Research Article

Synthesis, Molecular Docking Studies, *In Vitro* Antibacterial and Antifungal Activities of Some Novel N-4 Piperazinyl Derivatives of Gatifloxacin

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ABSTRACT

A series of gatifloxacin derivatives were synthesized (O1-O10) Via N-Piperazinyl linkage. The structural conformation was done by infra-red, nuclear magnetic resonance, mass spectrometry and elemental analysis technique. In present investigation, we screened docking simulation for synthesized compounds (O1-O10) to find out binding modes of derivatives with 5IWM and 3FV5. The compound O5 showed good antibacterial activity against gram positive (*S. Aureus*) and compound O6 showed good antibacterial activity against gram negative (*E.coli*) in comparison with standard drugs (Ciprofloxacin and Gatifloxacin). Compound O9 showed mild antifungal activity against (*A. niger*) and (*A. fumigatus*) in comparison with standard drug ketoconazole. The Zone of inhibition and MIC studies were performed to synthesized compounds. The correlation between experimental data (minimum inhibition concentration) versus docking score suggested that penetration for docking simulation were good in reproducing experimental orientation of these synthesized compounds.

Keywords: N-Piperazinyl derivatives, Ciprofloxacin, Gatifloxacin, DNA Gyrase, Anti-microbial, Topoisomerase-IV, Docking studies.

INTRODUCTION

Quinolone antibacterial agents have potent activity against gram-positive and gram-negative bacteria and are currently used as therapy for various bacterial infections. The antibacterial activities of quinolones are related in their inhibitory activities against DNA Gyrase (Topoisomerase-II) and topoisomerase-IV.[1,2] Both enzymes are members of the type-II topoisomerase family that controls bacterial DNA topology by passing a DNA double helix through another, by using a transient double -standard break.[3] It has recently been reported that the primary target of several quinolones in Escherichia coli is DNA gyrase and that in staphylococcus aureus is topoisomerase-IV.[2,4-11] Quinolones also inhibit mammalian type-II topoisomerase such as topoisomerase-IIVand their inhibitory potencies for topoisomerase-II have been correlated with their cytotoxicity.[12-16] Since the introduction of fluoroquinolones in late 1970s, they have generated great excitement, opportunities and applications in the antibacterial chemotherapeutic world, as these agents potentially offer's all the general attributes of ideal antibacterial agents. Over the last 15 years, researchers have attempted and proved these attributes as reality.[17]

Gatifloxacin, a newly developed quinolone, has shown potent activity against gram-negative bacteria and it has been reported that Gatifloxacin inhibits DNA gyrase of Escherichia Coli, Pseudomonas aeruginosa, Micrococcus luteus, and Staphylococcus aureus, like other quinolones, [18,19] Its structure and ball-stick three-dimensional model have shown in Figs.1 and 2, respectively.

It inhibits both bacterial DNA gyrase and topoisomerase-IV.[20, 21] The structure of gatifloxacin differs from earlier fluoroquinolones by the presence in the C-8 position of a methoxy group that enhances its antibacterial activity against gram positive bacteria improves its activity against DNA gyrase mutants of Escherichia coli.[22,23] The methoxy group in C-8 position also diminishes photosensitivity reactions.[24] Gatifloxacin, Ciprofloxacin, Levofloxacin inhibited members of the family Enterobacteriaceae comparably.[25,26] Gatifloxacin is more potent than ciprofloxacin against methicillin-resistant S. Aureus (MRSA),[26] although clinical significance of this still needs to be investigated. Gatifloxacin was recently investigated for the treatment of non-gonococcal urethritis.[27] Gatifloxacin is usually similar to moxifloxacin and levofloxacin and more active than ciprofloxacin against susceptible streptococci.[28-30] Dysglycemia has been noted as the life-threatening adverse effect of gatifloxacin, which led to its withdrawal from the market in the united states in 2006.[31] Gatifloxacin is reportedly highly active against atypical genitourinary pathogens such as chlamydia trachomatis and urea plasma urealyticum.[32,33] It is extensively distributed into many tissues and body fluids.[34-36] Due to structural characteristics, of gatifloxacin mechanism of killing action is not dependent on bacterial life cycle.[37]

The present study reports on the synthesis, spectroscopic analysis including IR and ^{1}H NMR, mass spectrometry and their biological activities of N-piperazinyl derivatives of gatifloxacin (O_1-O_{10}). Molecular docking was performed,

Molecular docking plays an important role in the rational design of drugs.

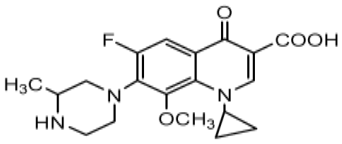


Figure 1: Gatifloxacin [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid]

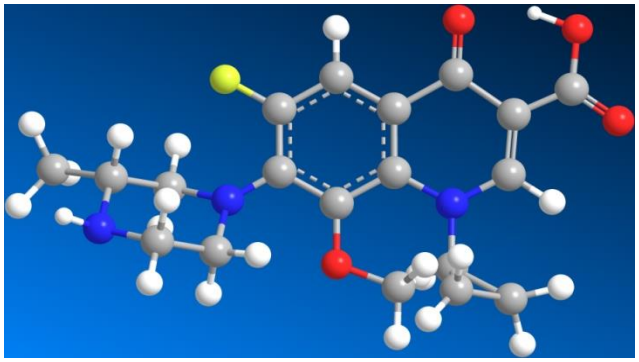


Figure 2: Gatifloxacin (ball and stick 3D model)

In the field of molecular modelling, docking 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.[38] Docking the goal of ligand and protein docking was mainly used to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure,[39] with invitro antimicrobial results in hand, we thought it worthwhile to perform studies to support the result.[40] The main aim was to evaluate the possible relationship between docking activity of the synthesized compounds (O_1-O_{10}) along with interaction with crystal structure of DNA gyrase of S.Aureus [PDB:5IWM] and topoisomerase-IV of E.Coli [PDB ID: 3FV5].

