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Research Article

SYNTHESIS, MOLECULAR BIOINFORMATICS MODELLING, AND ANTIMICROBIAL EVALUATION OF SOME NOVEL OXADIAZOLE FLUOROQUINOLONE DERIVATIVES

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ABSTRACT

Objective: The present study envisages a series of oxadiazole fluoroquinolone derivatives that were synthesized (D_1–D_{12}) with added derivatives such as phenyl, aminophenyl, amino hydroxyphenyl along with cyclopropyl, ethyl, piperazine, and imidazole.

Methods: All of the newly produced molecules were characterized by infrared, ^{1}H nuclear magnetic resonance, mass spectrometry, and elemental analysis technique and screened for docking stimulation to find out binding modes of synthesized derivatives with 3FV5, 5IMW, and 5ESE and evaluated for *in vitro* antimicrobial activity.

Results: From this study, it was found that the compound D_8 showed good antibacterial activity against Gram-positive (*Staphylococcus aureus*), compound D_9 showed good antibacterial activity against Gram-negative (*Escherichia coli*), and compound D_3 showed good antifungal activity against fungi (*Saccharomyces cerevisiae*) in comparison with standard drugs (Ciprofloxacin and fluconazole). The zone of inhibition and minimum inhibitory concentration studies was performed on synthesized compounds.

Conclusion: The analogs of oxadiazole flouroquinolone are suggested to be potent inhibitors with sufficient scope for further exploration.

Keywords: Ciprofloxacin, Norfloxacin, Fluconazole, DNA gyrase, Topoisomerase-IV, Docking studies.

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INTRODUCTION

Quinolones have risen to prominence as a significant class of antibacterial drugs in clinical trials. They appeal to scientists because of their high potency, quick bactericidal effects, and low-risk of resistance development [1].

Almost every quinolone antibiotic on the market used today is fluoroquinolones, which contain a fluorine atom and are effective against both Gram-positive and Gram-negative bacteria [2]. The potency, spectrum, and physicochemical characteristics of the latest fluoroquinolone generations have all improved significantly [3]. Because of their renal excretion and proclivity to be concentrated in the urine, the first members of the quinolone antibiotic class, such as nalidixic acid, were relatively low-potency drugs, which were primarily employed to treat infections of the urinary tract [4,5].

The fluorine atom and the 1-alkyl, 1,4-dihydro-4-oxo-quinolone-3-carboxylic acid skeleton of fluoroquinolones are responsible for their effectiveness in interacting with topoisomerase-II DNA gyrase and topoisomerase-IV enzymes, according to the SAR studies. Furthermore, the 6-fluoro and 7-piperazinyl groups are thought to be important for fluoroquinolones' broad-spectrum and antipseudomonal actions [6].

Ciprofloxacin is active against a large number of Gram-positive and Gram-negative bacteria *in vitro*. Ciprofloxacin kills bacteria by inhibiting the enzymes topoisomerase-II (DNA-gyrase) and topoisomerase-IV, which are needed for DNA replication, transcription, repair, and recombination in bacteria [7].

Norfloxacin is a fluoroquinolone antibiotic from the fluoroquinolone antibiotic class [8,9]. It is used to treat infections of the urinary system, gynecological infections, prostate inflammation, gonorrhea, and bladder infections [10-12]. *Staphylococcus aureus* is an opportunistic, anaerobic Gram-positive coccal pathogen that dwells on the mucous membranes of humans and skin and is extremely resistant to antibiotics [13]. *Escherichia coli* is a rod-shaped coliform bacteria that is Gram-negative and facultatively anaerobic found in the lower intestine of warm-blooded creatures (endotherms) [14,15]. Although the majority of *E. coli* strains are innocuous, some serotypes can cause significant food poisoning in their hosts and have been linked to product recalls due to food contamination [16,17].

The present study reports on the synthesis, spectroscopic analysis including infrared (IR) and ^{1}H nuclear magnetic resonance (^{1}H NMR), mass spectrometry, and their biological activities of oxadiazole flouroquinolone derivatives (D_1–D_{12}). In the rational design of medicines, molecular docking is critical. In the field of molecular modeling, 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 is an optimization problem that describes the "best-fit" orientation of a ligand when it binds to a certain protein of interest [18,19].

The main goal is to see if there is a link between the docking activity of the synthesized compounds (D_1–D_{12}) and their interactions with the crystal structures of *S. aureus* DNA gyrase (PDB: 5IWM), *E. coli* topoisomerase-IV (PDB ID: 3FV5), and *Saccharomyces cerevisiae* lanosterol 14-alpha demethylase (PDB ID: 5ESE).

