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Synthesis Docking Studies and Antitubercular Activity of Ofloxacin Scaffold using Blanc Reaction

C. RAMAKRISHNA[1,*] and G.V. SUBBAREDDY[2]

[1]Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur, Anantapuramu-515001, India
[2]Department of Chemistry, JNTUA College of Engineering, Pulivendula-516390, India

*Corresponding author: E-mail: rama0813@gmail.com

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Synthesis, molecular docking and characterization of ofloxacin derivatives and *in vitro* evaluation for their antitubercular action were carried out. Synthesis ofloxacin derivatives *viz*. O+TRI, O+MEL, O+NAP, O+SUL, O+METF, O+IBU, O+ASP, O+DNP, O+SSA, O+PH, O+MC (where TRI = trimethoprim, MEL = meloxicam, NAP = naproxen, SUL = sulfamethoxazole, METF = metformin, IBU = ibuprofen, ASP = aspirin, DNP = 2,4-dinitrophenyl hydrazine, SSA = 5-sulpho salicylic acid, PH = phenyl hydrazine, MC = 7-hydroxy-4-methyl coumarin) were carried using Blanc reaction and purified by using of ethanol by recrystallization. The reaction products consist of methylene group linkage between two moieties and the molecules were characterized by analytical methods TLC, solubility, melting point, spectroscopic methods (FT-IR, mass and ^{1}H NMR, ^{13}C NMR). *In silico* methods were adopted for synthetic derivatives by Autodock vina software. Determined physico-chemical parameters (*in silico* studies) and docking studies have been performed using protein (5BS8) with designed ligands, binding energy scores were noted for different derivatives. Derivatives O+TRI, O+NAP shown good activity at 0.8 µg/mL. In docking studies, all the compounds shown promising results when compared to standard drugs.

Keywords: Antitubercular activity, Autodock studies, Ofloxacin derivatives, Blanc reaction.

INTRODUCTION

Fluoroquinolones are useful, important antimicrobial agents and have a wide range to inhibit both Gram-negative and Gram-positive microorganisms. Some examples include ofloxacin, ciprofloxacin, pefloxacin, norfloxacin are the 2nd generation, levofloxacin is 3rd generation, while moxifloxacin is the 4th new generation. Most of the newer fluoroquinolones are entered the market almost every year [1]. In this study, ofloxacin, the second-generation fluoroquinolone antibiotic with a 7-piperazinyl substituent and a 6-fluoro substituent on the quinolone ring structure is focused. All the clinically important quinolones comprise a fluorine group at the C-6 position. These quinolones present an outstanding pharmacokinetic profile and achieve appreciable concentrations of higher than their MICs in a biological tissue [2].

The fluoroquinolones have been analyzed by various synthetic routes. The second-generation broad-spectrum fluoroquinolone antimycobacterial agents was developed by introducing a new functional groups at the 5th position [3,4]. Although many fluoroquinolones are approved for the treatments of different infections, the development of fluoroquinolones having specific properties, such as desired therapeutic index, pharmacokinetic profile and useful for overcoming increasing bacterial resistance, has received considerable attention [5]. The main occurrence is the increasing incidence of resistance. Fluoroquinolones, artificial antibacterial agents, are advantageous in the treatments of urinary tract infections, respiratory infections, typhoid fever, community-acquired pneumonia, soft tissue infections, sexually transmitted diseases, acute bronchitis, bone-joint infections and sinusitis [6,7].

Fluoroquinolones comprise a two-ring structure (bicyclic), where position N-1 undergoes a replacement. Most compounds exhibit carboxylic acid, ketonic, fluorine atom and nitrogen containing piperazinyl at the third position, fourth position, sixth position and C-7 position, respectively. Piperazine replacement at the C-7 position results in various clinically valuable antibacterial agents of fluoroquinolone [8]. Two main targets are identified for the levofloxacin activity: topoisomerase IV and topoisomerase II (DNA-gyrase) in high Gram-positive

bacteria, respectively [9,10]. With the inhibitory mechanisms of quinolones, a site near the C-7 substituent is regarded as the domain of drug-enzyme interactions and considered to control the spectrum and selectivity of quinolone molecules [11-13].

Mycobacterium tuberculosis is the bacilli bacteria that causes tuberculosis, which mainly attacks the lungs called pulmonary tuberculosis and other parts of the body called extrapulmonary tuberculosis [14]. During the active stage of tuberculosis, the disease is highly contagious and can infect people through inhalation of a small amount of *Mycobacterium tuberculosis* (MTB) [15,16]. After inhalation, the bacteria are mainly trapped by alveolar macrophages; however, they evade the immune system of a host for a long duration. Then, under immune compromised conditions of the host, they can be reactivated to the virulent form [17,18]. Tuberculosis can be effectively treated using the first-line drugs rifampicin, isoniazid, ethambutol, pyrazinamide and streptomycin. For several reasons, the first-line drugs often fail to cure tuberculosis. Replacement and disease spread contribute to the generation of drug-resistant bacteria. The emergence of multidrug-resistant tuberculosis (MDR-TB), *i.e.* resistant to isoniazid and rifampicin, is concerning because it leads to the requirement of second-line drugs, which are difficult to procure and are more expensive and toxic than first-line drugs.