MATERIAL AND METHODS

Chemistry

Chemicals, solvents and thin layer chromatography used in our work were obtained from chemical company E-Merck. The melting points were determined using the melting point apparatus (Bio-techno lab) in opened glass capillary tubes and were uncorrected.Infra-red spectra was listed as KBR powder using attenuated total reflectance (ATR) (Bruker). Mass spectroscopy was carried out to determine molecular weight of compound using GCMC QP5000 (shimadzu). ^{1}HNMR was carried out to assign structure of synthesized compound and were run on JEOL GSX400 spectrometer in DMSO-d_6.

Experimental

Procedure for synthesis of (compound O_1)7-(4-((4-amino-3-methylphenyl)sulfinyl)-3-methylpiperazin-1-yl)-1-cyclo propyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, (compound O_2) 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-((3-methyl-4-(methylamino) phenyl)sulfinyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline -3-carboxylic acid, (compound O_7) 1-cyclopropyl-7-(4-(3,4-dimethyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-3-methyl piperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydro quinoline-3-carboxylic acid, (compound O_8) 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(3-methyl-2,5-dioxo pyrrolidin-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, (compound O_9) 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(3-methyl-2-oxopyrrolidin-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid and (compound O_{10}) 7-(4-(3-(aminomethyl)-4-methyl pyrrolidin-1-yl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid fromgatifloxacin [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]:

To an equimolar amino methyl benzenesulfenyl (Derivative O_1) methyl (methylamino)benzenesulfenyl (Derivative of O_2), dimethyl oxopyrazol (Derivative of O_7), methyl dioxopyrrolidine (Derivative of O_8), methyl oxopyrrolidine (Derivative of O_9), N-Boc amino methyl methyl pyrrolidine (Derivative of O_{10}) and gatifloxacin were added to ethanol, required quantity of formaline (37%) was added and the reaction mixture was heated at reflux overnight, cooled. The resulting precipitate solid was filtered and recrystallized from 95% ethanol (100ml) to give the product.

Procedure for synthesis of (compound O_3) 7-(4-(4-amino-3-methylbenzoyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid and (compoundO_4)1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(3-methyl-4-nitrobenzoyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acidfromgatifloxacin [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]:

To an equimolar mixture of finely powdered amino methyl benzoyl chloride (Derivative of O_3), nitro methyl benzoyl chloride (Derivative of O_4) and Gatifloxacin was dissolved in 5% NaOH solution and was mixed vigorously. The reaction mixture was warmed for an hour and allowed to cool for crystallization. The precipitate was filtered off, washed and dried under vacuum in a desiccator. Gatifloxacin reacted with derivatives in the presence of THF and TEA.

Procedure for synthesis of (compound O_5)*1-cyclopropyl-7-(4-((3,4-dimethylphenyl)amino)-3-methylpiperazin-1-yl)-6-*fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carbo xylicacid, (compound O_6) 1-cyclopropyl-6-fluoro-8-methoxy-7-(4-((4-methoxy-3-methylphenyl)amino)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylicacidfromgatifloxacin [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]:

To an equimolar mixture of Gatifloxacin and sodium bicarbonate in 10ml of acetonitrile was stirred at 50ºc for 4hrs. Reaction mixture was cooled to 0ºc and dimethyl phenyl amino (Derivative of O_5) and methoxy methyl phenyl amino (Derivative of O_6) was added. The mixture was stirred at magnetic stirrer for 5hrs at 0-5ºc. Acetonitrile was removed, the precipitate was dried by sodium sulphate, recrystallized in hexane acetone mixture.

Spectral data

7-(4-((4-amino-3-methylphenyl)sulfinyl)-3-methyl piperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(**O_1**): yield: 64%; m.p: 265-267^{0}C.IR V_{max}(cm^{-1}ATR): 3373(N-H_2), 3011(CH Ar.), 2847 (C-H Cyclopropane), 2618 (O-H carboxyl), 1704(C=O carboxyl), 1623 (C=C), 1342 (SO Piperazine), 1342 (C-F), 1270 (C-N piperazine), 703 (C-S).^{1}H NMR (300 MHz; DMSO-d_6) δ:1.075-1.33(m,4H, Cyclopropane), 1.11(m,3H, methyl), 2.12(s,3H, methyl), 2.12(s,3H, methyl), 2.68(m,2H, methine), 2.73-2.83(m,2H, methylene), 3.135-3.17(m,2H, methylene), 3.28-3.54(m, 2H, methylene), 3.83(s,3H, methyl), 4.12(m, 1H, cyclopropane), 6.96(d, 1H, benzene), 7.24(d,1H, benzene), 7.51(s, 2H, amine), 7.69(d,1H, benzene), 8.01(s, 1H, ethylene), 15.12(s, 1H, Carboxylic acid). MS-ESI: m/z 528.18 (M+1), Elemental analysis (%): $C_{26}H_{29}FN_4O_5S$: C, 59.08; H, 5.53; F, 3.59; N, 10.60; O, 15.13; S, 6.07.

1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-((3-methyl-4-(methylamino)phenyl)sulfinyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (O_2): yield: 72%; m.p: 246-248^{0}C. IRV_{max}(cm^{-1}ATR): 2843 (CH Ar.), 1737 (C=O carboxyl), 1625 (C=C), 1374 (SO Piperazine), 1292 (Sec Amine), 1256 (C-N Piperazine), 710 (C-S).^{1}H NMR (300 MHz; DMSO-d_6) δ:1.075-1.33(m,4H, Cyclopropane), 1.11(m,3H, methyl), 2.12(s,3H, methyl),2.64(s,2H, methyl), 2.68(m,1H, methine), 2.73-2.83(m,2H, methylene), 3.135-3.17(m,2H, methylene), 3.28-3.54(q, 2H, methylene), 3.83(s,3H, methyl), 4.12(m, 1H, cyclopropane), 6.72(s, 1H, sec. amine), 6.83(d, 1H, benzene), 7.29(q, 2H, benzene), 7.69(d, 1H, benzene), 8.01(s, 1H, ethylene), 15.12 (s, 1H, Carboxylic acid). MS-ESI: m/z 542.20 (M+1), Elemental analysis (%): $C_{27}H_{31}FN_4O_5S$: C, 59.76; H, 5.76; F, 3.50; N, 10.33; O, 14.74; S, 5.91.