METHODS

Experimental

Synthetic procedure for ethyl7-chloro-6-fluoro-1, 4-dihydro-4-oxoquinoline-3- carboxylate (step-1)

The synthesis involves a "GOULD-JACOBS" reaction of appropriate aniline with diethyl ethoxy methylene malonate (EMME). Equimolar amounts of 3-chloro-4-fluoro aniline (0.01 mol) (white crystalline solid, m.p. 44–47°C) and diethyl EMME (0.01 mol), (almost colorless liquid, b.p 279–281°C) were collected in a beaker under the solvent-

free condition when a clear solution was obtained by shaking and was irradiated under microwave synthesis for 1–1.5 min at 540–750 watts. By this time, the entire reaction mixture had been turned into a semisolid mass with a white to pale yellow color, which had been washed with acetone to obtain an almost white solid, and had been recrystallized with N, N-dimethyl formamide (DMF). The purity of the compounds was checked by thin layer chromatography (TLC) using petroleum ether: Chloroform: Ether (85:15:5). Percentage yield is 92.0%.

Synthetic procedure for 7-chloro-6-fluoro-1, 4-dihydro-4-oxoquinoline-3-carboxylic acid (step-2)

The product of step-1 (an ethyl ester) (2.7 g, 0.01 mol) was dissolved in 50 ml of benzene and hydrolyzed to corresponding carboxylic acid using 50 ml of 5N aq.HCL. After that, the reaction mixture was stirred and heated under reflux for 5–6 h. The white solid was gradually precipitated at the bottom of the aqueous layer. To obtain the product, the substance was filtered and rinsed in water until it was completely neutral, dried, and recrystallized using acetone as the solvent.

Synthetic procedure for R1 substituted-7-chloro-6-fluoro-1, 4-dihydro-4-oxoquinoline-3-carboxylic acid (N1-substitution) (step-3)

The product of step-2 (0.01 mol) was added to 10 ml of DMF, followed by the addition of alkyl halides (Cyclopropyl iodide and ethyl iodide) (0.01 mol). The reaction mixture was heated to dissolve the acid which was partially soluble in cold conditions. Then, anhydrous potassium carbonate (0.02 mol) was added to the reaction mixture. The whole reaction mixture was heated to a temperature of 120–140°C and stirred for 5–8 h. The reaction mixture was then poured onto the crushed ice or ice-cold water, washed with cold water to remove DMF and potassium carbonate if any. The resultant solid was recrystallized from acetone to give the corresponding compounds. Percentage yield is 72%.

Synthetic procedure for R1 substituted-6-fluoro-1,4-dihydro-4-oxo-R7 substituted-quinoline-3-carboxylic acid (step-4)

The product of step-3 (R1 substituted–7–chloro-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (0.01 mol) was added to 10 ml of pyridine, 3 ml of triethylamine, and different heterocyclics such as piperazine (0.05 mol) and imidazole (0.05 mol) were taken in a double neck round bottom flask.

The composition was irradiated for 6 min in the microwave at 455 watts. After completion of the reaction, the reaction mixture was cooled to room temperature. The reaction mixture was then poured onto the crushed ice and neutralized with dilute hydrochloric acid. The solid product was filtered and dried.

Synthetic procedure for R_1 substituted-6-fluoro–1,4–dihydro-4-oxo-R_7 substituted quinoline-3-carbohydrazide (step-5)

A mixture of product step-4 (R1 substituted-6-fluoro–1,4-dihydro-4-oxo-R7 substituted quinoline-3-carboxylic acid) (20.0 g, 55.0 mmol) and 85% hydrazine hydrate (20.0 g, 340.0 mmol) was refluxed for 24 h. The resulting precipitate was filtered and recrystallized from 95% ethanol (100 ml) to give the product. The percentage yield is 67%. Melting point is 207–208°C.

Synthetic procedure for title compounds (D_1–D_{12}) R_1 substituted-6- fluoro –3-(5-substituted – 1,3,4–oxadiazol–2- yl) -R_7- substituted quinoline – 4(1H) – one

The mixture of product step-5 (R1 substituted-6- fluoro–1,4–dihydro-4-oxo-R7 substituted quinoline-3-carbohydrazide) (0.006 mol) and substituted aromatic acids (0.006 mol) in 15 ml of phosphorous oxychloride was refluxed for 8 h. TLC was used to monitor the reaction's progress, with ethyl acetate: acetone (9:1) as the eluent. The reaction liquid was cooled and carefully placed onto 200 g crushed ice, stirring constantly, before being neutralized with sodium bicarbonate solution (10% w/v). The yielding solid was then filtered, rinsed thoroughly in cold water, dried, and recrystallized from ethanol, DMF (2:1) to give title compounds D_1–D_{12}.

Spectral data

D_1 - 1-cyclopropyl-6-fluoro-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(piperazin-1-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3392.23 (N-H_2), 3060.55 (C-H Aromatic), 1708.33 (C=O carboxyl), 1624.81 (C=C), 1321.28 (C-F), 1253.71 (C-N piperazine), 3054 (C-H Cyclopropane), and 1133 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 0.28 (t,2H, methylene), 0.53 (q,2H, methylene), 1.35 (d, 1H, CH), 2.0 (s, 1H, NH), 2.78 (d, 4H, Ar-CH_2), 3.47 (d, 4H, Ar-CH_2), 5.93 (d, 4H, benzene), and 7.48 (s, 4H, benzene). MS-ESI: m/z 431.18 (M+1), Elemental analysis (%): C24H22FN5O2: C, 66.81; H, 5.14; F, 4.40; N, 16.23; and O, 7.42.