In present study, the Blanc reaction was adopted, ofloxacin and other medicinal compounds are interlinked using methylene bridge linkage. All these were synthesized, tested for inhibitory growth of mycobacterium.

EXPERIMENTAL

All pure drugs *viz*. trimethoprim, meloxicam, ibuprofen, metformin, naproxen, sulfamethoxazole, aspirin and other chemicals *viz*. 2,4-dinitrophenyl hydrazine, 5-sulpho salicylic acid, phenyl hydrazine, 7-hydroxy-4-methyl coumarin, methanal and conc. sulphuric acid used in the synthetic reactions were procured from the Sigma-Aldrich, USA. The melting points were estimated using the melting point apparatus and are reported herein uncorrected. The IR spectra of the com-pounds were recorded with the BRUKER FT-IR Spectrophoto-meter by using KBr discs. TLC with the plates of Merck 0.25 mm silica gel were used to monitor each reaction progress. The ^{1}H NMR spectra were measured on Bruker Avance III (USA) at 400 or 600 MHz.

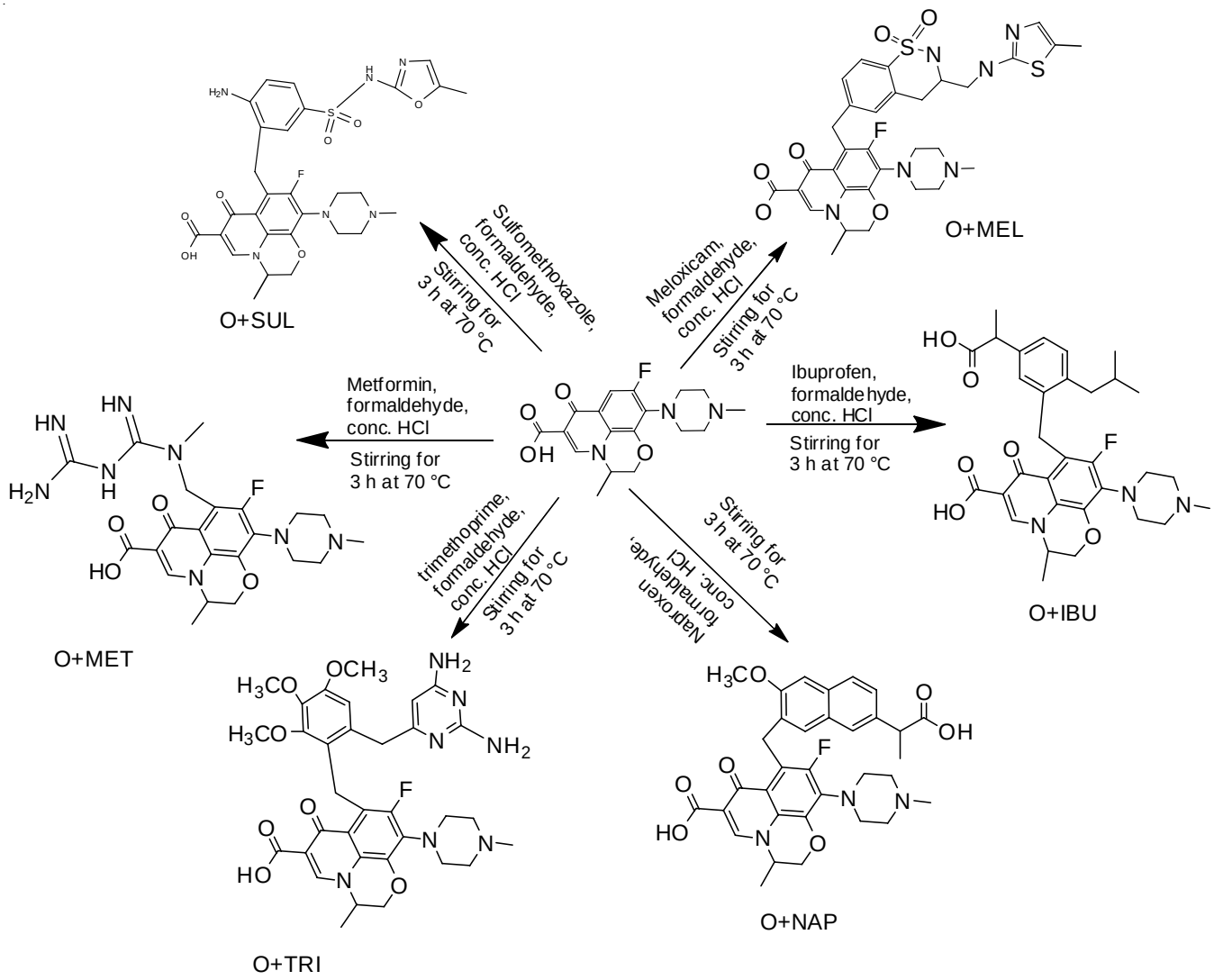
Pure ofloxacin drug was procured as a gift sample from Waksman Selman company, Anantapur, India. All the ofloxacin derivatives *viz*. (O+TRI, O+MEL, O+IBU, O+METF, O+NAP, O+SUL, O+ASP, O+DNP, O+SSA, O+PH and O+MC) were synthesized using Blanc reaction [19,20].

