N–NH_2), 7.9 (s, 8H, CH_2 piperazine), 4.4–4.6 (d, 2H, OCH_2 oxazine), 3.2–3.4 (m, 1H, CH oxazine), 2.3 (s, 3H,

N–CH_3), 1.2 (d, 3H, CH_3 oxazine). IR ν_{max} cm^{-1} (KBr): 3394.83, 3041.84, 1624.12, 1527.67, 1247.99, 1055.10, and 979.87. MS: 432.64 (M^+, 100%).

*General method of synthesis of Mannich bases: (**4a–c**, **5a–c**)*

A mixture of formaldehyde (40%, 1.5 ml), substituted ketone (0.001 mol) and compound 4/compound 5(0.001 mol) in ethanol was stirred at room temperature for 6 h. The precipitated solid was filtered under suction, washed with ethanol and recrystallized from hot ethanol.

*9-Fluoro-2,3-dihydro-6-(4,5-dihydro-4-(3-oxo-3-phenylpropyl)-5-sulfanylene-1,3,4-oxadiazol-2-yl)-3-methyl-l,10-(4-methylpiperazin-1-yl)-[1,4]oxazine[2,3,4-ij]quinolin-7-one (**4a**)*

Yield: 39%. m.p. 238–241°C. ^{1}H NMR (DMSO-d_6) δ: 7.8 (m, 1H, Ar–H), 7.5 (m, 4H, Ar–H), 7.9 (s, 8H, CH_2), 4.4–4.6 (d, 2H, OCH_2), 3.2–3.4 (m, 1H, CH), 2.8 (t, 2H, CH_2), 2.5 (t, 2H, CH_2), 2.3 (s, 3H, N–CH_3), 1.2 (d, 3H, CH_3 oxazine). IR ν_{max} cm^{-1} (KBr):3041.84, 1624.12, 1247.99, 1207.48 (COC), 1155.4, 1055.10, 979.87. MS: 550.64 (M^+, 100%).

*6-(4-3-(4-Aminophenyl)-3-oxopropyl)-4,5-dihydro-4-(3-oxo-3-phenylpropyl)-5-sulfanylene-1,3,4-oxadiazol-2-yl)-9-fluoro-2,3-dihydro-3-methyl l-10-(4-methylpiperazin-1-yl)-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**4b**)*

Yield: 30%. m.p. 242–245°C. ^{1}H NMR (DMSO-d_6) δ: 7.8 (m, 1H, ArH), 7.5 (m, 4H, ArH), 7.9 (s, 8H, CH_2), 4.4–4.6 (d, 2H, OCH_2 oxazine), 4.3 (s, 1H, NH_2), 3.2–3.4 (m, 1H, CH oxazine), 2.8 (t, 2H, CH_2), 2.5 (t, 2H, CH_2), 2.3 (s, 3H, N–CH_3), 1.2 (d, 3H, CH_3 oxazine). IR ν_{max} cm^{-1} (KBr): 3041.84, 1624.12, 1247.99, 1207.48, 1055.10, 698.4, 979.87. MS: 565.44 (M^+, 100%).

*9-Fluoro-2,3-dihydro-6-[3-(4,5-dihydro-4-(3-oxobutyl)-5-sulfanylene-1,3,4-oxadiazol-2-yl]-3-methyl-10-(4-methylpiperazin-1-yl)-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**4c**)*

Yield: 27%. m.p. 232–235°C. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.8(m, 1H, Ar–H), 7.5(m, 4H, Ar–H),7.9(s, 8H, CH_2), 4.4–4.6(d, 2H, OCH_2 oxazine), 4.3(s, 1H, NH2), 3.2–3.4(m, 1H, CH of oxazine), 2.8(t, 2H, CH_2), 2.5(t, 2H, CH_2), 2.3(s, 3H, N–CH_3), 1.2 (d, 3H, CH_3 of oxazine). IR ν_{max} cm^{-1} (KBr):3041.84, 1644.12, 1257.99, 1217.48, 1075.10, 678.4, and 959.87. MS: 511.54 (M^+, 100%).