D_2 - 3-(5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl)-1-cyclopropyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3499.13 (N-H_2), 3046.15 (C-H Aromatic), 1715.98 (C=O carboxyl), 1650.27 (C=C), 1288.57 (C-N piperazine), 1334.18 (C-F), 3055 (C-H Cyclopropane), and 1125 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 0.28 (t,2H, methylene), 0.53 (q,2H, methylene), 1.35 (d, 1H, CH), 2.0 (s, 1H, NH), 2.78 (d, 4H, Ar-CH_2), 3.47 (d, 4H, Ar-CH_2), 4.0 (s, 2H, NH_2), 5.93 (d, 4H, benzene), and 7.48 (s, 3H, benzene). MS-ESI: m/z 446.19 (M+1), Elemental analysis (%): C24H23FN6O2: C, 64.56; H, 5.19; F, 4.26; N, 18.82; and O, 7.17.

D_3 - 3-(5-(4-amino-2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-1-cyclopropyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3489.33 (N-H_2), 3061.34 (CH Ar), 1698.17 (C=O carboxyl), 1635.53 (C=C), 1345.12 (C-F), 1296.86 (C-N piperazine), 3218 (O-H Ar), 3050 (C-H Cyclopropane), and 1089 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 0.28 (t,2H, methylene), 0.53 (q,2H, methylene), 1.35 (d, 1H, CH), 2.0 (s, 1H, NH), 2.78 (d, 4H, Ar-CH_2), 3.47 (d, 4H, Ar-CH_2), 4.0 (s, 2H, NH_2), 5.0 (d, 1H, OH), 5.93 (d, 3H, benzene), and 7.48 (s, 3H, benzene). MS-ESI: m/z 462.18 (M+1), Elemental analysis (%): C24H23FN6O3: C, 62.33; H, 5.01; F, 4.11; N, 18.17; and O, 10.38.

D_4 - 1-ethyl-6-fluoro-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-7-(piperazin-1-yl) quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3468.31 (N-H_2), 3055.53 (CH Ar), 1686.17 (C=O carboxyl), 1620.31 (C=C), 1353.12 (C-F), 606.70 (C-Cl), 1274.64 (C-N piperazine), and 1139 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 1.13 (t, 3H methyl), 2.0 (s, 1H, NH), 2.78 (d, 4H, Ar-CH_2), 3.10 (t,2H, methylene), 3.47 (d, 4H, Ar-CH_2), 5.93 (d, 4H, benzene), and 7.48 (s, 4H, benzene). MS-ESI: m/z 419.18 (M+1), Elemental analysis (%): C23H22FN5O2: C, 65.86; H, 5.29; F, 4.53; N, 16.70; and O, 7.63.

D_5 - 3-(5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl)-1-ethyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3303.37 (N-H_2), 3010.32 (CH Ar.), 1748.53 (C=O carboxyl), 1676.94 (C=C), 1356.34 (C-F), 1282.69 (C-N piperazine), and 1126 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 1.13 (t, 3H methyl), 2.0 (s, 1H, NH), 2.78 (d, 4H, Ar-CH_2), 3.10 (t,2H, methylene), 3.47 (d, 4H, Ar-CH_2), 4.0 (s, 2H, NH_2), 5.93 (d, 4H, benzene), and 7.48 (s, 3H, benzene). MS-ESI: m/z 434.19 (M+1), Elemental analysis (%): C23H23FN6O2: C, 63.58; H, 5.34; F, 4.37; N, 19.34; and O, 7.36.

D_6 - 3-(5-(4-amino-2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-1-ethyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3492.32 (N-H_2), 3039.67 (CH Ar.), 3234.38 (O-H carboxyl), 1658.65 (C=O carboxyl), 1361.93 (C-F), 1675.78 (C=C), 1259.02 (C-N piperazine), and 1141 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 1.13 (t, 3H methyl), 2.0 (s, 1H, NH), 2.78 (d, 4H, Ar-CH_2), 3.10 (t,2H, methylene), 3.47 (d, 4H, Ar-CH_2), 4.0 (s, 2H, NH_2), 5.0 (d, 1H, OH), 5.93 (d, 3H, benzene), and 7.48 (s, 3H, benzene). MS-ESI: m/z 450.18 (M+1), Elemental analysis (%): C23H23FN6O3: C, 61.32; H, 5.15; F, 4.22; N, 18.66; and O, 10.65.

D_7 - 1-cyclopropyl-6-fluoro-7-(1H-imidazol-1-yl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3510.02 (N-H_2), 3044.56 (CH Ar.), 1744.80 (NO_2 Ar), 1629.28 (C=C), 3051.28 (C-H Cyclopropane), 1375.28 (C-F), 3051.24 (C-N imidazole), and 1076 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 0.28 (t,2H, methylene), 0.53 (q,2H, methylene), 1.35 (d, 1H, CH), 7.22 (d, 5H, benzene), and 8.03 (s, 6H, benzene). MS-ESI: m/z 413.13 (M+1), Elemental analysis (%): C23H16FN5O2: C, 66.82; H, 3.90; F, 4.60; N, 16.94; and O, 7.74.