General procedure: Ofloxacin (0.01 M) and other drugs (0.01 M) *viz*. trimethoprim, meloxicam, ibuprofen, metformin, naproxen, sulfamethoxazole, aspirin, 2,4-dinitro phenyl hydrazine, 5-sulpho salicylic acid, phenyl hydrazine, 7-hydroxy-4-methyl coumarin were transferred in separate beakers containing 10 mL ethanol and 10 mL 5% NaOH. The solutions were mixed stirred on a magnetic stirrer at 70 ºC. To the above solution 35 parts HCHO (formaldehyde) and 35% HCl were added dropwise with continued stirring. The reaction mixture was stirred for 3 h at 70 ºC using a magnetic stirrer. The resultant mixture was cooled and basified with the addition of NH_3 solution. The solid separated was collected by filtration and dried and recrystallized by using ethanol (**Schemes I and II**). All the synthesized derivatives are soluble in water, chloroform, DMSO, diethyl ether, *etc*.

8-(6-((2,6-Diaminopyrimidine-4-yl)methyl)-2,3,4-trimethoxybenzyl)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazin[2,3,4]quinolone-6-carboxylic acid (O+TRI): Milky white; yield 63.2%; m.p.: 218-220 ºC; TLC R_f value = 0.26 (8:2, chloroform:hexane). IR (KBr, ν_{max}, cm^{-1}): 3148 (C-H), 2768.79 (1º-amine), 2275.16 (C=N), 1662.22 (C=O), 1010.05 (C-F), 1105.70 (C-O); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.25 (3H, d, methyl), 2.50 (3H, s, N-CH$_3$), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.34 (4H, m, piperazine), 3.81 (4H, s, methylene bridge), 4.28 (2H, d, benzoxazine), 3.75 (9H, s, methoxy), 4.0 (4H, s, amine), 5.51 (1H, s), 5.90 (1H, s), 7.96 (1H, s), 11.0 (1H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.21, 43.20, 47.42, 54.15, 55.99, 62.09, 68.44, 79.90, 81.79, 103.82, 143.84. HRMS (*m/z*): $C_{33}H_{34}FN_7O_7$ (*m.w.* 663.71) calculated: 663.70; found: 663.73.

8-(2-Methyl-3-[(5-methyl-1,3-thiazol-2-yl)carbamoyl]-1,1-dioxo-1λ^6,2-benzothiazin-4-olate)-9-fluoro-3-methyl-10-(4-methylpiperazine-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazin[2,3,4-ij]quinoline-6-carboxylic acid (O+MEL): Pale yellow; yield 91.4%; m.p.: 217-220 ºC;TLC R_f value = 0.42 (8:2 chloroform:hexane). IR (KBr, ν_{max}, cm^{-1}): 3500.00 (N-H), 3140.42 (C-H), 2725.45 (1º amine), 2539.42 (S-H), 2273.16 (C=N), 1622.97 (C=O), 1400.88 (C-H), 1053.79 (C-F). ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.25-(3H, d, methyl), 2.35 (3H, s, thiazole methyl), 2.50 (3H, s, N-CH$_3$), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.24 (2H, d), 3.34 (4H, m, piperazine), 3.81 (2H, s, methylene bridge), 4.28 (2H, d, benzoxazine), 4.0 (2H, s, amine), 7.16-7.76 (2H, d, arom.), 7.17 (1H, s, thiazole ring), 7.96 (1H, s), 11.0 (1H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.30, 43.29, 47.43, 47. 47, 54.19, 56.13, 68.46, 69.90, 71.66, 79.92, 81.84, 103.40, 103.64, 124.89, 140.83, 140.90, 145.79, 154.86, 157.33, 176.47; HRMS (*m/z*) $C_{13}H_{33}FN_6O_6S_2$ calculated: 714.84; found: 715.80.

