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# DESIGN AND SYNTHESIS OF NOVEL 1,2,3–TRIAZOLE SCAFFOLDS: BIOLOGICAL ACTIVITY AND MOLECULAR DOCKING STUDIES

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**ABSTRACT**

The final targets are established by different types of functional groups on aryl azides with terminal alkynes of pefloxacin via click chemistry in conventional and microwave irradiation conditions. A series of novel derivatives are characterized by proton, carbon nuclear magnetic resonance technique and mass spectral techniques. From screening antibacterial results the 5c, 5d, 5e, 5f and 5g exhibited outstanding activity against Gram-positive and Gram-negative microorganisms compared to CPF. All the title compounds were subjected to molecular docking prediction and compounds 5h, 5a, 5g possesses the highest binding energy ΔG= –8.12, –7.61, –7.26 Kcal/mol, hydrogen bonding amino acid interactions Val40, Asp83, Lys93, Tyr23, Arg28 with the active site of Topoisomerase-IV from *S. pneumoniae* (4KPE). Further investigation, these compounds were evaluated to their molecular properties prediction by Molinspiration and Molsoft.

**Keywords**: Antibacterial Activity, Norfloxacin, 1,2,3-Triazole, Molecular Modeling, Molinspiration, 4KPE.
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## INTRODUCTION

Triazole is an important class of heterocyclic compounds, which shows a broad spectrum of biological activities and is widely employed as agrochemicals and pharmaceuticals. 1,2,3-Triazole based molecules are useful pharmacophore for many DNA-alkylating and cross linking agents. They have been evaluating as an interesting ingredient in terms of biological activity and also shown significant anticancer activities in many human cell lines.[1] In recent years, these agents have been considerably studied with regarding cancer chemotherapy, this has a guide to the development of many novel and further selective alkylating agents based on triazole scaffolds as anticancer agents.[2] The conventional synthetic route for triazoles by Huisgen 1,3-dipolar cycloaddition of azides with organic alkynes possessing electron-withdrawing substituents to provide 1,2,3-triazoles.[3,4] 1,2,3-Triazole derivatives have been found to show promising biological activity inducing antibacterial,[5,6] antiviral,[7] antifungal,[8] antimicrobial, anti-inflammatory and analgesic[9] activity have been explored. Especially, 1,2,3-triazole derivatives are found to show invasion, tumor proliferation, metastasis[10] antitubercular activity,[11] and anti-HIV activity.[12,13]

Fluoroquinolones (FQs) are generally used in adults for their broad spectrum of antibacterial activity towards Gram-(+ve) and Gram-(-ve) bacteria's, the high bioavailability, high tissue and intracellular penetration when administered orally, the half-life and good tolerability profile.[14,15] Fluoroquinolones interfere with two important bacterial enzymes like DNA gyrase (topoisomerase II) and topoisomerase IV.[16] These are acted by processing one region of duplex DNA through another, and during this process, these drugs are trapped a reaction intermediate containing quinolones, broken DNA, and enzymes. The resulting ternary complexes are blocking DNA replication, and some of the bacteria's death occurs within hours.[17] However, a wide range of central nervous system (CNS) effects have been reported with an estimated


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incidence of 1-2 % in patients taking fluoroquinolones.[18] Topoisomerase-IV and DNA-gyrase are bacterial type II topoisomerases essential for DNA replication and chromosome protection that act via a transient double stranded DNA fragment involving a covalent enzyme DNA cleavage complex. In spite of their mechanistic importance, the DNA cleavage determinants are not understood for any bacterial type-II enzymes. Further investigated DNA cleavage by *S. pneumoniae* topo-IV and gyrase stabilized by antibacterial drugs. In the present investigations, we solved the docking structures of a few 7-bridged FQs in the active site of topoisomerase-IV from *K. pneumoniae* and DNA binding site of E-site by the drug in the presence of topoisomerase-IV. Even though the degree of antibiotic resistance regarding FQs is much lower than that of β-lactams and there is a need to increase the diversity of these successful clinically used drugs. The 7,8-bridged fluoroquinolone moieties [ACHN-245 & ACHN-454] binds similar to that of Trovofloxacin, Levofloxacin, Clinafloxocin, and Moxifloxacin but the chemical modifications of cyclic heterocyclic derivatives provide the higher interactions with the active site of Topo-IV.[20] From the literature survey, we are investigating the novel fluoroquinolone-1,2,3-triazoles with the active site of *S. pneumoniae* from DNA gyrase [4KPE]. From a perusal of literature, it is understood that solvent-free synthesis of triazoles via azide-alkyne cycloaddition in conventional and microwave irradiation methods is relatively new and their antibacterial activities.