7-(4-(4-amino-3-methylbenzoyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydro quinoline-3-carboxylic acid (O_3): yield: 83%; m.p: 242-245^{0}C.IR V_{max}(cm^{-1}ATR):3376 (N-H), 2998(C-H Ar.), 2999(OH-Carboxyl), 1633(C=C), 1364(C-F), 1263(C-N Piperazine).^{1}H NMR (300 MHz; DMSO-d_6) δ:1.075-1.33(m,4H, Cyclopropane), 1.31(m,3H, methyl), 2.12(s,3H, methyl), 3.31-3.35(m,2H, methylene), 3.38-3.48(m,2H, methylene), 3.46-3.71(m, 2H, methylene), 3.65(m,1H, methine), 3.83(s,3H, methyl), 4.12(m, 1H, cyclopropane), 6.96(d, 1H, benzene), 7.44(d,1H, benzene), 7.51(s, 2H, amine), 7.66(s, 1H, benzene), 8.66(s,1H, ethylene), 14.93 (s, 1H, Carboxylic acid). MS-ESI: m/z 508.16 (M+1), Elemental analysis (%): $C_{27}H_{29}FN_4O_5$: C, 63.77; H, 5.75; F, 3.74; N, 1.02; O, 15.73.

1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(3-methyl-4-nitrobenzoyl)piperazin-1-yl)-4-oxo-1,4-dihydro quinoline-3-carboxylic acid (**O_4**): yield: 78%; m.p: 247-248ºC. IRV_{max}(cm^{-1}ATR): 3069(CH Ar.), 2870(CH Cyclopropane), 2656(O-H carboxyl), 2821 (OCH_3), 1677(C=C), 1580(NO_2), 1321(C-F), 1271(C-N Piperazine).^{1}H NMR (300 MHz; DMSO-d_6) δ:1.075-1.33(m,4H, Cyclopropane), 1.31(m,3H, methyl), 2.64(s,3H, methyl), 3.31-3.35(m,2H, methylene), 3.38-3.48(m,2H, methylene), 3.46-3.71(m,2H, methylene), 3.65 (m, 1H, methine), 3.83(s,3H, methyl), 4.12(m, 1H, cyclopropane), 7.69(d, 1H, benzene), 8.01(d,1H, benzene), 8.07(s, 1H, benzene), 8.30(d,1H, benzene), 8.66(s,1H, ethylene), 14.93 (s, 1H, Carboxylic acid). MS-ESI: m/z 538.19(M+1), Elemental analysis (%): $C_{27}H_{27}FN_4O_7$: C, 60.22; H, 5.05; F, 3.53; N, 10.40; O, 20.80.

1-cyclopropyl-7-(4-((3,4-dimethylphenyl)amino)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**O_5**): yield: 68 %; m.p: 270-272ºC.IR V_{max}(cm^{-1}ATR): 3010(CH Ar.), 2916(C-H Cyclopropane), 2545(OH Carboxyl), 1721(C=O carboxyl), 1628(C=C), 1332(C-F), 1271.15(C-N Piperazine), 1216.15(NH-amine).^{1}H NMR (300 MHz; DMSO-d_6) δ:1.075-1.33(m,4H, Cyclopropane), 1.27(m,3H, methyl), 2.19(s,3H, methyl), 2.21(s,3H, methyl), 2.73-2.83(m,2H, methylene), 2.88(m,1H, methine), 3.13-3.17(m,2H, methylene), 3.28-3.54(q,2H, methylene), 3.83(s, 3H, methyl), 4.12(m, 1H, cyclopropane), 6.75(d, 1H, benzene), 6.91(d,1H, benzene), 6.92(d, 1H, benzene), 7.69(d,1H, benzene), 8.66(s,1H, ethylene), 8.78(s,1H, sec. amine), 14.93 (s, 1H, Carboxylic acid).MS-ESI: m/z 494.23 (M+1), Elemental analysis (%): $C_{27}H_{31}FN_4O_4$: C, 65.57; H, 6.32; F, 3.84; N, 11.33; O, 12.94.

1-cyclopropyl-6-fluoro-8-methoxy-7-(4-((4-methoxy-3-methylphenyl)amino)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**O_6**): yield: 58%; m.p: 286-288ºC.IR V_{max}(cm^{-1}ATR): 3069(CH Ar.), 2870(CH Cyclopropane), 2821(OCH3), 2602(O-H carboxyl), 1657(C=O carboxyl), 1598(C-C), 1322(C-F), 1284(Sec Amine), 1271(C-N Piperazine).^{1}H NMR (300 MHz; DMSO-d_6) δ:1.075-1.33(m,4H, Cyclopropane), 1.27(m,3H, methyl), 2.15(s,3H, methyl), 2.73-2.83(m,2H, methylene), 2.88(m,1H, methine), 3.13-3.17(m,2H, methylene), 3.28-3.54(q,2H, methylene), 3.72 (s, 3H, methyl), 3.83(s,3H, methyl), 4.12(m, 1H, cyclopropane), 6.65(d, 1H, benzene), 6.80(d,1H, benzene), 6.97(s, 1H, benzene), 7.69(d,1H, benzene), 8.66(s,1H, ethylene), 8.78(s,1H, sec. amine), 14.93 (s, 1H, Carboxylic acid).MS-ESI: m/z 510.23 (M+1), Elemental analysis (%): $C_{27}H_{31}FN_4O_5$: C, 63.52; H, 6.12; F, 3.72; N, 10.97; O, 15.67.