D_8 - 3-(5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl)-1-cyclopropyl-6-fluoro-7-(1H-imidazol-1-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3448.32 (N-H_2), 3070.52 (CH Ar), 1682.47 (C=O carboxyl), 1644.64 (C=C), 1388.09 (C-F), 1180.22 (C-N imidazole), 3050.28 (C-H Cyclopropane), and 1076 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 0.28 (t,2H, methylene), 0.53 (q,2H, methylene), 1.35 (d, 1H, CH), 2.0 (s, 1H, NH), 2.78 (d, 4H, Ar-CH_2), 3.47 (d, 4H, Ar-CH_2), 4.0 (s, 2H, NH_2), 5.93 (d, 4H, benzene), and 7.48 (s, 3H, benzene). MS-ESI: m/z 428.14 (M+1), Elemental analysis (%): C23H17FN6O2: C, 64.48; H, 4.00; F, 4.43; N, 19.62; and O, 7.47.

D_9 - 3-(5-(4-amino-2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-1-cyclopropyl-6-fluoro-7-(1H-imidazol-1-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3426.32 (N-H_2), 3089.52 (CH Ar), 3256.26 (O-H carboxyl), 1684.58 (C=O), 1626.58 (C=C), 1393.15 (C-F), 2760.28 (C-S), 3052.28 (C-H Cyclopropane), 1194.25 (C-N imidazole), and 1172 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 0.28 (t,2H, methylene), 0.53 (q,2H, methylene), 1.35 (d, 1H, CH), 4.0 (s, 2H, NH_2), 5.0 (d, 1H, OH), 5.99 (d, 5H, benzene), and 8.03 (s, 4H, benzene). MS-ESI: m/z 444.13 (M+1), Elemental analysis (%): C23H17FN6O3: C, 62.16; H, 3.86; F, 4.27; N, 18.91; and O, 10.80.

D_{10} - 1-ethyl-6-fluoro-7-(1H-imidazol-1-yl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3478.32 (N-H_2), 3023.80 (CH Ar), 1685.58 (C=C), 1635.58 (C=O), 1323.00 (C-F), 1191.28 (C-O), and 1195.66 (C-N imidazole). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 1.13 (t, 3H methyl), 3.10 (t,2H, methylene), 7.22 (d, 6H, benzene), and 8.03 (s, 5H, benzene). MS-ESI: m/z 401.40 (M+1), Elemental analysis (%): C22H16FN5O2: C, 65.83; H, 4.02; F, 4.73; N, 17.45; and O, 7.97.

D_{11} - 3-(5-(4-aminophenyl)-1,3,4-oxadiazol-2-yl)-1-ethyl-6-fluoro-7-(1H-imidazol-1-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3296.32 (N-H_2), 3071.52 (CH Ar), 1694.58 (C=O), 1629.58 (C=C), 1333.15 (C-F), 1181.25 (C-N imidazole), and 1095.58 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 1.13 (t, 3H methyl), 3.10 (t,2H, methylene), 4.0 (s, 2H, NH_2), 6.52 (d, 5H, benzene), and 8.03 (s, 5H, benzene). MS-ESI: m/z 416.42 (M+1), Elemental analysis (%): C22H17FN6O2: C, 63.46; H, 4.12; F, 4.56; N, 20.18; and O, 7.68.

D_{12} - 3-(5-(4-amino-2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-1-ethyl-6-fluoro-7-(1H-imidazol-1-yl)quinolin-4(1H)-one

IR V_{max} (cm^{-1}ATR): 3512.32 (N-H_2), 3080.80 (CH Ar), 1689.33 (C=O), 1680.54 (C=C), 1312.00 (C-F), 1188.66 (C-N imidazole), 3280.38 (O-H carboxyl), and 1125 (C-O). ^{1}H NMR (300 MHz; DMSO-d_6) δ: 1.13 (t, 3H methyl), 3.10 (t,2H, methylene), 4.0 (s, 2H, NH_2), 5.0 (d, 1H, OH), 5.99 (d, 6H, benzene), and 8.03 (s, 3H, benzene). MS-ESI: m/z 432.13 (M+1), Elemental analysis (%): C22H17FN6O3: C, 61.11; H, 3.96; F, 4.39; N, 19.44; and O, 11.10.

Biological evaluations

Anti-microbial activity

The antibacterial and antifungal properties of all the title compounds were evaluated. Using nutrient agar medium, the antibacterial activity of the synthesized compounds was tested against two Gram-positive bacteria (*S. aureus* ATCC 6438P and Staphylococcus epidermidis ATCC 155), as well as two Gram-negative bacteria (*E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC29665). Using sabouraud dextrose agar, the antifungal properties of the compounds were evaluated against two fungus, *Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645. Antimicrobial tests were carried out using the paper disc diffusion method for preliminary screening. The agar streak dilution method was also used to assess the drugs' minimum inhibitory concentrations (MIC).

