International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(1): 208-216
© 2017 JEZS
Received: 20-11-2016
Accepted: 21-12-2016

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Quinoline appended 1, 2, 3-triazole analogues: Synthesis, antimicrobial and docking evaluation

R Gopi and S Senthil

Abstract
A series of quinoline appended 1, 2, 3- triazole derivatives were synthesized through cycloaddition of aromatic azides and 1, 4-diketone followed by pfitzinger reaction with isatin derivatives. Chemical structures of the derivatives were characterized by the various spectral techniques. The synthesized compounds were evaluated for their in vitro antimicrobial activity against three Gram positive and three Gram negative bacterial strains as well as four pathogenic fungal strains. The minimal inhibitory concentration and zone of inhibition were also determined. The compounds showed moderate to good biological activity**.** The obtained results encouraged the most active compounds could be useful as a template for future drug design, modification and exploration to produce more active analogs.

Keywords: 1,2,3-Triazole; Quinoline carboxylic acid; Antibacterial; Antifungal; Antimicrobial; Pfitzinger

Introduction

Bacterial and Fungal diseases are most dangerous threats against human health in the world. Life style nowadays increases the rate of bacterial and fungal diseases among the people. Although many of the natural and synthetic compounds used as antimicrobial drugs, the development and discovery of safer antimicrobial agents is still a demanding task for organic chemist world over. The need for new antimicrobial agents is greater than ever before because of the increase of multidrug resistance in common pathogens, the rapid emergence of new infections and increasing death rate of human people around the world. Despite a large number of antibiotics and chemotherapeutics available in the market for treatment, the need of more promising antimicrobial drug is more important in current situation [1, 2]. Since 2009, only 2 new antibiotics were approved in United States, and the number of new antibiotics annually approved for marketing continues to decline. The report could identify only seven antibiotics currently in phase 2 or phase 3 clinical trials to treat the GNB, which includes *E. coli*, *Salmonella*, *Shigella*, and the *Enterobacteriaceae* bacteria, and these drugs do not address the entire spectrum of the resistance developed by those bacteria [3, 5].

This situation makes medicinal chemist to search for new antimicrobial agents [5]. In current situation to discovery new antimicrobial agents is more difficult task [6, 7]. Development new antimicrobial drug involves chemical modification of existing drugs or combination of two or more biologically active heterocyclic molecule [8]. Some newly approved drugs which utilizes this strategy has allowed finding of more active and safe compounds with wide spectrum activity. Figure 1 presents some of potential investigational molecules which are in either phase II or Phase III of clinical trial derived from existing NCE's [9].

In this investigation, we choose quinoline structural and 1,2,3-triazole residues which are already reported bioactivity like antibacterial, antifungal, antimalarial, anticancer drugs and are under continuous evaluation for the development of potent bioactive molecules [10]. Some of the recent chemical modifications quinoline includes Bedaquiline (R207910) which has shown extraordinary activity against both drug susceptible and drug-resistant strains of M. tuberculosis, exhibiting MIC values of 30-120 mg/ml [11]. Laquinimod is an experimental immunomodulator drug and it is currently under investigation for oral treatment of multiple sclerosis (MS) [12]. GSK 299423 is an investigational compound which has shown potent activity against antibiotic-resistant strains of bacteria such as *Staphylococcus aureus,* including methicillin resistance S. aureus (MRSA) and against gram-negative bacteria like *E. coli, Pseudomonas, Klebsiella* and *Acinetobacter* [13].

The pyrimidine ethyl ester compound 1f prepared by a mixture of aromatic aldehyde (0.01mol), ethylacetoacetate (0.01mol) and urea (0.01mol) is mixed thoroughly with 0.15 mole of tin (II) chloride as catalyst in a conical flask. The content of the flask is irradiated in a domestic microwave oven (400W) for 6 minutes. The completion of the reaction is monitored by TLC Fascinated by multifarious bioactivity of quinoline various researchers and scientists are still engaged in developing potent molecule based on quinoline such as Saeed *et al.* [14] have reported the synthesis of conformationally constrained Analogs of N-Substituted Piperazinyl quinolones tested for antimicrobial activity. Impelled by these observations and in continuation of our research for bioactive molecules based on Quinoline appended 1, 2, 3 –triazole system [15, 17], we address here the synthesis and in-vitro antimicrobial studies and insilico studies of some newer differentiated Quinoline appended 1, 2, 3 –triazole derivatives.

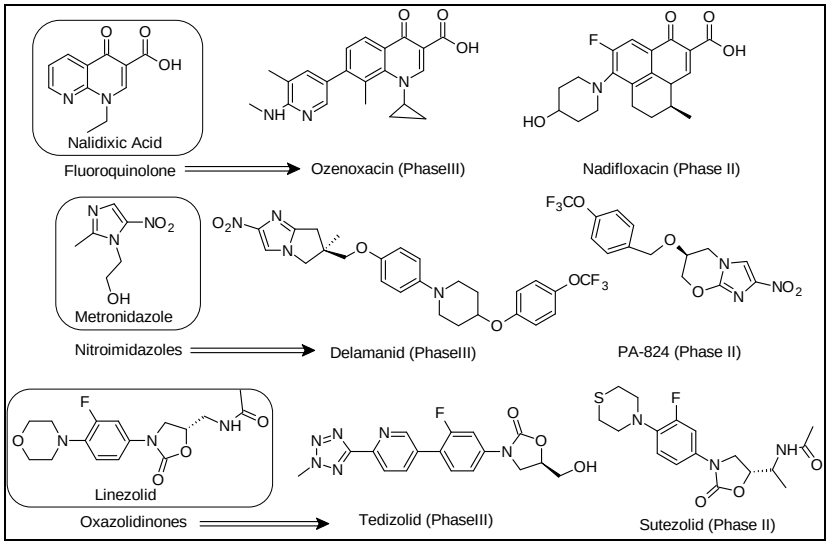
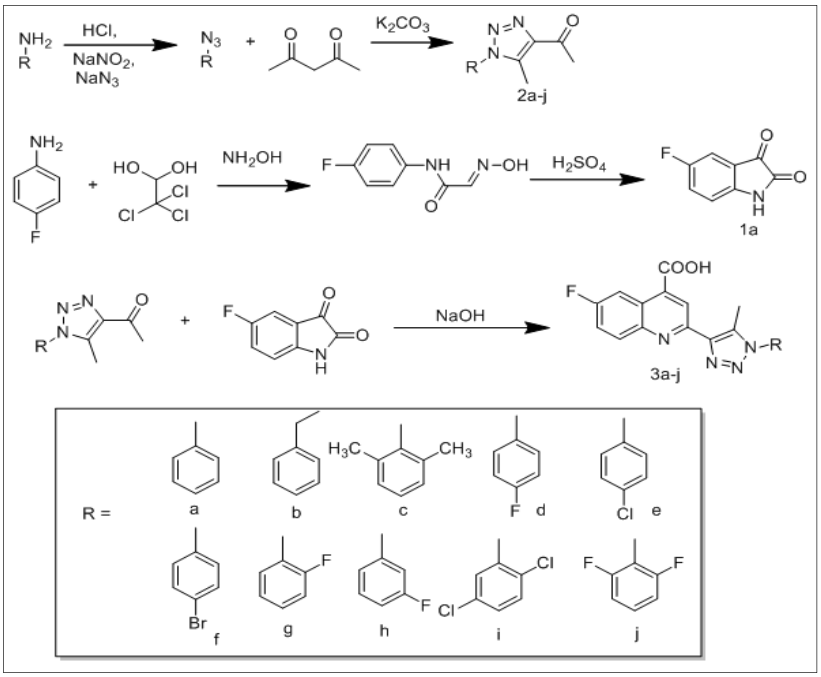