1-cyclopropyl-7-(4-(3,4-dimethyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**O_7**): yield: 66%; m.p.: 236-239ºC.IR V_{max}(cm^{-1}ATR): 3301(NH), 3050(CH Ar.), 2920(CH Cyclopropane), 2844(OCH3),

2781(O-H carboxyl), 1737(C=O carboxyl), 1625(C=C), 1374(C-F), 1256(C-N Piperazine).^{1}H NMR (300 MHz; DMSO-d_6) δ: 0.70(d,3H, methyl), 1.075-1.33(m,4H, Cyclopropane), 1.27(m,3H, methyl), 1.94(s,3H, methyl), 2.5(q,1H, methine), 2.73-2.83(m,2H, methylene), 2.88(m, 1H, methine), 3.13-3.17(m,2H, methylene), 3.28-3.54(q,2H, methylene), 3.83(s, 3H, methyl), 4.12(m, 1H, cyclopropane), 7.69(d,1H,benzene), 8.66(s,1H, ethylene), 14.93 (s, 1H, Carboxylic acid).MS-ESI: m/z 485.21 (M+1), Elemental analysis (%): $C_{24}H_{28}FN_5O_5$: C, 59.37; H, 5.81; F, 3.91; N, 14.42; O, 16.48.

1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(3-methyl-2,5-dioxopyrrolidin-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (O_8): yield: 74%; m.p: 242-245°C.IRV_{max}(cm^{-1}ATR): 3371(NH), 3069(CH Ar.), 2915(CH Cyclopropane), 2820(OCH_3), 2661(OH Carboxyl), 1720(C=O carboxyl), 1628(C=C), 1332(C-F), 1271(C-N Piperazine).^{1}H NMR (300 MHz; DMSO-d_6) δ: 1.075-1.33(m,4H, Cyclopropane), 1.17(d,3H, methyl), 1.27(m,3H, methyl), 2.33(s,3H, methyl), 2.73-2.83(m,2H, methylene), 2.83(m,1H, succinimide), 2.88(m, 1H, methine), 3.13-3.17(m,2H, methylene), 3.28-3.54(q,2H, methylene), 3.83(s, 3H, methyl), 4.12(m, 1H, cyclopropane), 7.69(d,1H,benzene), 8.66(s,1H, ethylene), 14.93 (s, 1H, Carboxylic acid).MS-ESI: m/z 486.17 (M+1), Elemental analysis (%): $C_{24}H_{27}FN_4O_6$: C, 59.25; H, 5.59; F, 3.91; N, 11.52; O, 19.73.

1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(3-methyl-2-oxopyrrolidin-1-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (O_9): yield: 82%; m.p: 268-270°C.IR V_{max}(cm^{-1}ATR): 3396(NH), 3010(CH Ar.), 2919(CH Cyclopropane), 2849(OCH_3), 2688(O-H carboxyl), 1703(C=O carboxyl), 1622(C=C), 1342(C-F), 1270(C-N Piperazine).^{1}H NMR (300 MHz; DMSO-d_6) δ: 1.075-1.33(m,4H, Cyclopropane), 1.12(d,3H, methyl), 1.27(m,3H, methyl), 1.91-2.17(m,2H, pyrrolidine-2-one), 2.33(m,3H, methyl), 2.33(m,1H, pyrrolidine-2-one), 2.73-2.83(m2H,methylene), 2.88(m,1H, methane), 3.13-3.17(m,2H, methylene), 3.24-3.34(m,2H, pyrrolidine-2-one), 3.28-3.54(q,2H, methylene), 3.83(s, 3H, methyl), 4.12(m, 1H, cyclopropane), 7.69(d,1H,benzene), 8.66(s,1H, ethylene), 14.93 (s, 1H, Carboxylic acid).MS-ESI: m/z 472.16(M+1), Elemental analysis (%): $C_{24}H_{29}FN_4O_5$: C, 61.01; H, 6.19; F, 4.02; N, 11.86; O, 16.93.

7-(4-(3-(aminomethyl)-4-methylpyrrolidin-1-yl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (O_{10}): yield: 76%; m.p: 260-263°C. IRV_{max}(cm^{-1}ATR): 3069.72(CH Ar.), 2872(CH Cyclopropane), 2820(OCH_3), 2661(O-H carboxyl), 1679(C=C), 1322(C-F), 1272(C-N Piperazine).^{1}H NMR (300 MHz; DMSO-d_6) δ: 0.93(d,3H, methyl), 1.075-1.33(m,4H, Cyclopropane), 1.27(d,3H, methyl), 1.44 (m,1H, pyrrolidine), 1.5(m, 2H, amine), 1.59 (m,1H, pyrrolidine), 2.44-2.69(q,2H, methylene), 2.73-2.83(m,2H, methylene), 2.815(m,4H, pyrrolidine), 2.88(m,1H, methine), 3.13-3.17(m,2H, methylene), 3.28-3.54(q,2H,methylene), 3.83(s, 3H, methyl), 4.12(m, 1H, cyclopropane), 7.69(d,1H,benzene), 8.01(s,1H, ethylene), 15.12 (s, 1H, Carboxylic acid).MS-ESI: m/z 487.26 (M+1), Elemental analysis (%): $C_{25}H_{34}FN_5O_4$: C, 61.59; H,7.03; F, 3.90; N, 14.36; O, 13.13.