8-((7-(1-Carboxyethyl)-3-methoxynapthalen-2-yl)methyl)-9-fluoro-3-methyl-10-(4-methylpiperazine-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazin[2,3,4-ij]quinoline-6-carboxylic acid (O+NAP): Cream; yield :81.25%; m.p.: 208-210 ºC; TLC R_f value = 0.57 (8:2 chloroform:hexane). IR (KBr, ν_{max}, cm^{-1}): 3500 (N-H), 3000 (C-H), 2834.45 (1º amine), 2725.74 (S-H), 1623.66 (C=O), 1009.64 (C-F), 978.84 (C-O); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.25-(3H, d, methyl), 1.53 (3H, m), 2.50 (3H, s, N-CH$_3$), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.34 (4H, m, piperazine), 3.81 (2H, s, methylene bridge), 3.85 (1H, d), 4.28 (2H, d, benzoxazine), 3.73 (3H, s, methoxy), 6.92-7.56 (5H, m, arom.), 7.96 (1H, s), 11.0 (2H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.32, 43.14, 43.30, 47.45, 54.20, 56.18, 68.48, 69.90, 71.61,



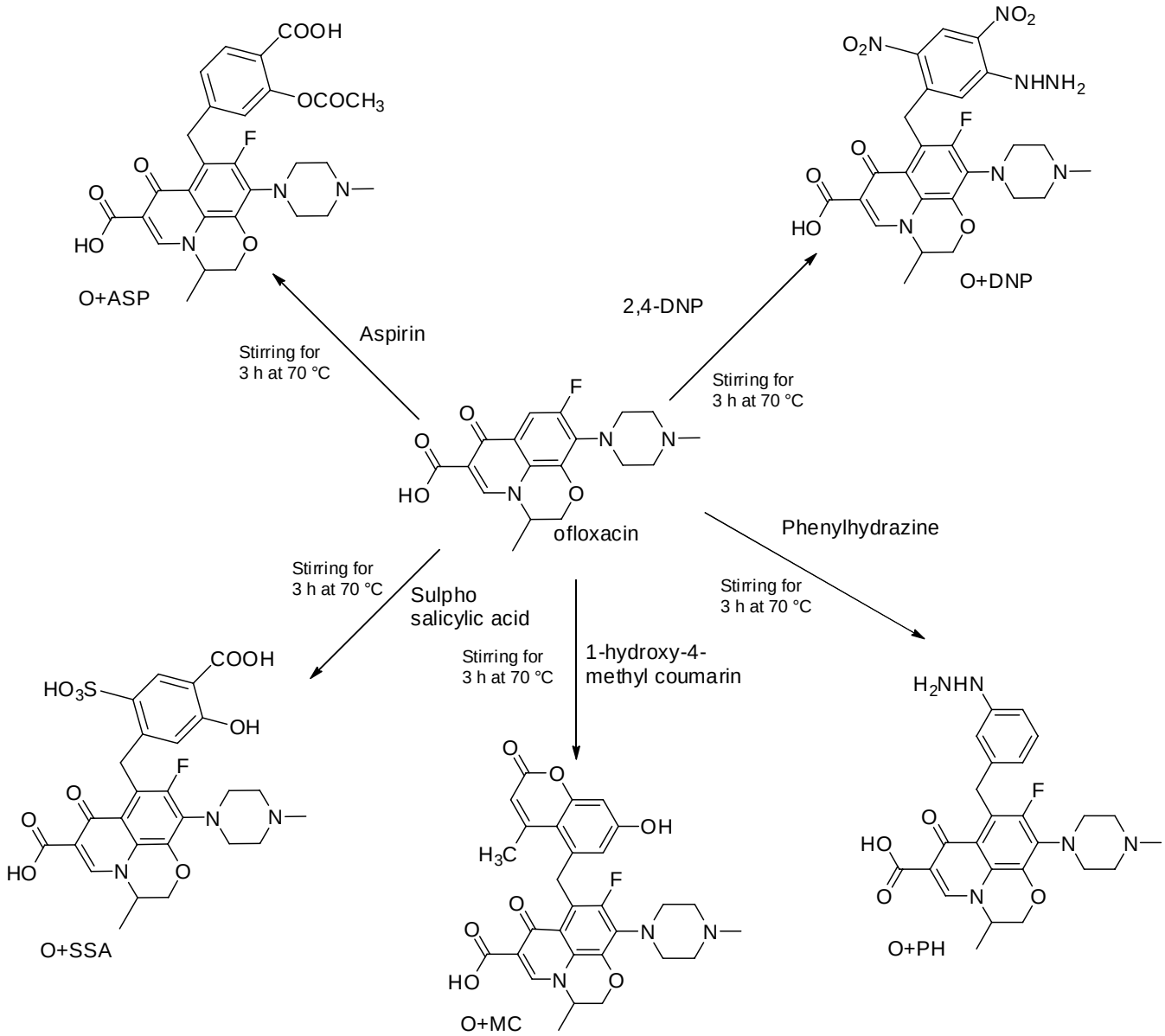
Scheme-I: Synthetic route

79.92, 81.85, 103.53, 124.99, 145.96; HRMS (*m/z*) $C_{33}H_{38}FN_3O_7$ (*m.w.* 603.65) calculated: 603.65, found: 604.67.