Fig 1: Chemical tailoring or chemical remodelling of existing antibacterial drugs classes, showing development of Gatifloxacin or Moxifloxacin from Nalidixic acid and investigational molecules PA-824 and OPC-67683 from Metronidazole etc.

2. Materials and Methods

Experimental Procedure

The objective molecule 3(a–j) were synthesized according to the Scheme 1 using fluorine substituted isatin (1a)and substituted 1,2,3-triazole 2(a-j).The yield of the desired derivatives was 85-90 % prior to purification. IR spectra were recorded on FT-IR Shimadzu 8300 spectrophotometer in KBr pellet and ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer in DMSO-d_6 using tetramethyl silane as the internal standard. The chemical shifts were noted in δppm units. Mass spectra were obtained on Shimadzu LCMS-2010EV. TLC was performed on aluminium-backed silica plate with visualization using UV-light and column chromatography using silica gel.



Scheme 1: General scheme for synthesis of target compounds

General procedure for the preparation and purification of compound 3(a–j):

Preparation of 5-Fluoroinolin-2,3-dione

N-(4-fluorophenyl)-2-hydroxyiminoacetamide

4-Fluoroaniline (0.50mol) was added slowly to 10% aqueous HCl solution. This suspension was added to a mixture of chloral hydrate (0.55mol) and sodium sulphate (50g) in 75 mL water with mechanical stirring. Hydroxylamine hydrochloride (1.63 mol) dissolved in 25mL water was added, and the resulting slurry was heated at 100˚C.After this temperature was reached, the heating mantle was removed immediately, and the solution was cooled to room temperature. The formed precipitate was collected by filtration, washed with water, and dried in a vacuum oven at 60˚C. Concentrated sulphuric acid (20mL) was heated at 50˚C, and N-(4-fluorophenyl)-2-hydroxyiminoacetamide was slowly added. The black solution was carefully heated at 90^0C.At this temperature, some slight cooling was necessary to keep the temperature at 90˚C. When no more heat had developed, the reaction mixture was heated at 90˚C for an additional half hour. The dark-red solution was cooled to room temperature and poured onto ice water. The precipitated solid was filtered and dried to get 5-Fluoroinolin-2, 3-Dione**.**

^{1}H NMR (400 MHz, DMSO-d6):6.89-6.92 (q, 1H, Ar-H), 7.39-7.46 (m, 1H, Ar–H), 11.40 (broad, NH). ESI–MS m/z: [M+1]: 165.90.

Synthesis of substituted azidobenzene

Aniline (20 g, 0.144 mol) was dissolved in (91.4 ml) 1:1 ratio of HCl, water and taken in a round bottom flask equipped with stirrer. The reaction was agitated at 0-5 C sodium nitrite (10 g, 0.144 mol) was dissolved in 51.30 ml of ice cold water and added drop wise, sodium azide (9.36 g 0.144 mol) dissolved in 113.40 ml of water and added dropwise, then reaction is allowed to continue for 30 min. The resultant precipitate was extracted with chloroform and washed successively with water. The organic layer was dried over anhydrous sodium sulphate, and the solvent stripped out in rotary evaporator to get azidobenzene, yield 93.5% (18.7 g).The similar procedure was followed for other substituted anilines.

Synthesis of benzyl azide

Benzyl chloride (6.72ml, 0.0584mol) was taken in a round bottom flask with 80 ml of dimethyl sulphoxide equipped with stirrer; sodium azide (7.6g, 0.1169mol) added and stirred overnight. The reaction mixture was quenched in ice-water and extracted with diethyl ether, washed successively with 5% bicarbonate solution and dried over anhydrous sodium sulphate. The solvent was removed by rotary evaporator to give benzyl azide, yield 71.4 % (4.8ml).

Synthesis of 1-[phenyl]-4-acetyl-5-methyl-1, 2, 3-triazole 2(a-j)

The azidobenzene (3 g, 0.018 moles), acetyl acetone (1.8 g, 0.018 moles), potassium carbonate (8 g, 0.057 moles), absolute ethanol (95%, 45 ml) were taken in a round bottom flask equipped with stirrer. The reaction mixture was agitated at 75 C for 30 min. The progress of the reaction was monitored by TLC. After the completion of the reaction, the solvent was removed under vacuum. The residual mass was quenched in the ice-water mixture and neutralized with 10% HCl solution. The product was extracted with diethyl ether dried over anhydrous sodium sulfate. Evaporation of the solvent yield crude product, which was purified by column chromatography using chloroform as eluent and recrystallized from absolute ethanol, yield 79.5% (3.9 g).The remaining substituted triazoles were prepared in the similar manner and their characterized.

1-[phenyl]-4-acetyl-5-methyl-1,2,3-triazole (2a)

^{1}H NMR (400MHz, $CDCl_3$): 2.59-2.63 (d, 3H, CH_3), 2.75 - 2.79(d, 3H, -$COCH_3$), 7.44-7.7.46 (m, 2H, Ar–H), 7.54-7.61 (m, 3H, Ar–H); IR (KBr, cm-1): 1238.8 (C–N), 1679.40 (C=O), 1505.3 (C-C); ESI–MS m/z: [M+1]: 201.90.

1-[benzyl]-4-acetyl-5-methyl-1,2,3-triazole (2b)

^{1}H NMR (400MHz, $CDCl_3$): 2.471 (s, 3H, CH_3), 2.63 -2.69 (d, 3H, -$COCH_3$), 5.523 (s, 2H, CH_2), 7.16-7.7.18 (d, 2H, Ar–H), 7.33-7.36 (m, 3H, Ar–H); IR (KBr, cm-1): 1215.7 (C–N), 1678.74 (C=O), 1557.65 (C-C); ESI–MS m/z: [M+1]: 216.05.