Biological Evaluation

Antibacterial and antifungal activity

All the title compounds were screened for their antibacterial and antifungal activities by paper disc diffusion method. The strain and test organisms were gram-positive bacteria (A)- such as (Staphylococcus Aureus ATCC-6483P, Staphylococcus Epidermidis ATCC 15) and two-gram negative bacteria (Escherichia Coli ATCC 2922 and Klebsiella pneumoniae ATCC 29665) using nutrient agar medium. Antifungal activities were tested against two fungal organism namely Aspergillus fumigatus ATCC 46645 using sabouraud dextrose agar (Apex laboratories Pvt. limited, Chennai, India). For primary screening antimicrobial test were carried out by paper disc diffusion method.

Paper-Disc Diffusion Technique

The sterilized medium was autoclaved at 120^{0}c for 30min at a temperature of 40-50^{0}c was inoculated (1ml/100ml of medium) with the suspension (10^5 cfu/ml) of the micro-organisms (matched to McFarland barium sulphate standard) and was poured into a Petri dish to give a depth of 3-4nm. The paper impregnated with the test compounds (100 µg/ml in dimethyl formamide) was placed on solidified medium. The plates were pre-incubated for 1hr at room temperature and incubated at 37^{0}c for 24 and 48hr under aseptic conditions for antibacterial and antifungal activities against standard antibacterial and antifungal antibiotics. Ciprofloxacin (100 µg/disc) was used as positive control over gram positive and gram-negative bacteria and Ketoconazole (100 µg/disc) was used for control over fungi MIC was also determined by agar streak dilution method and is presented in table-1.

Minimum inhibitory concentration (MIC)

MIC of the compound was determined by agar streak dilution method.[45,46] A stock solution of the synthesized compounds(100 µg/ml) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for anti- bacterial activity and sabouraud dextrose agar medium for anti-fungal activity). A specified quantity of the medium (40-50°C) containing the compound was poured into a Petri-dish to give a depth of 3-4 mm and was allowed to solidify. Suspension of the microorganism were prepared to contain approximately 10^5cfu/ml and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37 °C for 24 h and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance

exhibiting no visible growth of bacteria or fungi on the plate. The observed MIC is presented in Table-1.

Docking study

Molecular docking studies of synthesized compounds O_1-O_{10} with well established structure of *S. aureus* and *E. coli* was performed using Auto Dock vina 1.12 version and chimera 1.12 version. The binding pocket of the active site of DNA gyrase (PDB:5IWM) for gram positive bacteria like *S. aureus* and (PDB:3FV5) for gram negative bacteria like *E. coli*.[46,47] Docking method involves the following steps. First the ligand molecule was build, in second step required protein was downloaded from PDB, preparation and validation of macromolecule by x-ray crystallography. Third step is identification of binding affinity by the extent of binding of ligand to the protein of molecule.

RESULTS AND DISCUSSION

Chemistry

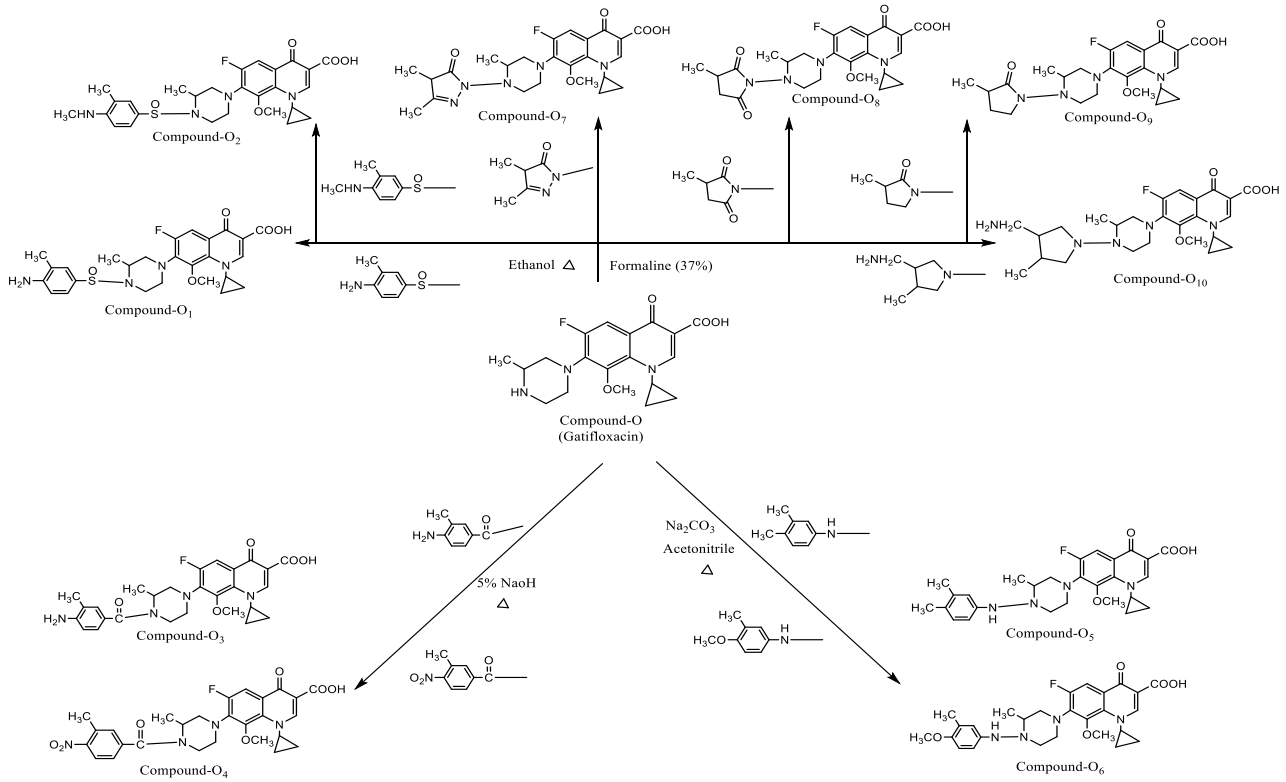
Syntheses of desired compounds O1-O10 were obtained with the help of various derivatives. The compounds O_1, O_2, O_7, O_8, O_9 and O_{10} were obtained by solvents like, formalin 37% and ethanol by reflux method.[41] The compounds O_3, O_4 were obtained by Schotten Baumann method by using reagents like 5% NaOH, tetrahydrofolate (THF) and Tri-ethylamine (TEA).[42] The compounds O_5 and O_6 were obtained by Na_2CO_3, acetonitrile, and ethanol by reflux method [43] as shown in scheme. IR of all compounds showed absorption bonds at 3250-3500cm^{-1} due to NH_2 group in addition of hydroxy groups at 2500-3000cm^{-1}, carboxyl groups at 1680-1744 and C-F functional group at 1321-1350cm^{-1}, respectively.[44] ^{1}H NMR of all compounds showed singlets at δ 1.0075-1.33 of CH_2 and δ 8.66 of ethylene group respectively. Their mass spectra were matched with the designed structures.