## EXPERIMENTAL

All the reactants, reagents, and solvents were obtained from commercially available sources and analytical grading. IR (KBr) spectrum was recorded on a Perkin–Elmer 400 FT-IR spectrometer ($\upsilon_{max}$ in $cm^{-1}$) or a Varian 670-IR in the frequency range of 600-4000 $cm^{-1}$. Melting points were determined by the open capillary method. $^1H$ NMR was recorded by using DMSO-$d_6$ solvent in 400 MHz and $^{13}C$ NMR was recorded by using DMSO-$d_6$ & $CDCl_3$ solvent 100MHz and TMS as an internal standard. The mass spectrum was recorded by using VG micro mass 70-70H instrument. The purity of these compounds was checked by TLC on silica gel and using n-hexane and ethyl acetate solvents. A mono wave 300 mass 24 (Anton par) microwave was used to carry out the microwave reactions in 30 mL microwave process vials with temperature control by the infrared detection temperature sensor.

### Preparation of Compound 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(prop-2-yn-1-yl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic Acid, 2

A mixture of 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid**1** (1.0 mmol), propargyl bromide (1.0 mmol) and trimethyl amine in tetrahedrofuran (15 mL) were stirred for 4 hr at ambient temperature under $N_2$ atmosphere. After removal of the solvent, the residue was purified by silica gel column chromatography eluted with 10:1 DCM to MeOH to give desired product **2** with good yield 70%.

### Synthesis of Substituted Aromatic Azides, 4

Aromatic substituted amines **3a-i** (1 mmol) dissolved in dry acetone (15 mL) to the magnetically stirred solution at room temperature and after that aq. solution of sodium azide (1.8 mmol) was added to the reaction vessel. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into crushed ice and extracted with $CHCl_3$ (3 x 25 mL). Finally, azide **4** was purified by column chromatography using chloroform: methanol (9:1).

### Synthesis of Final 1,2,3-triazole Derivatives

After establishing the required proportion of copper sulphate and sodium ascorbate, the CuAAC reaction was performed taking azides **4a-i**, and terminal alkyne 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(prop-2-yn-1-yl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid**2** in t-BuOH: $H_2O$ (1:1) (15 mL) at rt for 30 minto get the target molecules **5a-i** (Scheme-1). The solid crude products were purified by column chromatography (silica gel) eluted with DCM to MeOH to give the title compounds **5** in 70-81% yields.

### 1-ethyl-6-fluoro-7-(4-((1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid, 5a

$^1H$ NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.51 (3H, t, $J$ = 8 Hz, $CH_3$), 2.19 (1H, s, $CH_2$), 2.92 (4H, t, $J$ = 8 Hz, piperazine), 3.49 (4H, m, piperazine), 4.29 (2H, q, $J$ = 6 Hz, $NCH_2$), 6.77 (1H, d, $J$ = 7.0 Hz, Ar-H),

7.20 (2H, d, *J* = 7.0 Hz, Ar-H), 7.40 (1H, d, *J* = 7.0 Hz, Ar-H), 7.53 (1H, d, *J* = 7.0 Hz, Ar-H), 7.75 (1H, s, C5-H), 8.12 (1H, s, triazole-H), 8.42 (1H, s, C2-H), 8.81 (1H, s, C8-H), 15.12 (1H, s, COOH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 9.43, 14.92, 21.49, 34.84, 48.11, 51.02, 54.22, 57.43, 63.48, 107.01, 110.91, 116.38, 121.11, 125.09, 126.83, 132.50, 134.61, 142.52, 143.20, 150.98, 154.66, 157.14, 162.41, 166.12, 176.78. ESI-MS m/z: 522 $[M+H]^+$.