1-[2,6-dimethylphenyl]-4-acetyl-5-methyl-1,2,3-triazole (2c)

^{1}H NMR (400MHz, $CDCl_3$): 1.93 (m, 12H, CH_3), 7.12-7.26 (m, 3H, Ar–H); IR (KBr, cm-1): 1274.92 (C–N), 1686.79 (C=O), 1549.09 (C-C); ESI–MS m/z: [M+1]: 229.90.

1-[4-fluorophenyl]-4-acetyl-5-methyl-1,2,3-triazole (2d)

^{1}H NMR (400MHz, $CDCl_3$):2.57 (s, 3H, CH_3), 2.75 (s, 3H, -$COCH_3$), 7.26-7.30 (m, 2H, Ar–H), 7.43-7.46 (m, 2H, Ar–H); IR (KBr, cm-1): 1236.61 (C–N), 1681.99 (C=O), 1561.58 (C-C); ESI–MS m/z: [M+1]: 220.10.

1-[4-chlorophenyl]-4-acetyl-5-methyl-1,2,3-triazole (2e)

^{1}H NMR (400MHz, DMSO-d6):2.51 (s, 3H, CH_3), 2.63 (s, 3H, -$COCH_3$), 7.67-7.74 (m, 4H, Ar–H); IR (KBr, cm-1): 1236.6 (C–N), 1680.14 (C=O), 1560.3 (C-C); ESI–MS m/z: [M+1]: 235.95.

1-[4-bromophenyl]-4-acetyl-5-methyl-1,2,3-triazole (2f)

^{1}H NMR (400MHz, DMSO-d6): 2.51-2.512 (d, 3H, CH_3), 2.63 (s, 3H, -$COCH_3$), 7.60-7.62 (m, 2H, Ar–H), 7.85-7.87 (m, 2H, Ar–H); IR (KBr, cm-1): 1274.7 (C–N), 1686.23 (C=O), 1547.9 (C-C); ESI–MS m/z: [M+1]: 279.95.

1-[2-Fluorophenyl]-4-acetyl-5-methyl-1,2,3-triazole (2g)

^{1}H NMR (400MHz, DMSO-d6): 2.41 (s, 3H, CH_3), 2.64 (s, 3H, -$COCH_3$), 7.48-7.52 (m, 1H, Ar–H), 7.59-7.64 (m, 1H, Ar–H), 7.71-7.77 (m, 2H, Ar–H); IR (KBr, cm-1): 1287.75 (C–N), 1682.63 (C=O), 1512.5 (C-C); ESI–MS m/z: [M+1]: 220.

1-[3-chlorophenyl]-4-acetyl-5-methyl-1,2,3-triazole (2h)

^{1}H NMR (400MHz, DMSO-d6): 2.50(s, 3H, CH_3), 2.64(s, 3H, -$COCH_3$), 7.63-7.74 (m, 3H, Ar–H), 783 (s, 1H, Ar–H); IR (KBr, cm-1): 1278.6 (C–N), 1689.6 (C=O), 1587.1(C-C); ESI–MS m/z: [M+1]: 236.00.

1-[3,5-dichlorophenyl]-4-acetyl-5-methyl-1,2,3-triazole (2i)

^{1}H NMR (400MHz, DMSO-d6): 2.36(s, 3H, CH_3), 2.63(s, 3H, -$COCH_3$), 7.79-7.86 (m, 2H, Ar–H), 7.95 (s, 1H, Ar–H); IR (KBr, cm-1): 1277.9 (C–N), 1681.70 (C=O), 1557.4 (C-C); ESI–MS m/z: [M+1]: 270.05.

1-[2,6-difluorophenyl]-4-acetyl-5-methyl-1,2,3-triazole (2j)

^{1}H NMR (400MHz, DMSO-d6): 2.53-2.56 (d, 6H, CH_3), 5.62 (s, 2H, Ar–H), 7.16-7.20 (t, 2H, Ar–H), 7.49-7.51 (m, 1H, Ar–H); IR (KBr, cm-1): 1234.2 (C–N), 1687.13 (C=O), 1561.9 (C-C); ESI–MS m/z: [M+1]: 232.05.

Synthesis of 6-Fluoro-2-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid 3(a-j)

1-(1-phenyl-1H-1,2,3-triazol-4-yl)ethanone (0.0099 mol) was dissolved in ethanol(20ml). 4-FluoroIsatin (0.0099mol) was added in lot wise. The reaction mixture was stirred at 25-28^{0}C. Sodium hydroxide (0.0792mol) dissolved in water (20ml) and added drop wise to the reaction mixture. Heated to reflux for 24 hrs (till completion of reaction). Cooled to room temperature and the solvent was stripped out in rotary evaporator. The residue was adjusted to pH-3.5 using cooled dil. HCl (10ml). The precipitated solid was dried at 50^{0}C. The compound was further purified by column chromatography using cyclohexane and THF mixtures. The remaining compounds were prepared in the similar manner and their characterization data are as follows:

6-Fluoro-2-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid (3a)

IR (KBr, cm^{-1}): 2924.7 (OH), 1240.2 (C–N), 1717.38 (C=O), 1502.9 (C-C); ^{1}H NMR (400 MHz, DMSO-d6): 2.817 (s, 3H–CH3), 7.63-7.77 (m,7H,Ar-H), 8.13-8.16 (d, 1H, Ar–H), 8.49-8.53 (d, 1H, Ar–H), 8.80 (s, 1H, Ar–H), 14.11 (broad, OH); ^{13}C NMR (DMSO-d6) d (ppm): 167.35 (C=O), 159.78 (C=C), 145.98 (Ar–C), 142.12 (Ar–C), 130.39 (Ar–C), 125.91 (Ar–C), 120.76 (Ar–C), 120.51 (Ar–C), 109.97 (Ar–C), 109.728 (Ar–C), 10.87 (C–C, CH3). ESI–MS m/z: [M+1]: 349.05. Anal. Calcd. for C19H13FN4O2: C,65.51; H,3.76; F,5.45; N, 16.08; O,9.19; Found: C,65.43; H,3.45; F,5.25; N,16.07; O,9.07.

2-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-6-fluoroquinoline-4-carboxylic acid (3b)

IR (KBr, cm^{-1}): 2925.7 (OH), 1219.7 (C–N), 1711.8 (C=O), 1455.73 (C-C); ^{1}H NMR(400 MHz, DMSO-d6): 2.78 (s, 3H–CH3), 5.72 (s,2H,) 7.25-7.44 (m, 5H,Ar-H), 7.75-7.79 (q, 1H, Ar–H), 8.14-8.19 (t, 1H, Ar–H),8.50-8.54 (d,1H,Ar-H),8.7 (s, 1H, Ar–H),14.45 (broad, OH); ^{13}C NMR (DMSO-d6) d (ppm): 167.40 (C=O), 159.73 (C=C), 145.96 (Ar–C), 142.24 (Ar–C), 132.75 (Ar–C), 127.87 (Ar–C), 120.73 (Ar–C), 120.47 (Ar–C), 109.69 (Ar–C), 109.95 (Ar–C), 51.47 (Ar-CH$_2$), 27.88 (C–C, CH3). ESI–MS m/z: [M+1]: 363.10. Anal. Calcd. for C20H15FN4O2: C, 66.29; H, 4.17; F,5.24; N,15.46; O,8.83; Found: C,66.17; H,4.05; F,5.25; N,15.07; O,9.07.