*9-Fluoro-2,3-dihydro-6-[3-(4-amino-4,5-dihydro-1-(3-oxobutyl-5-sulfanylene-1H-1,2,4-triazol-3-yl)]-3-methyl-1,10-(4-methylpiperazin-1-y)-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**5a**)*

Yield: 27%. m.p. 246–249°C. ^{1}H NMR (DMSO-d_6) δ: 7.9(s, 8H, CH_2), 7.7(m, 1H, Ar–H), 6.9 (s, 2H, N–NH_2), 4.4–4.6 (d, 2H, OCH_2 oxazine), 3.2–3.4 (m, 1H, CH oxazine), 2.8 (t, 2H, CH_2), 2.5(t, 2H, CH_2), 1.2(d, 3H, CH_3 oxazine), 2.3(s, 3H, N–CH_3). IR ν_{max} cm^{-1} (KBr): 3041.84, 2850.88, 1624.12, 1361.74, 1055.10, 698.4, 802.41. MS: 501.44 (M^+, 100%).

*9-Fluoro-2,3-dihydro-6-[3-(4-amino-4,5-dihydro-1-(2-hydroxy-3-oxo-3-phenylpropyl-5-sulfanylene-1H-1,2,4-triazol-3-yl)]-3-methyl-10-(4-methylpiperazin-1-yl)-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**5b**)*

Yield: 29%, m.p. 240–243°C. ^{1}H NMR (DMSO-d_6) δ:7.9 (s, 8H, CH_2 piperazine), 7.7 (m, 1H, Ar–H quinolone) 6.9 (s, 2H, N–NH_2), 4.4–4.6 (d, 2H, OCH_2 oxazine), 3.2–3.4(m, 1H, CH oxazine), 2.8 (t, 2H, CH_2), 2.5 (t, 2H, CH_2), 2 (m, 1H, OH), 1.2(d, 3H, CH_3 of oxazine), 2.3 (s, 3H, N–CH_3). IR ν_{max} cm^{-1} (KBr): 3041.84, 2850.88, 1624.12, 1552.27, 1361.74, 1157.33, 1055.10, 648.4, 802.41. MS: 580.43 (M^+, 100%).

*9-Fluoro-2,3-dihydro-6-[3-(4-amino- 4,5-dihydro-1-(3-(4-nitrophenyl)-3-oxobutyl-5-sulfanylene-1H-1,2,4-triazol-3-yl)]-3-methyl-10-(4-methylpiperazin-1-yl)-[1,4]oxazino[2,3,4-ij]quinolin-7-one (**5c**)*

Yield: 29%. m.p. 240–243°C. ^{1}H NMR (DMSO-d_6) δ: 8.27(m, 4H, Ar–H), 7.9(s, 8H, CH_2 piperazine), 7.7 (m, 1H, Ar–H quinolone) 6.9 (s, 2H, N–NH2), 4.4–4.6 (d, 2H, OCH_2 oxazine), 3.2–3.4 (m, 1H, CH oxazine), 2.8 (t, 2H, CH_2), 2.5(t, 2H, CH_2), 1.2(d, 3H, CH_3 oxazine), 2.3(s, 3H, N–CH_3). IR ν_{max} cm^{-1} (KBr): 3041.84, 2850.88, 1624.12, 1055.10, 698.4, 802.4. MS: 618.34 (M^+, 100%).

In-silico molecular docking

The 3D structure of Topoisomerase II chain A (3ILWA) was extracted from protein data bank at the NCBI (National Centre for biotechnology information, http://www.ncbi.nib.hlm.gov). 3ILWA is a crystal structure of the DNA Gyrase. The synthesized compounds which are analogues of ofloxacin were taken for prediction of 3D structure and energy was minimized for flexible docking using Argus lab (Argus Lab 4.0.1, Mark A. Thompson, Planaria Software LLC, Seatle, WA, http://www.arguslab.com). The