**1-ethyl-6-fluoro-7-(4-((1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid, 5b**

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.52 (3H, t, *J* = 8.0 Hz, $CH_3$), 2.53 (2H, s, $CH_2$), 3.27 (2H, t, *J* = 8 Hz, piperazine), 3.69 (4H, m, piperazine), 4.17 (2H, t, *J* = 6.5 Hz, piperazine), 4.44 (2H, q, *J* = 6.0 Hz, $NCH_2$), 6.87 (2H, s, Ar-H), 7.35 (1H, d, *J* = 7.0 Hz, Ar-H), 7.60 (2H, m, Ar-H), 7.74 (1H, s, C5-H), 8.03(1H, s, triazole-H), 8.69 (1H, s, C8-H), 14.96 (1H, s, COOH).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ ppm 9.47, 16.07, 29.48, 47.32, 51.04, 54.26, 57.42, 63.43, 110.36, 119.68, 121.18, 123.33, 130.99, 134.59, 139.53, 143.25, 146.20, 150.93, 154.75, 161.14, 166.17, 176.78, 179.07.ESI-MS m/z: 522 $[M+H]^+$.

**1-ethyl-6-fluoro-7-(4-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid, 5c**

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.32 (3H, t, *J* = 8 Hz, $CH_3$), 2.72 (4H, t, *J* = 8 Hz, piperazine), 3.70 (4H, t, *J* = 8 Hz, piperazine), 4.12 (3H, s, $OCH_3$), 4.62 (2H, s, $CH_2$), 5.12 (2H, q, *J* = 6 Hz, $NCH_2$), 6.87 (1H, s, Ar-H), 7.18 (2H, d, *J* = 7.0 Hz, Ar-H), 7.30 (1H, d, *J* = 7.0 Hz, Ar-H), 7.39 (1H, d, *J* = 7.0 Hz, Ar-H), 7.72 (1H, s, C5-H), 7.76 (1H, s, triazole-H), 8.03 (1H, s, C2-H), 8.69 (1H, s, C8-H), 11.84 (1H, s, COOH). $^{13}$C NMR (**100** MHz, $CDCl_3$) δ ppm 9.45, 16.04, 41.26, 45.27, 51.01, 54.25, 57.42, 63.42, 106.11, 110.45, 113.28, 117.44, 123.27, 128.95, 130.27, 134.58, 139.09, 144.13, 148.22, 152.93, 154.15, 157.84, 161.13, 166.19, 176.76, 179.14. ESI-MS m/z: 507 $[M+H]^+$.

**7-(4-((1-(2,4-dimethoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid, 5d**

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.30 (3H, t, *J* = 8 Hz, $CH_3$), 2.65 (4H, t, *J* = 8 Hz, piperazine), 3.25 (4H, t, *J* = 8 Hz, piperazine), 3.65 (3H, s, $OCH_3$), 3.76 (1H, q, *J* = 6 Hz, $NCH_2$), 4.10 (3H, s, $OCH_3$), 4.65 (2H, s, $CH_2$), 6.96 (1H, s, Ar-H), 7.23 (1H, d, *J* = 7.0 Hz, Ar-H), 7.41 (1H, s, C5-H), 7.72 (1H, d, *J* = 7.0 Hz, Ar-H), 7.81 (1H, s, triazole-H), 7.98 (1H, s,C2-H), 8.68 (1H, s,C8-H), 12.05 (1H, s, COOH). $^{13}$C NMR (**100** MHz, $CDCl_3$) δ ppm 8.55, 16.04, 41.25, 47.26, 48.15, 51.29, 54.25, 57.41, 63.42, 106.87, 108.19, 117.43, 121.18, 124.94, 130.26, 134.57, 139.09, 143.25, 146.21, 150.93, 154.74, 157.83, 160.23, 166.18, 176.75, 179.13. ESI-MS m/z: 537 $[M+H]^+$.