Paper disc diffusion technique

The sterilized [19] (autoclaved for 30 min at 120°C) medium (40–50°C) was injected (1 ml/100 ml of medium) with the microbe suspension (105 cfu/ml) and inserted to a depth of 3–4 mm into a petri dish. The test substances were impregnated paper (100 g/ml in dimethylformamide) and were positioned on the solidified medium. The plates were pre-incubated at room temperature for 1 h before being incubated at 37°C for 24 and 48 h, respectively, for antibacterial and antifungal activity. For antibacterial and antifungal activity, ciprofloxacin (100 g/disc) and ketoconazole (100 g/disc) were chosen as standards. The observed zone of inhibition is presented in Table 1.

MIC

The compound's MIC [20,21] was obtained using the agar streak dilution method. A stock solution of the synthesized chemical in dimethylformamide (100 g/ml) was produced, and graded amounts of the test compounds were mixed into a preset amount of sterile agar that has been melted (nutrient agar for anti-bacterial activity and sabouraud dextrose agar medium for anti-fungal activity). A specific amount of the compound-containing medium (40–50°C) was poured into a petri dish to a depth of 3–4 mm and allowed to solidify. Suspensions of microorganisms were produced to contain roughly 105 cfu/ml and applied to plates with serially diluted substances in dimethylformamide to be evaluated, and then incubated at 37°C for 24 h and 48 h, respectively, for bacteria and fungi. The MIC was taken as the lowest concentration of the test item that resulted in no visible bacteria or fungal growth on the plate. The observed MIC is presented in Table 1.

Molecular docking studies

Molecular docking studies of synthesized compounds (D_1–D_{12}) with the well-established structures of *S. aureus* and *E. coli* were performed using Auto Dock vina 1.12 version and chimera 1.12 version [22]. The binding pocket of the active site of DNA gyrase (PDB: 5IWM) for Gram-positive bacteria like *S. aureus*, topoisomerase-IV (PDB: 3FV5) for Gram-negative bacteria like *E. coli* and lanosterol 14-alpha demethylase (PDB: 5ESE) for fungal-like *S. cerevisiae*. The phases in the docking procedure are as follows: First, the ligand molecule was built, in the second step, required protein was downloaded from PDB, preparation and validation of macromolecule by X-ray crystallography. The third step is the identification of binding affinity by the extent of binding of ligand to the protein of molecule.

RESULTS AND DISCUSSION

Chemistry

The synthetic route to obtain the necessary derivative from commercially available reagent is briefly outlined in the Scheme 1. The synthesized compounds (D_1–D_{12}) were obtained with the help of various derivatives and reagents such as 85% hydrazine hydrate, 40% sodium hydroxide, and glacial acetic acid. All reactions of synthesized compounds occurred at optimum temperature. All of the compounds that were synthesized had their structures validated by IR, ^{1}HNMR, and mass spectral elemental analysis techniques.

Antibacterial activity

All of the compounds that have been produced (D_1–D_{12}) 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. pneumoniae*) bacteria. Against both Gram-positive and Gram-negative bacteria, all of the compounds showed mild to moderate activity. Compounds D_8, D_3,

Table 1: Anti-microbial activity of the synthesized compounds (D1–D12) (100 µg/ml)

Compounds	*In vitro* activity - zone of inhibition in mm (MIC in µg/ml)					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Aspergillus niger*	*Aspergillus fumigatus*
D_1	28 (1.2)	25 (3.4)	23 (1.2)	24 (4.2)	25 (11.3)	23 (11.2)
D_2	29 (1.3)	28 (2.9)	26 (0.8)	27 (2.3)	29 (10.6)	24 (10.5)
D_3	26 (0.5)	24 (0.6)	26 (0.3)	22 (0.4)	31 (9.8)	23 (10.2)
D_4	31 (1.0)	26 (0.9)	23 (1.0)	24 (2.8)	18 (13.9)	20 (14.8)
D_5	23 (0.8)	27 (0.8)	22 (2.2)	20 (3.9)	26 (11.2)	23 (11.0)
D_6	34 (1.2)	31 (2.2)	30 (0.4)	31 (0.5)	29 (10.7)	21 (10.5)
D_7	24 (2.4)	26 (3.8)	27 (0.5)	24 (0.6)	25 (11.3)	25 (11.3)
D_8	21 (0.4)	25 (0.5)	28 (1.7)	26 (3.5)	25 (12.9)	24 (13.1)
D_9	29 (0.8)	27 (0.8)	29 (0.2)	23 (0.3)	26 (12.7)	27 (13.8)
D_{10}	28 (2.5)	21 (3.1)	28 (1.9)	22 (3.8)	22 (14.1)	21 (14.8)
D_{11}	30 (1.3)	27 (2.6)	27 (1.0)	28 (2.5)	27 (12.5)	26 (13.5)
D_{12}	33 (1.2)	29 (2.2)	31 (0.7)	29 (0.7)	24 (10.8)	25 (10.7)
Ciprofloxacin[a]	37 (0.5)	35 (0.12)	35 (0.06)	36 (0.06)	–	–
Ketoconazole[b]	–	–	–	–	29 (10.8)	33 (11.4)
DMF[c]	–	–	–	–	–	-