2-(1-(2,6-dimethylphenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6-fluoroquinoline-4-carboxylic acid (3c)

IR (KBr, cm^{-1}): 2926.9 (OH), 1216.2 (C–N), 1708.52 (C=O), 1477.3 (C-C); ^{1}H NMR (400 MHz, DMSO-d6):1.96 (s, 6H–CH3), 2.61 (s, 3H–CH3), 7.33-7.47 (m, 2H,Ar-H),7.49-7.51 (m, 1H, Ar–H), 7.78-7.83 (m, 1H, Ar–H), 8.20-8.23 (m, 1H, Ar–H), 8.55-8.58 (m, 1H, Ar–H),8.88 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d6) d (ppm): 167.38 (C=O), 159.79 (C=C), 146.04 (Ar–C), 141.58 (Ar–C), 131.18 (Ar–C), 124.87 (Ar–C), 120.58 (Ar–C), 120.84 (Ar–C), 109.75 (Ar–C), 9.79 (C–C, CH3), 17.25 (C–C, CH3). ESI–MS m/z: [M+1]: 377.11. Anal. Calcd. for C21H17FN4O2: C, 67.01; H, 4.55; F,5.05; N,14.89; O,8.50; Found: C, 67.17; H, 4.08; F,5.25; N,15.07; O,8.19.

6-Fluoro-2-(1-(4-fluorophenyl)-5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid (3d)

IR (KBr, cm^{-1}): 2925.2 (OH), 1238.5 (C–N), 1720.7 (C=O), 1515.9 (C-C); ^{1}H NMR (400 MHz, DMSO-d6): 2.82 (s, 3H–CH3), 7.48-7.15 (m, 3H,Ar-H),7.76-7.81 (m, 4H, Ar–H), 8.18-8.22 (q, 1H, Ar–H), 8.52-8.55 (d, 1H, Ar–H), 8.82 (s,1H,Ar-H).^{13}C NMR (DMSO-d6) d (ppm): 167.37 (C=O), 159.8 (C=C), 146.10 (Ar–C), 142.06 (Ar–C), 131.82(Ar–C), 124.84 (Ar–C), 120.84 (Ar–C), 120.58 (Ar–C), 109.99 (Ar–C), 109.74 (Ar–C), 10.77 (C–C, CH3). ESI–MS m/z: [M+1]: 367.10. Anal. Calcd. for C19H12F2N4O2: C,62.30; H,3.30; F,10.37; N,15.29; O,8.74; Found: C,62.15; H,3.37; F,10.07; N,15.07; O,8.53.

2-(1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6-fluoroquinoline-4-carboxylic acid (3e)

IR (KBr, cm^{-1}): 2926.2 (OH), 1213.3 (C–N), 1717.51 (C=O), 1499.1 (C-C); ^{1}H NMR (400 MHz, DMSO-d6): 2.85 (s, 3H–CH3), 7.60-7.62 (d, 1H,Ar-H),7.69-7.72 (m,2H, Ar–H), 7.85-7.88 (m, 3H, Ar–H), 8.20-8.24 (m, 1H, Ar–H), 8.53-8.56 (m,1H,Ar-H), 8.83 (s,1H,Ar-H).^{13}C NMR (DMSO-d6) d (ppm): 167.36 (C=O), 159.8 (C=C), 146.02 (Ar–C), 142.22 (Ar–C), 130.25 (Ar–C), 124.79 (Ar–C), 120.87 (Ar–C), 120.62 (Ar–C), 110.05 (Ar–C), 109.75 (Ar–C), 10.82 (C–C, CH3). ESI–MS m/z: [M+1]: 381.15 Anal. Calcd. for C19H12ClFN4O2: C, 59.62; H,3.16;Cl,9.26; F,4.96; N,14.64;O,8.36; Found: C,59.15; H,3.37; Cl,9.07; F,4.37; N, 14.07; O,8.53.

2-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6-fluoroquinoline-4-carboxylic acid (3f)

IR (KBr, cm^{-1}): 2925.3 (OH), 1213.4 (C–N), 1715.99 (C=O), 1495.63 (C-C); ^{1}H NMR (400 MHz, DMSO-d6): 2.85 (s, 3H–CH3), 7.67-7.82 (m, 6H,Ar-H),8.2-8.23 (m,1H, Ar–H), 8.35-8.56 (m, 1H, Ar–H), 8.83 (s, 1H, Ar–H).^{13}C NMR (DMSO-d6) d (ppm): 167.35 (C=O), 159.8 (C=C), 146.00 (Ar–C), 142.23 (Ar–C), 132.80 (Ar–C), 124.86 (Ar–C), 120.86 (Ar–C), 120.60 (Ar–C), 109.99 (Ar–C), 109.74 (Ar–C), 10.82 (C–C, CH3). ESI–MS m/z: [M+1]: 426.05 Anal. Calcd. for C19H12BrFN4O2: C, 53.42; H,2.83; Br,18.70; F,4.45; N,13.11; O,7.49; Found: C, 53.15; H, 2.37; Br,18.07; F,4.37; N, 13.07; O,7.53.

6-Fluoro-2-(1-(2-fluorophenyl)-5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid (3g)

IR (KBr, cm^{-1}): 2926.8 (OH), 1238.02 (C–N), 1707.19 (C=O), 1510.13 (C-C); ^{1}H NMR (400 MHz, DMSO-d6): 2.67 (s, 3H–CH3), 7.52-7.64 (m, 1H,Ar-H), 7.66-7.75 (m,1H, Ar–H), 7.66-7.83 (m, 3H, Ar–H), 8.20-8.22 (m, 1H, Ar–H), 8.24-8.54 (m, 1H, Ar–H), 8.84 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d6) d (ppm): 167.33 (C=O), 159.83 (C=C), 151.06 (Ar–C), 141.82 (Ar–C), 132.70 (Ar–C), 124.90 (Ar–C), 124.79 (Ar–C), 120.83 (Ar–C), 109.97 (Ar–C), 109.73 (Ar–C), 10.15 (C–C, CH3). ESI–MS m/z: [M+1]:367.07 Anal. Calcd. for C19H12F2N4O2: C, 62.30; H,3.30; F,10.37; N,15.29; O,8.74; Found: C, 62.10; H,3.04; F,10.04; N, 15.07; O,8.53.