**1-ethyl-6-fluoro-4-oxo-7-(4-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1,4-dihydro quinoline-3-carboxylic Acid, 5e**

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 0.98 (3H, t, *J* = 8.0 Hz, $CH_3$), 2.67 (4H, t, *J* = 8.0 Hz, piperazine), 3.20 (4H, t, *J* = 8.0 Hz, piperazine), 3.51 (3H, s, $CH_3$), 4.10 (2H, s, $CH_2$), 4.69 (2H, q, *J* = 6.0 Hz, $NCH_2$), 6.81 (2H, d, *J* = 6.5 Hz, Ar-H), 7.10 (1H, d, *J* = 7.0 Hz, Ar-H), 7.35 (1H, s, C5-H), 7.55 (1H, s, triazole-H), 8.45 (1H, s,C2-H), 9.02 (1H, s, C8-H), 11.68 (1H, s, COOH). $^{13}$C NMR ($CDCl_3$, 100 MHz) *δ* ppm: 14.54, 18.26, 54.26, 56.02, 59.67, 61.50, 113.41, 114.66, 115.33, 120.32, 122.32, 123.21, 130.06, 130.49, 140.05, 145.24, 147.93, 151.67, 158.51, 159.80, 165.82. ESI-MS m/z: 491 $[M+H]^+$.

**7-(4-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid, 5f**

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.22 (3H, t, *J* = 8.0 Hz, $CH_3$), 2.35 (2H, t, *J* = 8.0 Hz, piperazine), 2.96 (2H, m, piperazine), 3.21 (2H, s, $CH_2$), 3.70 (2H, m, piperazine), 4.21 (2H, m, piperazine), 4.67 (2H, q, *J* = 6.0 Hz, $NCH_2$), 7.01 (1H, d, *J* = 7.0 Hz, Ar-H), 7.18 (1H, d, *J* = 7.0 Hz, Ar-H), 7.51 (1H, s, C5-H), 7.53 (1H, d, *J* = 7.0 Hz, Ar-H), 7.74 (1H, d, *J* = 7.0 Hz, Ar-H), 8.06 (1H, s, triazole-H), 8.69 (1H, s,C2-H), 8.77 (1H, s, C8-H), 14.97 (1H, s, COOH).$^{13}$C NMR ($CDCl_3$, 100 MHz) *δ* ppm:174.93, 173.62, 162.31,

153.82, 150.64, 149.48, 137.51, 131.36, 129.03, 124.88, 124.46, 117.87, 113.50, 113.16, 110.72, 56.47, 55.82, 50.33, 49.55, 29.66, 9.12. ESI-MS m/z: 512 $[M+H]^+$.

**1-ethyl-6-fluoro-7-(4-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid, 5g**

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.21 (3H, t, $CH_3$), 2.42 (2H, t, *J* = 8.0 Hz, piperazine), 2.92 (2H, s, $CH_2$), 3.21 (2H, m, piperazine), 3.65 (2H, m, piperazine), 4.18 (2H, m, piperazine), 4.56 (2H, q, *J* = 6.0 Hz, $NCH_2$), 7.03 (1H, d, *J* = 7.0 Hz, Ar-H), 7.21 (1H, d, *J* = 7.0 Hz, Ar-H), 7.52 (1H, s, C5-H), 7.63 (1H, d, *J* = 7.0 Hz, Ar-H), 7.76 (1H, d, *J* = 7.0 Hz, Ar-H), 7.96 (1H, s, triazole-H), 8.15 (1H, s,C2-H), 8.69 (1H, s, C8-H), 14.92 (1H, s, COOH).$^{13}$C NMR ($CDCl_3$, 100 MHz) *δ* ppm: 14.4, 23.94, 28.92, 30.56, 44.91, 49.74, 54.62, 67.75, 108.40, 114.72, 118.94,121.55,129.82,134.20, 138.17,142.38, 146.28,154.76,165.24, 172.24,174.04. ESI-MS m/z: 522 $[M+H]^+$.