8-(4-Amino-N-(5-methylthiazol-2-yl)benzene sulfonamide)-9-fluoro-3-methyl-10-(4-methylpiperazine-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazin[2,3,4-ij]quinoline-6-carboxylic acid (O+SUL): Yellowish brown; yield :69.32%; m.p.: 188-190 ºC; TLC R_f value = 0.37 (8:2 chloroform:hexane); IR (KBr, ν_{max}, cm^{-1}): 3500 (N-H), 3039.97 (C-H), 2855.82 (1º amine), 2728.27 (S-H), 1622.97 (C=O), 1053.63 (C-F), 979.90 (C-O); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.25-(3H, d, methyl), 2.35 (3H, s, oxazole methyl), 2.50 (3H, s, N-CH_3), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.34 (4H, m, piperazine), 3.81 (2H, s, methylene bridge), 4.0 (3H, s, amine), 4.28 (2H, d, benzoxazine), 7.10 (1H, s, oxazole), 7.96 (1H, s), 7.48-8.82 (3H, d, arom.), 11.0 (1H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.27, 43.24, 47.44, 54.17, 56.25, 68.45, 103.47, 103.71, 124.93, 140.93, 145.99, 169.22, 176.70; HRMS *m/z*: $C_{28}H_{29}FN_6O_7S$ calculated:626.66; found:626.67.

8-((3-Carbamimidoyl-1-methyl guanidino)methyl)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (O+MET): Milky white; yield:84.94%; m.p.: 207-210 ºC; TLC R_f value = 0.30 (8:2 chloroform:hexane); IR (KBr, ν_{max}, cm^{-1}): 3040.83 (C-H), 1132.53 (C-O), 1707.27 (C=O), 3137.94 (N-H), 1204.65 (C-N), 1009.52 (C-F), 2539.85 (S-H), 2725.41(1º amine); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.25 (3H, d, methyl), 2.0 (5H, s, amines), 2.30 (3H, s, N-CH_3), 2.50 (3H, s), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.34 (4H, m, piperazine), 3.81 (2H, s, methylene bridge), 4.28 (2H, d, benzoxazine), 7.96 (1H, s), 11.0 (1H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.26, 43.25, 47.37, 47.42, 54.15, 56.14, 68.40, 69.88, 71.53, 79.90, 81.81, 103.22, 103.46, 106.07, 120.28, 124.14, 128.74, 130.72, 130.87, 140.65, 145.64, 154.68, 157.15, 168.87, 176.19; HRMS *m/z* $C_{22}H_{29}FN_8O_4$ calculated 488.92; found:489.75.

8-(5-(1-Carboxyethyl)-2-isobutylbenzyl)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (O+IBU): Whitish brown; yield:77.10%; m.p.: 198-200 ºC; TLC R_f value = 0.43 (8:2 chloroform:hexane); IR (KBr, ν_{max}, cm^{-1}): 3000 (C-H), 2273.08 (C-N), 1719.82 (C=O), 2955.99 (1º amine), 1461.60 (C-H, aliphatic), 1401.39 (C-O), 1009.58 (C-F); ^{1}H



Scheme-II: Synthetic route

NMR (600 MHz, DMSO-d_6): δ 1.0 (6H, s, CH_3),1.25-(3H, d, methyl), 1.5 (3H, d, methyl), 2.30 (3H, s, N-CH_3), 2.50 (2H, d), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.34 (4H, m, piperazine), 3.81 (3H, s), 4.28 (2H, d, benzoxazine), 6.90 (3H, m, aromatic), 7.96 (1H, s), 11.0 (2H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.34, 43.16, 43.33, 47.42, 47.46, 54.20, 56.17, 68.46, 69.90, 71.56, 79.92, 81.85, 103.25, 103.25, 106.27, 120.37, 120.46, 124.80, 130.73, 130.88, 140.69, 140.76, 145.71, 154.70, 157.17, 169, 176.25; HRMS *m/z* $C_{32}H_{38}FN_3O_6$ calculated: 579.66; found:580.67.

8-(3-Acetoxy-4-carboxybenzyl)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazino[2,3,4-*i,j*]quinoline-6-carboxalic acid (O+ASP): Pale yellow; yield:75%; m.p.: 175-180 ºC;TLC R_f value = 0.28 (8:2 chloroform:hexane); IR (KBr, ν_{max}, cm^{-1}): 2950.64 (C-H), 1617.39 (C-C arom.), 1668.34 (C=O), 3033.95 (C-H, arom.), 699.04 (C-F), 2593.74 (O-H, carboxylic acid), 952.53 (O-H, bending, carboxylic acid); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.25 (3H, d, methyl), 2.10 (3H, s, acetyl), 2.50 (3H, s, N-CH_3), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.34 (4H, m, piperazine), 3.81 (3H, s), 4.28 (2H, d, benzoxazine), 7.0-7.8 (3H, m, arom.), 7.96 (1H, s), 11.0 (2H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.21, 43.06, 43.22, 47.36, 47.40, 54.14, 56.13, 63.21, 68.37, 69.87, 70.04, 71.35, 77.79, 79.90, 81.80, 103.36, 103.60, 105.93, 116.28, 119.35, 120.35, 120.44, 124.73, 128.77, 130.38, 130.71, 130.86, 131.96, 134.27, 140.04, 140.71, 145.66, 154.72, 157.19, 159.58, 168.93, 176.36; HRMS *m/z* $C_{28}H_{28}FN_3O_8$ calculated (553.54); found: 553.72.