Ciprofloxacin[a]: Standard antibacterial drugs, Ketoconazole[b]: Standard antifungal drug, DMF[c]: Control, MIC: Minimum inhibitory concentrations

D_5, D_9, and D_4 were found to possess significant antibacterial activity against Gram-positive organisms when compared to the standard drug (Ciprofloxacin) and exhibited MIC values in the range of 0.4–2.9 µg/ml. Compounds D_9, D_3, D_6, D_7, and D_{12} were discovered to have significant antibacterial activity against Gram-negative organisms when compared to standard drugs (Ciprofloxacin) and exhibited MIC values in the range of 0.5–3.9 µg/ml, as shown in Table 1.

Compound D_8 exhibited good or equal Gram-positive antibacterial activity against *S. aureus* and *S. epidermis* with MIC values in range of (0.4 µg/ml) and (0.5 µg/ml) when compared to standard ciprofloxacin (0.5 µg/ml) and (0.215 µg/ml), respectively. This good antibacterial activity is may be due to the addition of a new derivative of the aminophenyl group at the third position and the imidazole ring at the seventh position.

Compound D_9 exhibited good Gram-negative antibacterial activity against *E. coli* and *K. pneumoniae* with MIC values in the range of (0.2 µg/ml) and (0.3 µg/ml), respectively, when compared to standard ciprofloxacin (0.06 µg/ml). This good antibacterial activity is may be due to the addition of a new derivative of amino hydroxyphenyl group at third position and imidazole ring at seventh position.

Antifungal activity

All of the compounds that have been produced (D_1–D_{12}) were tested zone of inhibition and MIC values against two fungi organisms (*A. niger* and *A. fumigatus*). All the compounds exhibited good activity. Compounds D_3, D_2, D_6, D_{12}, D_5, D_1, and D_7 were found to possess significant antifungal activity against *A. niger* when compared to standard ketoconazole. Compounds D_3, D_2, D_6, and D_{12} were found to possess significant antifungal activity against *A. fumigatus* when compared to standard drug ketoconazole. The range of MIC values was found to be 9.8–14.7 µg/ml against *A. niger* and 10.2–16.2 µg/ml against *A. fumigatus,* respectively, as shown in Table 1.

Compound D_3 exhibited excellent antifungal activity against *A. niger* and *A. fumigates* with MIC values of (9.8 µg/ml) and (10.2 µg/ml) when compared to the standard ketoconazole (10.8 µg/ml) and (11.4 µg/ml). This mild antifungal activity is may be due to the addition of a new derivative of amino hydroxyphenyl group at third position and piperazine ring at seventh position.

Docking study

Molecular docking studies were used in the investigation with a training set made up of our produced compounds with unknown inhibitory efficacy. Molecular docking experiments were carried out in an attempt to identify the molecular facilities responsible for biological action. From the docking studies, we predicted that all the synthesized compounds (D_1–D_{12}) possess better antibacterial activity than the standard drug (ciprofloxacin). By having a good binding affinity with target protein, it could be used as a potential drug as antibacterial.

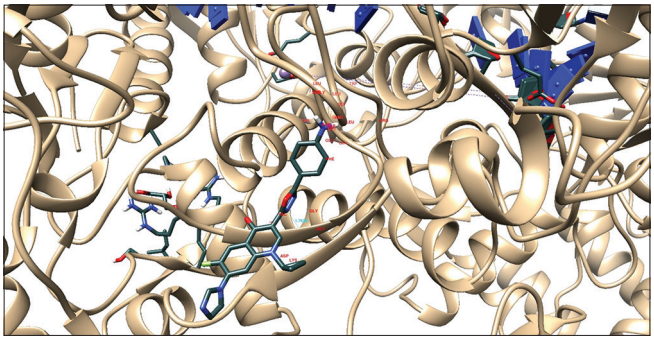


Fig. 1: H-bonds interactions between compound (D_8) with topoisomerase-II DNA gyrase enzyme of Gram-positive *Staphylococcus aureus* bacteria (5IWM)