Antibacterial activity

All the synthesized compounds (O_1-O_{10}) were tested zone of inhibition and MIC values against two gram-positive (*S. aureus* and *S. epidermidis*) and two gram-negative (*E. coli* and *K. pneumonia*) bacteria. All the compounds exhibited good activity against both gram-positive and gram-negative bacteria. Compounds O_5, O_2, O_6 and O_1 were found to possess significant antibacterial activity against gram positive organisms when compared to standard drugs (ciprofloxacin and gatifloxacin). Compounds O_6 and O_5 were found to possess significant antibacterial activity against gram negative organisms when compared to standard drugs (ciprofloxacin and gatifloxacin). The synthesized compounds exhibited MIC values in the range of 0.2-4.0 µg/ml shown in Table 1.

Compound O_5 exhibited excellent antibacterial activity with MIC value in range of (0.6µg/ml) when compared to standard drug Gatifloxacin (0.8µg/ml) and Ciprofloxacin (0.6µg/ml). This excellent antibacterial activity was may be due to addition of new derivative 3,4-dimethylbenzenamine group at 7th position of piperazinyl ring.

Compound O_6 exhibited good gram negative antibacterial with MIC values in range of (0.2µg/ml) when compared to standard drug Gatifloxacin (0.19µg/ml) and Ciprofloxacin (0.13µg/ml). This good antibacterial activity was may be due to addition of new derivative 4-methoxy-3-methylbenzenamine at 7th position of piperazinyl ring.



Scheme: Synthesis of N-Piperazinyl derivatives of Gatifloxacin

Anti-fungal activity

All the synthesized compounds (O_1-O_{10}) were tested zone of inhibition and MIC values against two fungi organisms (A. Niger and A. fumigatus). All the compounds exhibited mild activity. Compound O_9 was found to possess significant anti-fungal activity against A. Niger and A. fumigatus when compared to standard drug ketoconazole. The synthesized compounds exhibited MIC values in the range of 12.7-17.2µg/ml shown in Table 1.

Compound O_9 exhibited mild antifungal activity with MIC values of (12.8µg/ml) and (12.7µg/ml) when compared to standard drug Ketoconazole (11.2µg/ml) and (10.8µg/ml). This mild antifungal activity was maybe due to addition of new derivative 3-methylpyrrolidin-2-one group at 7th position of Piperazinyl ring.

Table 1: Anti-microbial activity of the synthesized compounds (O_1-O_{10}) (100 µg/ml)

Compounds	*Invitro* activity - Zone of inhibition in mm (MIC in µg/ml)					
	Gram-Positive bacteria		Gram-Negative bacteria		Fungi	
	S.aureus	*S.epidermidis*	*E.coli*	*K.pneumoniae*	*A.Niger*	*A.fumigatus*
O1	30(1.1)	25(3.4)	28(1.5)	25(4.0)	21(16.6)	18(14.1)
O2	33(0.8)	24(3.3)	25(1.6)	25(2.6)	22(15.9)	19(13.6)
O3	27(1.9)	26(3.8)	30(1.2)	35(0.4)	21(16.2)	17(13.8)
O4	29(1.3)	26(2.6)	32(0.9)	32(2.0)	19(15.8)	18(13.9)
O5	36(0.6)	31(2.2)	34(0.3)	21(3.7)	22(16.6)	23(14.2)
O6	32(0.8)	28(2.9)	34(0.2)	34(0.5)	22(17.2)	21(14.7)
O7	28(1.4)	24(3.1)	24(2.1)	25(3.9)	24(15.3)	25(13.2)
O8	27(1.8)	25(3.3)	30(1.2)	28(2.1)	23(14.1)	25(12.9)
O9	24(2.1)	27(2.5)	24(1.7)	27(3.3)	29(12.8)	26(12.7)
O10	25(2.0)	21(3.1)	27(2.1)	24(2.4)	22(15.8)	22(14.1)
Gatifloxacin[a]	34(0.8)	33(0.8)	32(0.19)	33(0.5)	-	-
Ciprofloxacin[a]	35(0.6)	35(0.12)	35(0.13)	36(0.09)	-	-
Ketoconazole[b]	-	-	-	-	32(11.2)	29(10.8)
DMF[c]	-	-	-	-	-	-

Key words: Gatifloxacin[a], Ciprofloxacin[a]: Standard antibacterial drugs, Ketoconazole[b]: Standard antifungal drug and DMF[c]: Control. *S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus Epidermidis, E.coli: Escherichia Coli, K. pneumonia: Klebsiella pneumonia, A.Niger: Aspergillus Niger, A. Fumigatus: Aspergillus Fumigatus*.

Docking study

Molecular docking studies was employed for the analysis with training set composed of our synthesized compounds whose inhibitory activity was unknown. In order to find out the molecular facilities responsible for biological activity molecular docking studies were performed. From the docking studies, we predicted that all the synthesized compounds (O_1-O_{10}) possessed better antibacterial activity than the standard drugs (Ciprofloxacin and Gatifloxacin). By having good binding affinity with target protein and it could be used as potential drug as antibacterial.