8-(5-Hydrazino-2,4-dinitrobenzyl)-9-fluoro-3-methyl-10-(4-methyl piperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]-oxazino[2,3,4-*i,j*] quinoline-6-carboxylic acid (O+DNP): Brownish red; yield:71%; m.p.: 220 ºC; TLC R_f value = 0.23 (8:2 chloroform:hexane); IR (KBr, ν_{max}, cm^{-1}): 1620.15 (C=O), 3394.83 (N-H, *str.*), 822.06.04 (C-F), 693.73 (N-H, wagging),

2955.44 (C-H, *str.*),1620.15 (N-O, symm. *str.*), 1456.49 (N-O, asymm. *str.*), 1H NMR (600 MHz, DMSO-d_6): δ 1.25-(3H, d, methyl), 2.0 (2H, s, 1º amine), 2.50 (3H, s, N-CH_3), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.34 (4H, m, piperazine), 3.81 (3H, s), 4.0 (1H, s, 2º amine), 4.28 (2H, d, benzoxazine), 7.96 (1H, s), 8.90 (2H, s, arom.), 11.0 (1H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.24, 17.25, 43.06, 43.23, 47.38, 47.42, 54.15, 56.22, 68.42, 69.88, 79.90, 103.37, 103.61, 105.98, 120.52, 124.85, 128.79, 130.77, 130.92, 131.99, 140.78, 145.86, 154.86, 157.31, 169.01, 176.51; HRMS *m/z* $C_{25}H_{26}FN_7O_8$ calculated: 571.18; found: 571.71.

8-(3-Carboxy-2-hydroxy-5-sulfobenzyl)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]-oxazino[2,3,4-*i*]quinolone-6-carboxylic acid (O+SSA): Pale yellow; yield: 67%; m.p.: 214 ºC; TLC R_f value = 0.24 (8:2 chloroform:hexane); IR (KBr, ν_{max}, cm^{-1}): 683.76 (C=O, carbonyls), 621.61 (C=F *str.*), 1472.68 (C-H bending alkane), 981.08 (O-H bending carboxylic acid), 3407.24 (O-H *str.* phenolic), 1618.26 (C-C *str.* arom.), 3062.27 (C-H *str*. arom.), 683.53 (S-H *str.*); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.25 (3H, d, methyl), 2.0 (OH, s, sulphonic acid), 2.50 (3H, s, N-CH_3), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.34 (4H, m, piperazine), 3.81 (3H, s), 4.28 (2H, d, benzoxazine), 5.0 (1H, s, phenol), 7.0, 8.5 (2H, s, arom.), 7.96 (1H, s), 11.0 (2H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.25, 43.06, 43.22, 47.38, 47.42, 54.15, 56.22, 68.41, 69.87, 79.90, 103.37, 103.61, 105.97, 120.57, 124.85, 130.77, 130.92, 140.77, 145.85, 154.85, 157.32, 169.01, 176.49; HRMS *m/z*: $C_{26}H_{26}FN_3O_{10}S$ calculated: 591.56; found: 591.70.

8-(3-Hydrazinobenzyl)-9-fluoro-3-methyl-10-(4-methyl piperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazino[2,3,4-*i,j*]quinoline-6-carboxylic acid (O+PH): Light brown; yield: 65%; m.p.: 217 ºC; TLC R_f value = 0.20 (8:2 chloroform: hexane); IR (KBr, ν_{max}, cm^{-1}): 675.13 (C-F *str.*), 767.97 (N-H wagging 1º amines), 1477.85 (C-H bending alkenes), 1359.27 (C-H rocking alkanes) 1636.01 (C=O *str.* carbonyls), 3364.17 (N-H *str.* 2º amines), 3073.84 (C-H *str.*, arom.), 2762.94 (O-H stretch carboxylic acid), 2952.86 (C-H *str.* alkanes); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.25-(3H, d, methyl), 2.0 (2H, d, 1º amine), 2.50 (3H, s, N-CH_3), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.34 (4H, m, piperazine), 3.81 (3H, s), 4.0 (1H, t, 2º amine), 4.28 (2H, d, benzoxazine), 6.40-7.10 (3H, m, arom.), 7.96 (1H, s), 11.0 (1H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.22, 43.20, 47.39, 47.43, 54.14, 56.20, 68.42, 69.87, 79.90, 81.79, 103.52, 103.76, 124.94, 145.98, 155.01, 169.35; HRMS *m/z* $C_{25}H_{28}FN_5O_4$ calculated: 481.52 found: 481.73.