2-(1-(3-chlorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6-fluoroquinoline-4-carboxylic acid (3h)

IR (KBr, cm^{-1}): 2927.23(OH), 1217.99 (C–N), 1707.92 (C=O), 1592.83 (C-C); ^{1}H NMR (400 MHz, DMSO-d6): 2.86 (s, 3H–CH3), 7.71-7.83 (m, 4H,Ar-H),7.91 (s,1H, Ar–H),8.20-8.24 (m,1H, Ar–H), 8.53-8.56 (m, 1H, Ar–H), 8.83 (s, 1H, Ar–H), 14.24 (broad, OH). ^{13}C NMR (DMSO-d6) d (ppm): 167.32 (C=O), 159.81 (C=C), 151.18 (Ar–C), 142.15 (Ar–C), 136.00 (Ar–C), 124.86 (Ar–C), 124.78 (Ar–C), 120.57 (Ar–C), 109.97 (Ar–C), 109.72 (Ar–C), 10.80 (C–C, CH3). ESI–MS m/z: [M+1]: 383.05 Anal. Calcd. for C19H12ClFN4O2: C, 59.62; H,3.16;Cl,9.26; F,4.96; N,14.64;O,8.36; Found: C, 59.25; H, 3.27; Cl,9.07; F,4.37; N, 14.07;O,8.65.

2-(1-(2,5dichlorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6-fluoroquinoline-4-carboxylic acid (3i)

IR (KBr, cm^{-1}): 2926.19 (OH), 1216.30 (C–N), 1707.82 (C=O), 1475.78 (C-C); ^{1}H NMR (400 MHz, DMSO-d6): 2.70 (s, 3H–CH3), 7.79-7.90 (m, 4H,Ar-H), 7.92 (s,1H, Ar–H), 8.09-8.10 (d,1H, Ar–H), 8.20-8.23 (m, 1H, Ar–H), 8.55-8.58 (m, 1H, Ar–H), 8.82 (s, 1H, Ar–H), 14.34 (broad, OH). ^{13}C NMR (DMSO-d6) d (ppm): 167.31 (C=O), 159.86 (C=C), 151.06 (Ar–C), 141.70 (Ar–C), 136.44 (Ar–C), 124.97 (Ar–C), 124.86 (Ar–C), 120.92 (Ar–C), 109.80 (Ar–C), 109.72 (Ar–C), 10.16 (C–C, CH3). ESI–MS m/z: [M+1]:417.05 Anal. Calcd. for C19H12Cl2N4O2: C, 54.70; H,2.66;Cl,16.99; F,4.55; N,13.43;O,7.67; Found: C, 54.25; H, 2.27; Cl,16.37; F,4.37; N, 13.07;O,7.65.

2-(1-(2,6-difluorobenzyl)-5-methyl-1H-1,2,3-triazol-4-yl)-6-fluoroquinoline-4-carboxylic acid(3j)

IR (KBr, cm^{-1}): 2928.78 (OH), 1220.29 (C–N), 1705.05 (C=O), 1474.43 (C-C); ^{1}H NMR (400 MHz, DMSO-d6): 2.94 (s, 3H–CH3), 5.66 (s, 2H–CH_2) 7.16-7.49 (q,2H,Ar-H), 7.51-7.53 (m,1H, Ar–H), 7.71-7.76 (q,1H, Ar–H), 8.12-8.16 (m, 1H, Ar–H), 8.44-8.48 (m, 1H, Ar–H), 8.68 (s, 1H, Ar–H). ^{13}C NMR (DMSO-d6) d (ppm): 167.39 (C=O), 159.72 (C=C), 151.50 (Ar–C), 141.62 (Ar–C), 135.88 (Ar–C), 124.64 (Ar–C), 124.75 (Ar–C), 120.44 (Ar–C), 109.93 (Ar–C), 109.68 (Ar–C), 9.56 (C–C, CH3). ESI–MS m/z: [M+1]:399.11 Anal. Calcd. for C19H11F3N4O2: C, 59.38; H,2.88; F,14.83; N,14.58;O,8.33; Found: C, 59.25; H,2.27; F,14.37; N, 14.07;O,8.65.

3. Antimicrobial studies

The zone of inhibition of the synthesized compound 3(a-j) were determined by agar diffusion technique [18, 19]. The organism tested were Staphylococcus aureus (MTCC 96), Pseudomonas aeruginosa (MTCC 2488), Escherichia coli (MTCC 1687), Bacillus subtilis (MTCC 441), Proteus vulgaris (MTCC 744), Enterococcus faecalis (ATCC 29212) for antibacterial activity and Aspergillus niger (MTCC 1344), Candida albicans (MTCC 854), Curvularia lunata (MTCC 2030), Trichophyton simii (110/02) for antifungal activity. The agar media were inoculated with test organism and a solution of test compound 50µg/ml in DMSO. DMSO 10 µg/ml was separately in cups (8 mm diameter) in the agar medium. Chloramphenicol (10mcg/ disc), and Fluconazole (25 mcg/ disc), were used as a reference for antibacterial and antifungal activity respectively. The zones of inhibition were measured after 24 hrs incubation.

MIC values were determined by disc diffusion method. The stocks of each sample were prepared in the concentration 50 µg/ml. This was done by weighing 0.01mg of the sample and dissolving in 2 ml of DMSO. From stock solution different concentrations 5 µg /ml, 10 µg /ml, 20 µg /ml, 30 µg /ml, of each compound were prepared. Thus, proper amounts of the different concentrations of compounds were pipette on the blank disks, which were placed on the plates. The plates were incubated at $37^{0}C$ for 24 h. The minimum inhibitory concentrations (MICs), the lowest concentration (µg /ml) of the test compound that gives no visible growth on the plates were recorded as shown in figure 3.2.1. DMSO was used as a solvent control to ensure that solvent had no effect on bacterial growth. Chloramphenicol and Fluconazole were used as a control drug. The results of the anti microbial activities are summarized in table 1-5.