Gram positive bacteria Docking Studies

Among all the docked compounds O_5, O_2, O_6 and O_1 showed good binding affinity and interaction with topoisomerase-II (DNA gyrase enzyme) (5IWM) with reference to standard drugs Ciprofloxacin and Gatifloxacin. The interactions of H bonds with ligands and bacterial enzymes explained, Compound O_5 at 3rd position carboxylic hydrogen forms hydrogen bond with GLY960 and 4th position oxygen forms hydrogen bond with GLY958 as shown in figure 3 & interactions are shown in figure 5.

Compound O_5 having higher dock score (-9.5) towards bacterial S. Aureus enzyme than the standard Ciprofloxacin (-7.8) and Gatifloxacin (-7.8) drugs. We may declare that the higher docking score was due to addition of 3,4-dimethylbenzenamine group at 7th position of gatifloxacin structure. The remaining compounds docking score and hydrogen bond interactions are described in Table 2.

Gram negative bacteria Docking Studies

Among all the docked compounds O_6 and O_5 showed good binding affinity and interaction with topoisomerase-IV enzyme (3FV5) with reference to standard drugs ciprofloxacin and gatifloxacin. Compound O_6 at 3rd position carboxylic hydrogen forms two hydrogen bonds with LEU80 & ARG79 as shown in figure 4 & interactions are shown in figure 5.

Compound $O_6$was having higher affinity (-7.4) towards E. Coli enzyme than the standard Ciprofloxacin (-7.3) and

Gatifloxacin (-6.7) drugs. We may declare that the higher docking score was due to addition of 4-methoxy-3-methylbenzenamine group at 7th position of gatifloxacin structure. The remaining compounds docking score and hydrogen bond interactions were described in Table 2. The correlation between experimental data (MIC) versus docking score of S.aureus and E.coli are displayed 0.928 r^2 and 0.935 r^2 (fig 6 & 7) which suggests that, parameters for docking simulation were good for both S.aureus and E.coli in reproducing experimental orientation of synthesized compounds.

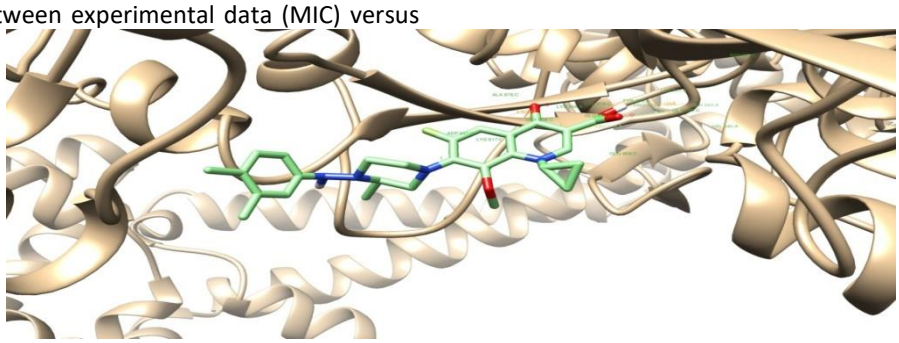


Figure 3: H-bonds interactions between compound (O_5) with topoisomerase-II DNA gyrase enzyme of gram positive *S. aureus* bacteria (5IWM).

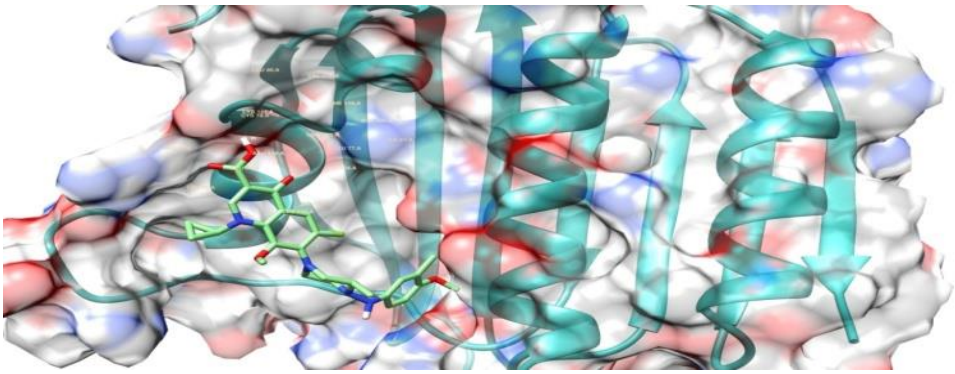


Figure 4: H-bonds interactions between compound (O_6) with Topoisomerase-IV enzyme of gram negative *E.coli* bacteria (3FV5)

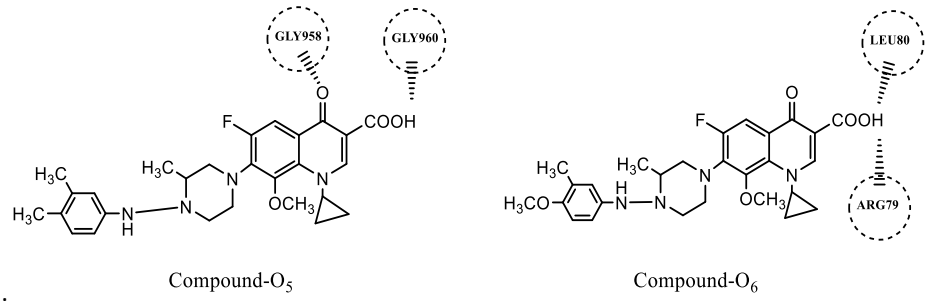


Figure 5: Two-Dimensional (2D) ligand interaction diagram of compounds O_5 with DNA gyrase and O_6 with E.Coli, the amino acid residues at the binding site are tagged in circles.