9-Fluoro-8-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-5-yl)methyl)-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazino[2,3,4-*i,j*]quinoine-6-carboxylic acid (O+MC): White; yield: 80%; m.p.: 202-205 ºC; TLC R_f value = 0.27 (8:2 chloroform:hexane); IR (KBr, ν_{max}, cm^{-1}): 677.49 (C-F *str.*), 1557.96 (C-C *str.* arom.), 2769.48 (O-H *str.* carboxylic acid), 3400, 3666 (O-H *str.*, phenol), 3066.17 (C-H *str.*, arom.); ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.25 (3H, d, methyl), 1.75 (3H, s, methyl), 2.50 (3H, s, N-CH_3), 2.72 (4H, t, piperazine), 3.17 (1H, m), 3.34 (4H, m, piperazine), 3.81 (3H, s), 4.28 (2H, d, benzoxazine), 5.0 (OH, s, phenol), 5.90-6.30 (3H, s, arom.), 7.96 (1H, s), 11.0 (1H, s, OH, carboxylic acid); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.24, 43.22, 47.38, 47.43, 54.15, 56.14, 68.41, 69.88, 71.54, 79.91, 81.80, 103.40, 103.64, 106.34, 124.80, 130.69, 130.84, 145.76, 154.82, 157.29, 169.12, 176.43; HRMS *m/z* $C_{29}H_{28}FN_3O_7$ calculated: 549.55; found: 549.72.

Antitubercular activity (*in vitro*): Antimycobacterial activity of the compounds against *M. tuberculosis* was studied using microplate Alamar Blue assay (MABA). This method is non-toxic, presents a strong correlation with BACTEC radiometric and proportional methods and employs a thermally stable reagent. First, during incubation, 200 µL of sterile deionized water was added to each outer perimeter well of sterile 96 wells plate to reduce medium evaporation in test wells. Then, 100 µL of the Middlebrook 7H9 broth was added to 96 wells plate and the serial dilutions of the compounds were prepared on a plate directly. The final drug concentrations were 100-0.800 µg/mL. The plates were covered and sealed with parafilm and incubated at 37 ºC for 5 days. After 5 days, 25 µL of freshly prepared 1:1 mixture of 10% Tween 80 and the Alamar Blue reagent was added to the plate, and the solution was incubated for 24 h. In the well, a blue colour indicated no bacterial growth, and pink colour revealed growth. MIC was the lowest drug concentration prevented the change in colour from blue to pink [21].

Molecular docking studies: Autodock vina program was used in molecular docking studies to predict the binding affinity of the synthesized compounds towards antitubercular activity. The crystal structure of high-resolution structures of *Mycobacterium tuberculosis* DNA Gyrase-inhibitor complexes (PDB Id: 5BS8) and structure were selected and downloaded from RCSB-PDB. The 5BS8 protein belongs to isomerase classification expressed from *Mycobacterium tuberculosis* resolution 2.40 Å [22].

RESULTS AND DISCUSSION

The ofloxacin as starting material was used for the synthesis of its derivatives using some medicinal drugs (trimethoprim, meloxicam, naproxen, sulfamethoxazole, metformin, ibuprofen and aspirin) and organic compounds (2,4-dinitrophenyl hydrazine, 5-sulpho salicylic acid, phenyl hydrazine, 7-hydroxy-4-methyl coumarin) were linked with methylene bridge *via* Blanc reaction. The synthesized derivatives were recrystallized by suitable solvents like ethanol.

Antitubercular activity (*in vitro*): The synthesized derivatives were evaluated for antitubercular activity using MABA method. The synthesized ofloxacin derivatives showed the promising activity as that of standard isoniazid (INH) at the concentration of (100, 50, 25, 6.25, 3.12, 1.6 and 0.8 µg/mL) (Table-1).

Docking studies: In this study, we virtually docked selected synthesized ofloxacin derivatives against X-ray crystallographic structures which include the structure of *M. tuberculosis* DNA Gyrase domain (5BS8) using autodock vina docking software (Fig. 1) [22]. The detailed molecular docking results are shown in Table-2.