Table 1: Antibacterial studies of 6-Fluoro-2-(5-methyl-1-substituted-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid derivative against gram (- ve bacteria). Zone of inhibition is expressed in mm at 5 µg/ml,10 µg/ml,20 µg/ml,30 µg/ml

Compounds	*E. coli (µg/ml)*				*P. vulgarise (µg/ml)*				*P.aeruginosa(µg/ml)*			
	5	**10**	**20**	**30**	**5**	**10**	**20**	**30**	**5**	**10**	**20**	**30**
3a	8	9	10	11	11	15	16	18	12	13	15	17
3b	8	10	11	13	8	11	14	16	8	11	12	13
3c	10	11	13	14	12	13	14	16	10	12	13	15
3d	11	12	15	16	6	8	9	12	8	11	12	13
3e	8	9	11	14	0	13	14	16	13	14	14	15
3f	9	11	15	16	11	12	12	13	11	12	13	15
3g	8	9	10	10	11	12	13	14	11	12	14	15
3h	12	13	13	14	11	13	15	15	11	13	13	14
3i	10	11	12	14	11	12	13	15	11	12	12	14
3j	9	11	12	13	7	8	11	15	9	11	11	12
Chloramphenicol	21	21	21	21	14	14	14	14	23	23	23	23

Table 2: Antibacterial studies of 6-Fluoro-2-(5-methyl-1-substituted-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid derivative against gram (+ ve bacteria). Zone of inhibition is expressed in mm at 5 µg/ml,10 µg/ml,20 µg/ml,30 µg/ml

Compounds	*S. aureus*(µg/ml)				*B. Subtilis*(µg/ml)				*E. Faecalis*(µg/ml)			
	5	**10**	**20**	**30**	**5**	**10**	**20**	**30**	**5**	**10**	**20**	**30**
3a	11	12	13	14	8	11	12	14	11	12	13	15
3b	11	14	15	18	0	6	12	13	12	14	15	17
3c	11	12	13	15	12	13	14	16	11	12	12	13
3d	8	10	13	16	12	13	14	16	0	8	10	11
3e	11	11	13	14	11	13	13	15	11	13	15	15
3f	11	12	14	15	11	13	16	18	10	11	13	14
3g	8	9	11	12	8	10	11	13	7	8	12	15
3h	9	11	11	12	0	8	9	11	8	11	12	13
3i	11	12	14	15	11	12	12	14	9	10	11	12
3j	9	10	10	12	7	9	10	11	7	10	12	14
Chloramphenicol	22	22	22	22	15	15	15	15	19	19	19	19

Table 3: Mics (µg /ml) value of 6-Fluoro-2-(5-methyl-1-substituted-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid derivative against gram (+ ve bacteria) & gram (- ve bacteria)

Compounds	E. coli (µg/ml)	S. aureus (µg/ml)	P.aeruginosa (µg/ml)	B. subtilis (µg/ml)	P. vulgaris (µg/ml)	E. faecalis (µg/ml)
3a	5	<5	<5	5	<5	<5
3b	5	<5	5	10	5	<5
3c	<5	<5	<5	<5	<5	<5
3d	<5	5	5	<5	5	10
3e	5	<5	10	<5	<5	<5
3f	5	<5	<5	<5	<5	<5
3g	5	5	<5	5	<5	5
3h	<5	5	<5	10	<5	5
3i	<5	<5	<5	<5	<5	5
3j	5	5	5	5	5	5
Chloramphenicol	14	16	14	15	14	13
DMSO	NI	NI	NI	NI	NI	NI

Table 4: Antifungal studies of 6-Fluoro-2-(5-methyl-1-substituted-1H-1,2,3-triazol-4-yl)quinoline-4-carboxylic acid derivative against fungi. Zone of inhibition is expressed in mm at 5 µg/ml,10 µg/ml,20 µg/ml,30 µg/ml

Compounds	*A.niger*(µg/ml)				*C.albicans*(µg/ml)				*T.simii*(µg/ml)				*C.lunata*(µg/ml)			
	5	10	20	30	5	10	20	30	5	10	20	30	5	10	20	30
3a	10	10	12	12	0	10	11	13	0	0	10	10	0	10	10	12
3b	13	13	14	14	12	13	14	15	8	12	13	14	10	11	12	15
3c	9	10	12	14	12	12	13	14	8	9	13	13	11	11	12	14
3d	8	9	10	10	9	10	12	13	9	10	12	12	7	8	10	13
3e	10	11	11	13	11	13	13	14	7	9	10	10	9	9	14	14
3f	9	10	14	15	7	11	13	13	11	11	12	13	9	10	13	15
3g	7	9	10	12	9	10	11	12	9	10	11	12	10	12	13	13
3h	11	11	12	13	10	11	13	13	8	9	10	11	7	8	11	13
3i	11	11	12	12	7	9	10	11	13	13	14	15	10	10	11	12
3j	10	10	11	12	8	9	9	10	9	10	10	12	11	12	12	14
Fluconazole	17	17	17	17	16	16	16	16	20	20	20	20	19	19	19	19

Docking studies

The ligand dataset was virtually screened with the protein targets using V Life molecular design suite version4.5 (V Life MDS 4.5) and the binding affinity were analyzed for each docked conformation (Reyes and Kollman, 2000). V Life MDS 4.5 is validated software designing for calculating the accurate binding interaction energies of the 3-D structures of a known protein receptor with ligand or another protein molecule. The V Life MDS docking program approximated a complete systematic search of the conformational, orientational and positional space of the docked ligand molecules into the receptor (protein) binding pocket. Considering the well obtained *in vitro* results, it was worthwhile to go for docking to study the binding inside the active site of DNA Gyrase enzyme, potential target for antibacterial agents and support the *in vitro* results. Conformations having low energy and exhibited favourable hydrogen bonding with the amino acids side chain and its amide nitrogen were considered. Binding affinity of the protein–ligand interactions are of significant importance because they tell us how well the ligand binds to the target macromolecule. Docking simulations of 1, 2, 3-Triazole appended quinoline against DNA Gyrase enzyme target (PDB Code-5CDQ) lead to the identification of few potential compounds.

4. Results and Discussion

General synthetic methods

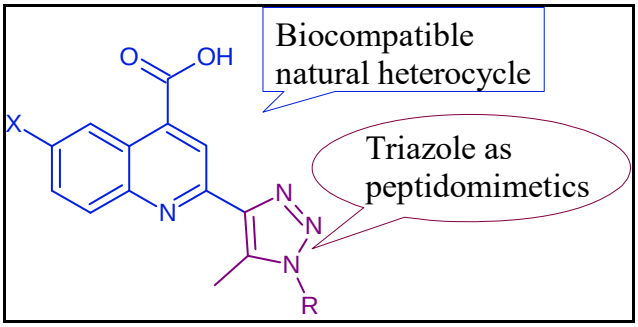


Fig 2: Target compound

The core structure shown in figure 2 represents the final test compound for synthesis and antimicrobial studies. The route of synthesize of quinoline appended 1,2,3-triazole derivatives is outlined in scheme 1.