**1-ethyl-6-fluoro-7-(4-((1-(2-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid, 5h**

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.20 (3H, t, *J* = 8.0 Hz, $CH_3$), 3.02 (4H, t, *J* = 8.0 Hz, piperazine), 3.41 (4H, t, *J* = 8.0 Hz, piperazine), 4.01 (2H, s, $CH_2$), 4.44 (2H, q, *J* = 6.0 Hz, $NCH_2$), 7.20 (1H, d, *J* = 7.0 Hz, Ar-H), 7.43 (1H, d, *J* = 7.0 Hz, Ar-H), 7.55 (2H, d, *J* = 7.0 Hz, Ar-H), 7.80 (1H, s, C5-H), 8.05 (1H, s, triazole-H), 8.63 (1H, s, C2-H), 9.32 (1H, s, C8-H), 9.89 (1H, s, Ar-OH), 11.75 (1H, s, COOH). $^{13}$C NMR ($CDCl_3$, **100** MHz) *δ* ppm: 21.32, 24.96, 30.64, 39.58, 42.71, 50.02, 59.61, 117.33, 121.74, 125.68, 127.44, 128.32, 129.57, 130.60, 135.10, 135.61, 136.28, 144.61, 150.84, 154.26, 166.21, 171.02. ESI-MS m/z: 493 $[M+H]^+$.

**7-(4-((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid, 5i**

$^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 1.58 (3H, t, *J* = 8.0 Hz, $CH_3$), 2.60 (2H, t, *J* = 8.0 Hz, piperazine), 2.89 (2H, s, $CH_2$), 3.25 (2H, m, piperazine), 3.60 (2H, m, piperazine), 3.98 (2H, m, piperazine), 4.28 (2H, q, *J* = 6.0 Hz, $NCH_2$), 6.97 (1H, s, Ar-H), 7.15 (1H, d, *J* = 7.0 Hz, Ar-H), 7.36 (1H, s, C5-H), 7.53 (1H, d, *J* = 7.0 Hz, Ar-H), 7.70 (1H, d, *J* = 7.0 Hz, Ar-H), 7.91 (1H, s, triazole-H), 8.09 (1H, s,C2-H), 8.44 (1H, s, C8-H), 14.90 (1H, s, COOH).$^{13}$C NMR ($CDCl_3$, 100 MHz) *δ* ppm: 19.58, 31.86, 46.53, 54.04, 56.57, 59.26, 106.83, 120.29, 124.77, 125.29, 127.29, 127.43, 128.43, 130.98, 131.94, 132.09, 134.96, 136.45, 144.92, 156.51, 158.92, 175.67. ESI-MS m/z: 512 $[M+H]^+$.

**Antibacterial Activity**

In the present study a collection of five microorganisms were used in clinical isolates including Gram-(+) organisms *Streptococcus pneumoniae, Bacillus subtilis*, *Staphylococcus aureus*, Gram-(–) strains *Escherichia coli*, *Pseudomonas aeruginosa* strains were produced from Microbiology laboratory, Global Hospital, Hyderabad. All the strains were tested for their purity by standard microbiological techniques. The bacterial stock cultures were maintained on Mueller Hinton Agar (MHA) slants and stored at 4 °C. An agar-well diffusion method was used for the evaluation of antibacterial sensitivity.[21, 22] The bacterial organisms were reactivated from stock culture by being transferred into Mueller Hinton Broth and incubated at 37 °C for overnight. DMSO was used as a negative control. A final inoculum containing 1 x $10^6$ CFU/mL forming was added to MHA medium and poured into sterile Petri dishes. Our prepared compounds were added at a concentration of 1.0 mg/50 μL to wells punched on agar surface. Agar plates were incubated overnight at 37 °C and the diameter zone of inhibition around each well was measured in millimeters. These experiments were performed in triplicates and norfloxacin antibiotic was used as a positive reference.