TABLE-1
ANTITUBERCULAR RESULTS OF SYNTHESIZED COMPOUNDS

Compound derivative	Sample	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL	0.8 µg/mL
O+TRI	01	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
O+MEL	02	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
O+NAP	03	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
O+SUL	04	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
O+MET	05	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
O+IBU	06	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
O+ASP	07	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
O+DNP	08	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
O+SSA	09	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
O+PH	10	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
O+MC	11	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
Standard	Isoniazid	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant

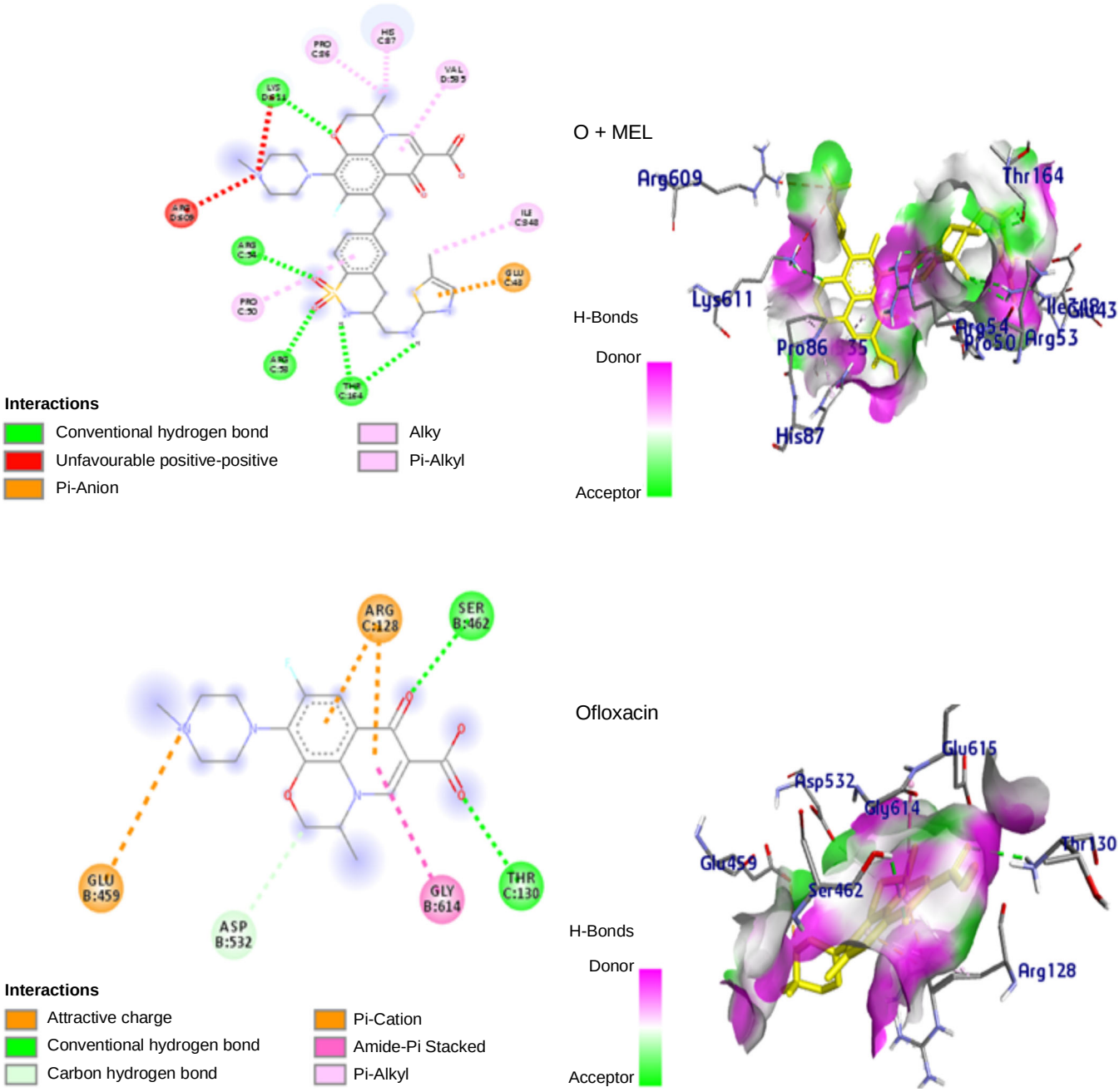


Fig. 1.

TABLE-2

Compound derivative	Binding energy (Kcal/mol)
O+SUL	-9.8
O+MEL	-10.5
O+IBU	-9.1
O+NAP	-9.7
O+TRI	-9.6
O+MET	-10.2
O+ASP	-9.2
O+DNP	-10.0
O+SSA	-9.4
O+PH	-9.3
O+MC	-10.0
Ofloxacin standard	-9.0

Conclusion

In this study, ofloxacin derivatives were synthesized and their antitubercular activity was evaluated using the MABA method. Future work can focus on the derivatives of ofloxacin. The advantageous effects of the drugs revealed that the ofloxacin derivatives present a great potential for the use as antitubercular drugs. The *in silco* study presented many interesting results. On the basis of the theoretical predictions, the ofloxacin moiety is a promising pharmacophore available in various pharmacologically active agents and can be chemically modified for feasible permeability and oral bioavailability.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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