The target compound is divided into two subunits: The quinoline part and triazole part as shown in Figure 3.1 The quinoline structure derived from isatin by reacting under pfitzinger reaction conditions with triazole containing keto derivative to achieve both the functionalities in the target compound

We tried different inorganic base such as Lithium hydroxide, Sodium hydroxide, Potassium hydroxide etc for pfitzinger reaction. The rate of reaction was very slow in Lithium hydroxide, Sodium hydroxide, for halogen substituted molecule. (~48hrs). In Potassium hydroxide reaction goes to completion in ~18 hrs. The better purity of compound achieved using adjusting pH to 3-3.5. Most of the substituent gives good yield between ~79-92% and reaction time between ~18-21 hrs. Generally reaction time is faster for electron withdrawing groups compare to electron releasing groups. The mass spectrum of compound **3a** showed molecular ion peak at m/z 365.05 is agreed with the molecular formula $C_{19}H_{14}N_4ClO_2$. Similarly a series of compounds with varying substitutions with aromatic ring attached to triazole ring were synthesized.

Antimicrobial studies

Antibacterial studies

All compounds inhibits bacterial growth against Gram positive bacteria (S. Aureus, B. Subtilis, E. Faecalis) and Gram negative bacteria (E.coli, P.vulgaris, P. aeruginosa). Compound b shows no inhibition against gram negative bacteria E.coli. Compound 3b,3g,3j shows moderate inhibition (4-9mm) against gram negative bacteria in various concentration whereas Compound 3b,3j shows moderate inhibition (4-10mm) against gram negative bacteria in various concentration. Control (Chloramphenicol) shows good inhibition (23-14 mm) against all strains whereas negative control (DMSO) shows zero inhibition as shown in table 1-2.

Minimum Inhibition Concentration of most of compounds are <5 µg/ml which shows good inhibition against all strains except compounds 3b,3d,3e,3h where MIC at 10 µg/ml shows low inhibition against B. Subtilis, E. faecalis, P.aeruginosa, B. Subtilis respectively. Remaining compounds 3g, 3j shows moderate MIC 5 µg/ml against all the strains.

Antifungal studies

All compounds inhibit fungal growth against A. Niger. C. Albicans, T. Simii, C. Lunata. Except Compound a shows zero inhibition against C. Albicans, T. Simii, C. Lunata at low concentration (5,10 µg/ml) whereas at higher concentration(20,30 µg/ml) inhibits in the range of 10 to 12mm.Compounds 3d,3e,3f,3g,3h,3i shows moderate inhibition 7mm against C.lunata, T.simii, C.albicans A.niger at low concentration (5 µg/ml) whereas at higher concentration (10,20,30 µg/ml) inhibits in the range of 8 to 13mm. Control (Fluconazole) shows good inhibition (16-20mm) against all strains whereas negative control (DMSO) shows zero inhibition as shown in table 3

Minimum Inhibition Concentration of most of compounds are <5 µg/ml which shows good inhibition against all strains except compounds 3a where MIC at 10 µg/ml,20 µg/ml, 10 µg/ml shows low inhibition against C. Albicans, T. Simii, C. Lunata. Remaining compounds 3d, 3 g, 3j shows moderate MIC at 5 µg/ml against all strains. A representative zone of inhibition in (mm) of comp 3c and3d against bacterial strains *B. Subtilis and fungal strains C. albicans* in various concentrations is shown in figure 3.

Fig 3: Zone of inhibition in (mm) of comp 3c and 3d against bacterial strains *B. Subtilis and fungal strains C.albicans in various concentrations*

A closer look at the results revealed that the title compounds possess better antibacterial activity as compared to antifungal activity. Among all the prepared compounds, 3c, 3h and 3i were found to be the most promising antibacterial and antifungal agents. Core structures consists of quinoline and 1,2,3-triazole,substituting electron withdrawing fluorine atom in sixth position of quinoline ring and fourth position carboxylic acid group greatly increase antibacterial activity similarly in 1,2,3-triazole, substitution of electron withdrawing and electron releasing group in first position of 1,2,3-triazole greatly increase antifungal activity.

Docking studies

The results of docking against DNA Gyrase topoisomerase II (PDB Code-5CDQ) are listed in Table 6. Docking scores of almost all synthesized compound were similar to the internal ligand value. The docking scores of tested compounds range between 97.8956 and 144.1034. The maximum number of hydrogen bond interactions shown by tested compounds was 9 comparable to internal ligand interaction values i.e., 30 The compounds which showed maximum docking score values are 3g (-144.103) (Table 9) in comparison with internal ligand value (-147.0043 Ciprofloxacin). The binding mode of compound, 3g and ciprofloxacin against the DNA Gyrase topoisomerase II is illustrated in figure 4 and figure 5. For Compound 3g One hydrogen bonding between acid(0) with N(ASP83C) at a bond distance of 4.848 A ˚shown in dark blue colour, Three π-π interaction between aromatic ring and N(MET121A) at a bond distance of 5.818 A ˚shown in light blue colour and Three π-π interaction between triazole ring and N(ALA68A) at a bond distance of 4.517 A ˚shown in light blue colour and two interaction between acetyl oxygen and N(ASP83C) at a bond distance of 5 A ˚shown in light blue colour(Figs. 3.3.1) From the docking results (Table 6) and antimicrobial activity it was observed that 1, 2, 3-Triazole appended quinoline showed similar mol dock score in comparison with ciprofloxacin, it may be due to increase in hydrophobicity and due to substitution of hydrogen atom of 1, 2, 3-Triazole appended quinoline with aromatic ring. On comparing the in vivo and in silico activity results of synthesized compounds, it was observed that the whole fluoro series 1, 2, 3-Triazole appended quinoline (3a–j) was most effective as antimicrobial agents as they exhibited maximum MIC values and maximum mol dock score, respectively.

Table 6: The yield, reaction time and dock score of various substituted quinolones

	R	Yield	Reaction Time	Mol dock score
3a	Phenyl	90	20	-97.895696
3b	Benzyl	92	21	-103.896265
3c	2,6-dimethyl	88	21	-112.324960
3d	4-Fluorophenyl	85	18	-115.977937
3e	4-Chlorophenyl	84	18	-112.841869
3f	4-bromophenyl	79	18	-125.794167
3g	2-Fluorophenyl	80	20	-144.103400
3h	3-Chlorophenyl	92	20	-109.454788
3i	2,5-dichlorophenyl	81	20	-114.305520
3j	2,6-difluorophenyl	85	20	-104.429644
Control	Ciproflaxacin	-	-	-147.004314