**Docking**

**Identification of Active Site Pockets**

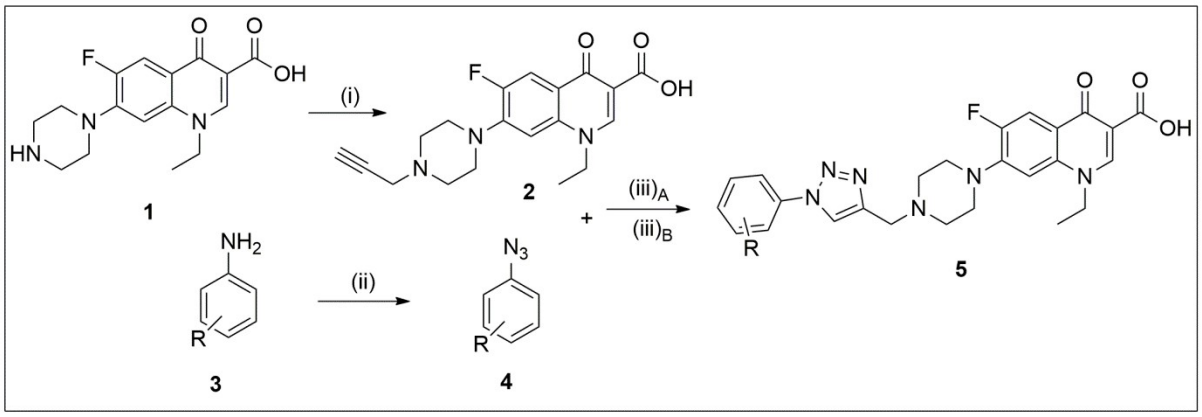
Nine potent molecules were allowed for docking study with new fluoroquinolones in complex with topo-IV from *S. pneumoniae* and E-site G-gate (4KPE) protein. The active site calculations were carried out by

using the online tool PdbSum.[23] It showed two hydrogen bond interactions with Arg456 (C), Ser79 (A), and DC which are present in the active site. The ligand present in the protein are AF5 -(7ar,8r)-8-Amino-4-Cyclopropyl-12-Fluoro-1-Oxo-4,7,7a, 8,9,10-Hexahydro-1h-Pyrrolo[$1^1 2^1$:1,7] azepino[2,3-H]quinoline-2-Carboxylic acid. **9** synthesized compounds were sketched in chemsketch[24] and converted into .mol2 format using open babel software. Molecular modeling was performed to all the ligands separately by using AutoDock4.2, Lamarckian Genetic Algorithm (LGA) and implemented empirical free energy function.[25] Initially, the protein was downloaded from RCSB and loaded in autodock, further the hydrogens were added and saved it in PDBQT format. The grid parameters were selected and the calculations were evaluated by using Auto-Grid. For all grid-point spacing of 0.375 Å was applied and grid map with 60 X 60 X 60 points was used. X, Y, Z-coordinates were selected on the basis of the amino acids present in the active site.