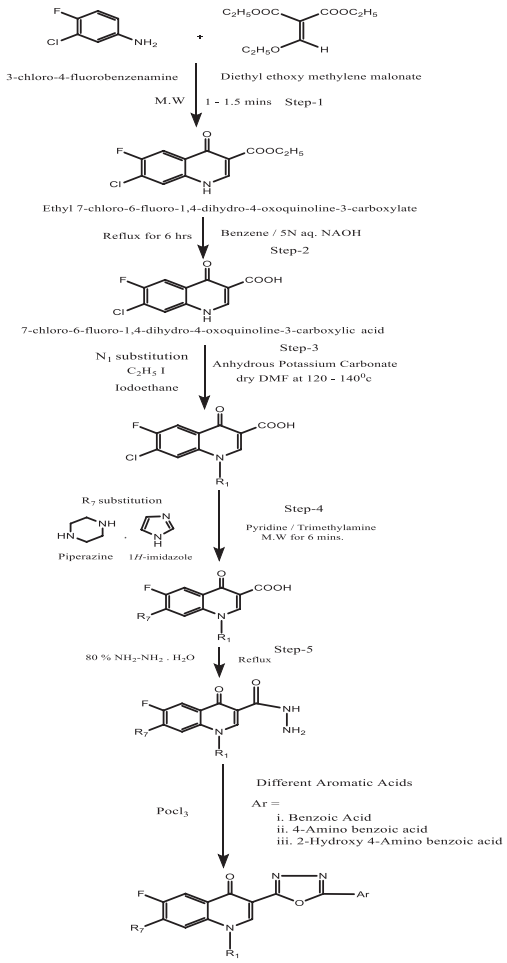
Gram-positive bacteria docking studies

Among all the docked compounds D_8, D_3, D_5, and D_9 show good binding affinity and interaction with topoisomerase-II DNA gyrase enzyme (5IWM) with reference to standard drug ciprofloxacin. Compound D_8 has a higher dock score (–10.1) toward bacterial *S. aureus* enzyme than the standard ciprofloxacin (–7.8) drug as shown in Fig. 1. We may declare that the higher docking score is due addition of the aminophenyl group at the third position oxadiazole ring of the ciprofloxacin structure. The remaining compound's docking score is described in Table 2.

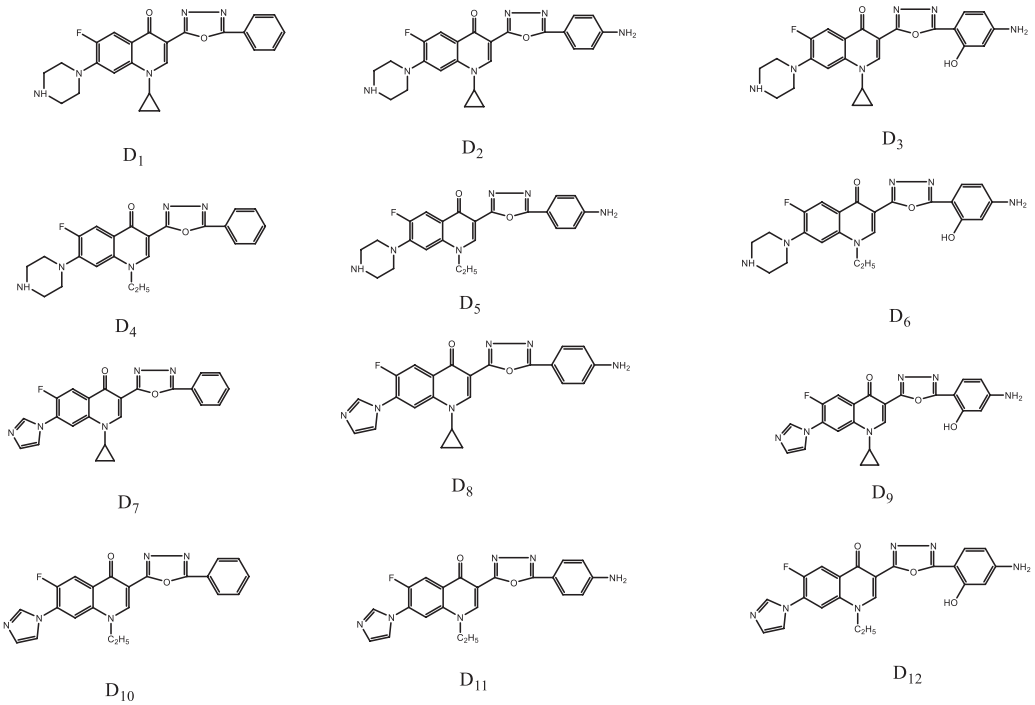
Gram-negative bacteria docking studies

Among all the docked compounds D_9, D_3, D_6, and D_7 show good binding affinity and interaction with topoisomerase-IV enzyme (3FV5) with reference to standard ciprofloxacin.