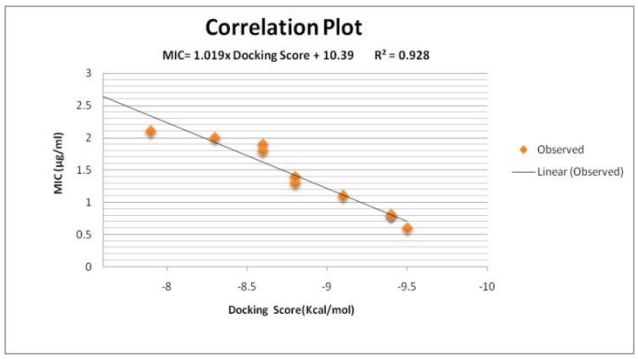


Figure 6: Gram Positive (*S.aureus*) Correlation plot between MIC (µg/ml) and Docking Scores (Kcal/mol)

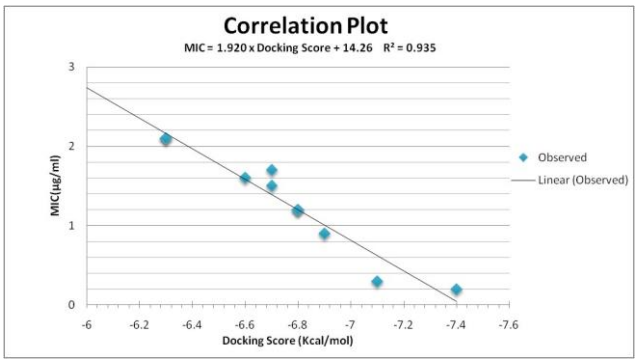


Figure 7: Gram Negative (*E.coli*) Correlation plot between MIC (µg/ml) and Docking Scores (Kcal/mol)

Table-2 Docking result of Synthesized Compounds (O_1-O_{10}) with MIC Values (µg/ml)

Compounds	Gram-Positive bacteria (S.aureus)			Gram-Negative bacteria (E.coli)		
	Docking Score[a]	MIC µg/ml	H Bonds with *5IWM* Protein	Docking Score[a]	MIC µg/ml	Interactions with *3FV5* Protein
O1	-9.1	1.1	Amino methyl benzene sulfenyl Oxygen with GLY934 & Amino hydrogen with GLU691.	-6.7	1.5	4th Position Oxygen with ILE81.
O2	-9.4	0.8	3rd position COOH oxygen with ALA138 & hydrogen with GLY960.	-6.6	1.6	3rd position COOH oxygen with SER82 & THR13.
O3	-8.6	1.9	3rd position COOH hydrogen with ILE983 & Amino methyl benzoyl hydrogen with ASP139.	-6.8	1.2	Amino methyl benzoyl oxygen with SER82.
O4	-8.8	1.3	Nitro methyl benzoyl oxygen with ASP139 & 3rd position COOH oxygen with ILE983.	-6.9	0.9	Nitro methyl benzoyl oxygen forms hydrogen bond with SER82.
O5	-9.5	0.6	3rd position COOH hydrogen with GLY960 & 4th Position Oxygen with GLY958.	-7.1	0.3	No interactions.
O6	-9.4	0.8	4th Position Oxygen with ALA138 & Methoxy (methyl phenyl) amino oxygen with LYS982.	-7.4	0.2	3rd position COOH hydrogen forms two hydrogen bonds with LEU80 & ARG79.
O7	-8.8	1.4	Dimethyl oxopyrazol oxygen with ASP693.	-6.3	2.1	3rd position COOH Oxygen with THR126 & Hydrogen with ARG124.
O8	-8.6	1.8	Methyl dioxo pyrrolidine Piperzine Oxygen with LEU473.	-6.8	1.2	3rd position COOH Oxygen with THR126 & Hydrogen with THR126
O9	-7.9	2.1	Methyl oxo pyrrolidine Piperzine oxygen with SER694.	-6.7	1.7	3rd position COOH Oxygen with THR126 & Hydrogen with ARG124.
O10	-8.3	2.0	3rd position COOH hydrogen with LEU843 &Aminomethylmethylpyrrolidine hydrogen with ARG885.	-6.3	2.1	3rd position COOH Oxygen with SER82 & Hydrogen with ILE76, 4th Position oxygen with SER82 &Aminomethylmethylpyrrolidine Two hydrogen bonds with GLY10 & THR13
Gatiflo-xacin[b]	-7.8	0.8	3rd position COOH oxygen with PHE962 & 4TH Position Oxygen with PHE962.	-6.7	0.19	3rd position COOH oxygens with ILE81 & SER82.
Ciproflo-xacin[b]	-7.8	0.6	7th PositionPiperzine hydrogen with VAL1120 & 3rd position COOH oxygen with ALA790.	-7.3	0.13	7th Position Piperzine hydrogen with ASP56.

[a]Based on Auto Dock Vina Score, [b]Gatifloxacin, Ciprofloxacin (Standard Drugs), *S. aureus: Staphylococcus aureus, E.Coli: Escherichia coli*

CONCLUSION

In conclusion we have synthesized and characterized ten new derivatives of gatifloxacin. All the compounds were fully characterized by spectral data and elemental analysis. All the compounds were studied for their interactions with DNA gyrase (topoisomerase-II) and topoisomerase-IV enzymes by molecular docking protocol. Among tested compounds O_5, O_2, O_6 and O_1 exhibited good docking score for gram positive bacteria and compounds like O_6 and O_5 for gram negative bacteria. Invitro antibacterial activity of tested compounds showedgood activity against microorganisms. In particular compounds O_5, O_2, O_6 and O_1 possessed significant gram-positive activity and compound O_6 and O_5 possessed significant gram-negative activity. The results of antibacterial activity were supported by docking analysis. These results provided the essential information for developing new derivatives of Gatifloxacin with improved activity.

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