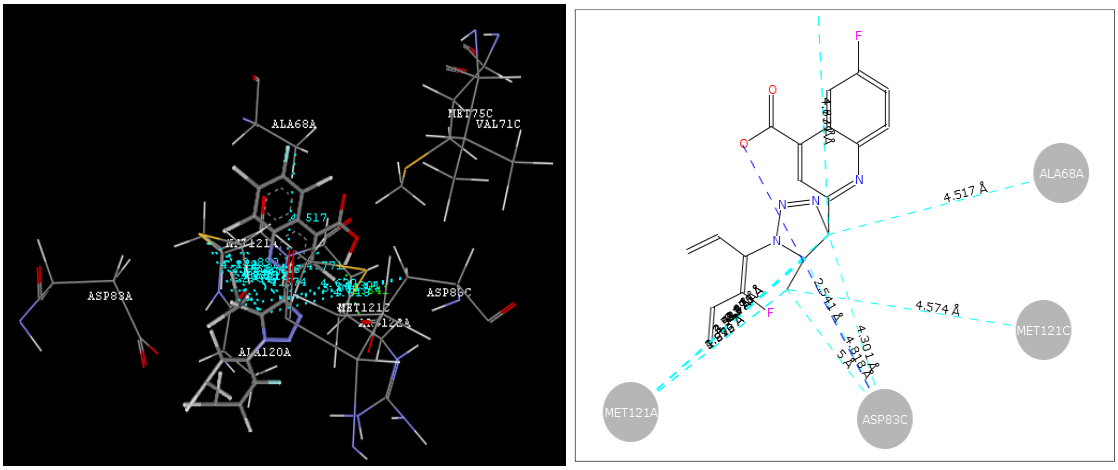


Fig 4: *-Molecular docking pose (3D-Image) of Compound 3g into the active binding site of DNA Gyrase (DNA Topoisomerase-II) PDB Code-5CDQ)

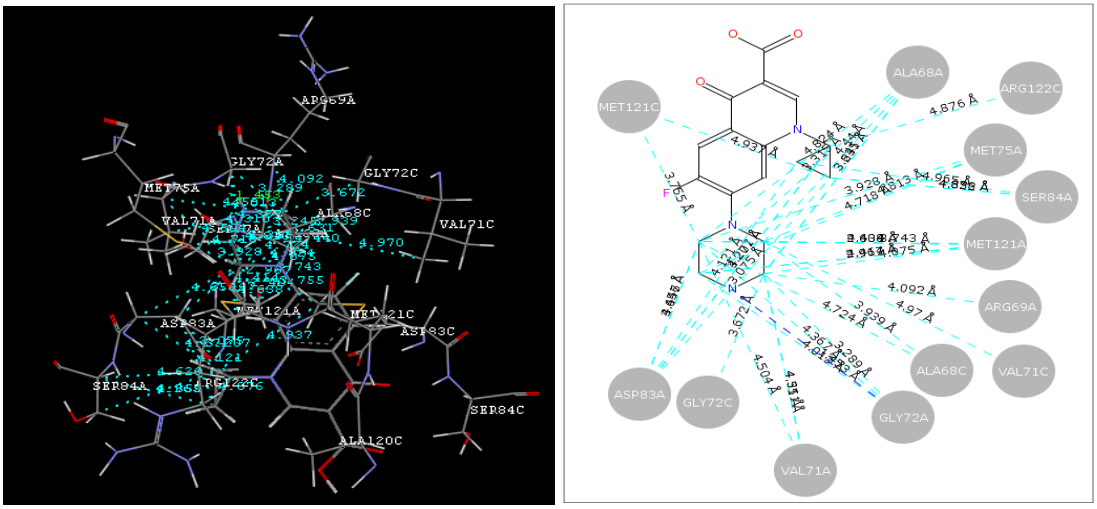


Fig 5: *-Molecular docking pose (3D-Image) of standard Ciprofloxacin into the active binding site of DNA Gyrase (DNA Topoisomerase-II) PDB Code-5CDQ)

*The dotted Dark blue lines indicate H-Bond interactions and light sky blue dotted lines indicate the VanDerrWaals interactions.

5. Conclusion

A series of quinoline carboxylic acid derivative using pfitzinger reaction synthesized from isatin and various 1,2,3-triazole containing keto functional group. Structures of the synthesized compounds were investigated by usual spectral techniques to ascertain the chemical structure. On comparing the in vivo and in silico activity results of synthesized compounds, it was observed that 1, 2, 3-Triazole appended quinoline derivatives with electron withdrawing substituent's were most effective antimicrobial agents as they exhibited maximum MIC values and maximum mol dock score, compared to market available standards.

6. Acknowledgements
One of author thankful to the Head, Department of Chemistry, Government Arts College, Salem for providing research facilities.

7. References

1. Steenhuysen Julie, Drug pipeline for worst superbugs on life support: report 2013. *Reuters*.
2. Boucher HW, Talbot GH, Benjamin DK, Bradley J, Guidos RJ, Jones RN. *et al.* Clin Infect Dis 2013;56:1685-94.
3. Moellering RC. Int J Antimicrob Agents. 2011; 37:2-9.
4. Spellberg B, Powers JH, Brass EP, Miller LG, Edwards JE. Clin Infect Dis. 2004; 38:1279-86.
5. Wenzel RP, Edmond MB. N Engl J Med. 2000; 343:1961-3.
6. Rolston KV, Hematol Oncol. 2009; 27:107-14.
7. Abbanat D, Morrow B, Bush K. Curr Opinion Pharmacol. 2008; 8:582-92.
8. Dos Santos JL, Dutra LA, Ferreira de Melo TR, Chin CM. In Tech. 2012, 69-86.
9. Butler MS, Blaskovich MA, Cooper MA. J Antibiot. 2013; 66:571-91.
10. Kumar S, Bawa S, Gupta H. Mini Rev Med Chem. 2009; 09:1648-54.
11. Diacon AH, Pym A, Grobusch M, Patientia R, Rustomjee R *et al.* N Engl J Med. 2009; 360:2397-405.
12. Haggiag S, Ruggieri S, Gasperini C. Ther Adv Neurol Disord. 2013; 6:343-52.
13. Bax BD, Chan PF, Eggleston DS, Fosberry A, Gentry DR *et al.* Nature. 2010; 466:935-40.
14. Emami S, Foroumadi A, Samadi N, Faramarzi MA, Rajabalian S. Arch Pharm (Weinheim). 2009; 342:405-11.
15. Kumar S, Bawa S, Kaushik D, Panda BP. Arch Pharm (Weinheim). 2011; 344:474-80.
16. Kumar S, Bawa S, Drabu S, Panda BP. Med Chem Res. 2011; 20:1340-8.
17. Bawa S, Kumar S. Indian J Chem B. 2009; 48:142-5.
18. Carrod LP, Grady FD. Antibiotics and Chemotherapy, third ed, Churchill Livingstone Edinburg. 1972, 477.
19. Habib NS, Soliman R, Tombary AE, Hawash SAE, Omaima G, Shaaban OG. Arch Pharm Res. 2007; 30:1511-1520.
20. Sarah Zeghada, Ghenia Bentabed-Ababsa, Acha Derdour, Safer Abdelmounim, Luis R Domingo *et al.* Organic and Biomolecular Chemistry, Royal Society of Chemistry. 2011; 9:4295-4305.