Compound D_9 is has a higher affinity (–7.9) toward *E. coli* enzyme than the standard ciprofloxacin (–7.3) drug, as shown in Fig. 2. We may declare that the higher docking score is due to the addition of a new derivative the amino hydroxyphenyl group at the third position



Derivatives of Fluoroquinolones from D_1-D_{12}



Scheme 1: Synthesis of (D_1–D_{12}) derivatives of fluoroquinolones

Table 2: Docking result of synthesized compounds (D1–D12)

Compounds	Docking result of synthesized compounds (D_1–D_{12})		
	Gram-positive bacteria (*Staphylococcus aureus*)	**Gram-negative bacteria (*Escherichia coli*)**	**Fungi (*Saccharomyces cerevisiae*)**
D_1	−8.9	−7.2	−10.8
D_2	−8.9	−7.4	−11.1
D_3	−9.3	−7.8	−11.3
D_4	−9.1	−7.4	−10.6
D_5	−9.2	−7.3	−10.9
D_6	−9.0	−7.8	−11.1
D_7	−8.9	−7.8	−10.8
D_8	−10.1	−7.6	−9.6
D_9	−9.2	−7.9	−9.5
D_{10}	−8.5	−7.5	−10.6
D_{11}	−8.5	−7.0	−9.7
D_{12}	−9.0	−7.7	−11.0
Ciprofloxacin[a]	−7.8	−7.3	–
Fluconazole[b]	–	–	−7.5

Ciprofloxacin[a]: Standard antibacterial drug, Fluconazole[b]: Standard antifungal drug

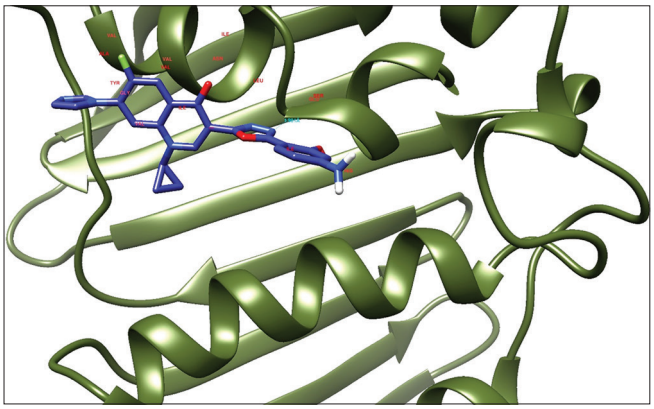


Fig. 2: H-bonds interactions between compound (D_9) with topoisomerase-IV enzyme of Gram-negative *Escherichia coli* bacteria (3FV5)

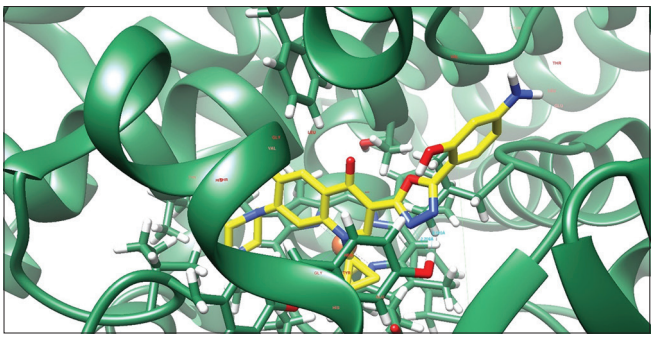


Fig. 3: H-bonds interactions between compound (D_3) with lanosterol 14-alpha demethylase enzyme of *Saccharomyces cerevisiae* fungi (5ESE)

oxadiazole ring of ciprofloxacin. The remaining compound's docking score is described in Table 2.

Fungal docking studies

Among all the docked compounds D_3, D_2, D_6, D_{12}, and D_5 show good binding affinity and interaction with lanosterol 14-alpha demethylase enzyme (PDB:5ESE) with reference to standard drug fluconazole.

Compound D_3 has a higher dock score (−11.3) toward fungal *S. cerevisiae* enzyme than the standard fluconazole (−7.5) drug, as shown in Fig. 3.

We may declare that the higher docking score is due to the addition of a new derivative amino hydroxyphenyl group at the third position oxadiazole ring of ciprofloxacin. The remaining compound's docking score is described in Table 2.

CONCLUSION

We have synthesized and characterized 12 new derivatives of fluoroquinolones. All the molecules were studied for their interactions with topoisomerase-II DNA gyrase, topoisomerase-IV, and lanosterol 14-alpha demethylase enzymes by molecular docking protocol. Among the tested molecules, compounds D_8, D_3, and D_5 exhibited good docking scores for Gram-positive bacteria, compounds D_9, D_3, D_6, and D_7 for Gram-negative bacteria, and compounds D_3, D_2, D_6, D_{12}, and D_5 exhibited good docking scores for fungal. *In vitro* antibacterial activity of tested compounds shows mild activity against micro-organisms used. In particular compounds D_8, D_3, D_5, D_9, and D_{20} possess the significant Gram-positive activity, compounds D_9, D_3, D_6, D_7, and D_{12} possess significant Gram-negative activity, and compounds D_3, D_2, D_6, D_{12}, and D_5 possess the significant fungal activity. The results of antibacterial activity are supported by docking analysis only for *S. aureus* and *S. cerevisiae*.

AUTHORS CONTRIBUTION

The first author makes contributions to the conception, design, and implementation of the research, and analysis of the results. The second author helped to supervise the project and gave final approval of the written manuscript, and the third author helped to gather, write, and align the manuscript.

CONFLICT OF INTERESTS

The authors confirm that the content of the article has no conflict of interests.

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