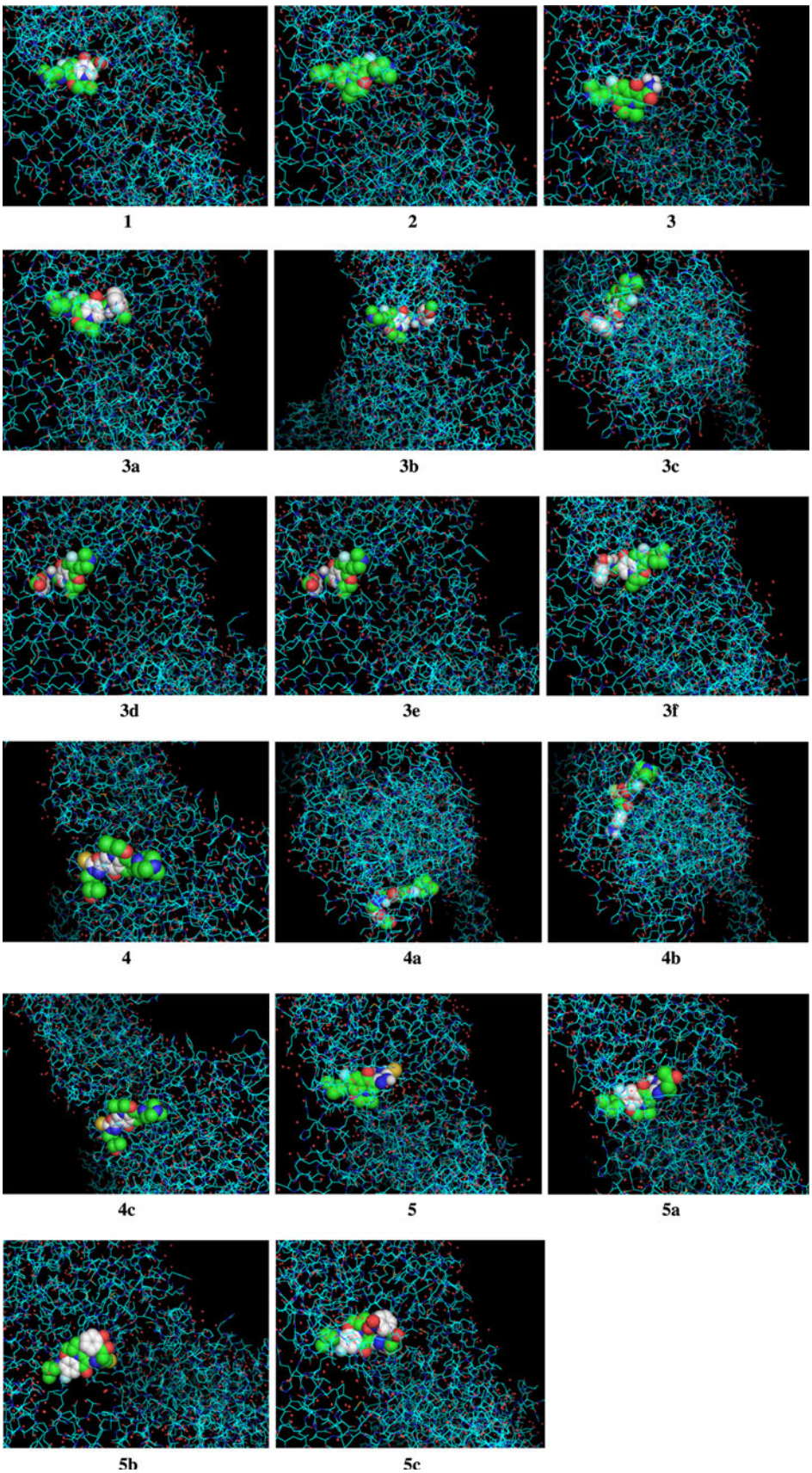


Fig. 1 Best affinity mode of synthesized compounds

structures of these synthesized compounds and enzyme are shown in Fig. 1. In the docking study receptor was treated as a rigid body and a grid potential was used to evaluate the scoring function. Here 3D structure of protein Topoisomerase chain A was used as receptor and all the synthesized compounds were used as ligands. In Autodock vina 4.0 (Trott and Olson 2010), nonpolar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. The grid calculation were set up, 4.9 × −20.8 × 64.6 Å grid originating at 40, 40, 40 with resolution of 0.375 Å, respectively, was generated around the compound.

Antibacterial screening

The MIC of synthesized compounds was determined by using serial two folds dilution method. A series of test tubes were prepared containing the same volume of medium inoculated with the test organism (the inoculam may vary from 103 to 106 cells per milliliters). Decreasing concentration of drug were added to the tubes, usually a stepwise dilution of by a factor of 2 (two fold serial dilution) was used (i.e., if the concentration of drug in the first tube is 500 μg/ml, in the second tube it will be 250 μg/ml and in the third 125 μg/ml and so as). One tube was left without drug, to serve as a positive control for the growth of the organism. The culture was incubated at a room temperature optimal for the test organism for a period sufficient for the growth of at least 10–15 generators (usually 24 h for bacteria at 37°C and 48 h for fungi at 27°C. The tubes were inspected visually to determine the growth of the organism indicated by turbidity (In fact, turbidity of the culture medium is indicative of the presence of a large number of cells). The tubes containing the antimicrobial agent in concentration sufficient to inhibit the growth remaining clear. In experimental terms, the MIC is the concentration of the drug present in the last clear tube, i.e., the tube having the lowest antibiotic concentration in which growth is not observed (Ramesh 2002).

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