## RESULTS AND DISCUSSION

### Chemistry

These target compounds were synthesized as shown in Scheme-1**,** compound 1 on reacted with propargyl bromide in the presence of $Et_3N$ in dry THF to obtained intermediate 2. Further, the different substituted amines converted into azides in the presence of $NaN_3$, acetone as a solvent at ambient temperature to give 4 in excellent yields. The final compounds were obtained via Click Chemistry by using terminal alkyne 2 and azide 4 in conventional method as well as microwave irradiation technique in the presence of $CuSO_4.5H_2O$, sodium ascorbate, t-BuOH:$H_2O$. The microwave irradiation (MW) method has been offering a standard shift in the target synthesis due to their peculiar direct 'in core' heating of the reactions thus decreasing the reaction times. Moreover, it offered myriad advantages such as acceleration of sluggish transformation, higher yields, environmentally greener conditions and explosively promoting the cyclo condensation reaction. Under the nonclassical reaction rates are faster than classical heating conditions, for example, the synthesis of triazole compounds was obtained in 7 hr by conventional method whereas, under microwave conditions, it was acquired in 6-16 min (5a-i, Table-1). Substituted azides 4 and terminal alkyne 2**,** $CuSO_4.5H_2O$ in t-BuOH were taken in a closed vessel and irradiated under the microwave (MW) at 85 ºC for 6-16 min. The obtained crude product was recrystallized from methanol to furnish 5a-i in 68-75%. All the reactions were performed in the presence of the 80-90 watts of microwave irradiation. The structure of compound 5a was confirmed based on the following analytical data as $^1$H NMR δ ppm at 1.51 triplet for 3H, 2.19 singlet for 1H belongs to $CH_2$, CH; δ 2.92-3.49 triplet for piperazine ring, and 6.77, 7.20, 7.40, 7.53 doublet for aromatic hydrogens. Further δ 7.75, 8.12, and 8.42 singlets for C-5, triazole ring, and C-2 protons, 15.12 singlet for OH protons of acid respectively. $^{13}$C NMR δ in ppm 176.78, 166.12 for CO of quinolinone ring, and acid moiety. Signal δ ppm at 157.14-110.91 for aromatic carbons, δ at 63.48, 57.43 for $NCH_2$ and δ at 48.11 for NCH carbons respectively. *m/z* (ESI): 522 $(M+H)^+$ fragmentation patterns were in accordance with the assigned structure.



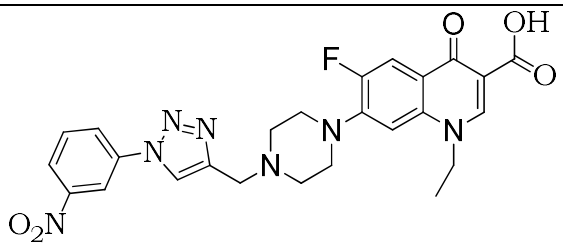
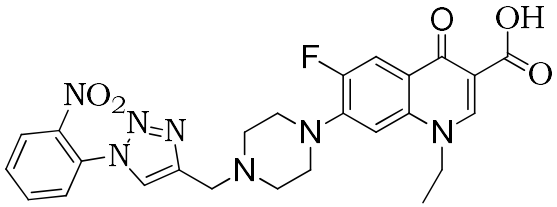
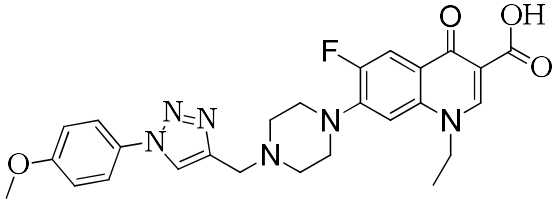
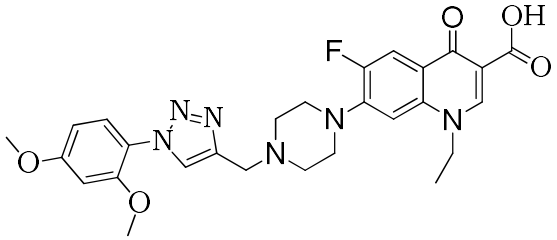
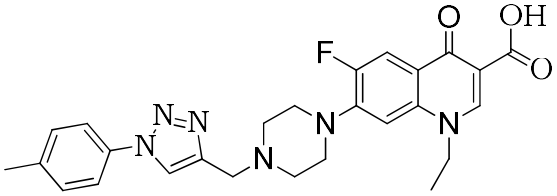
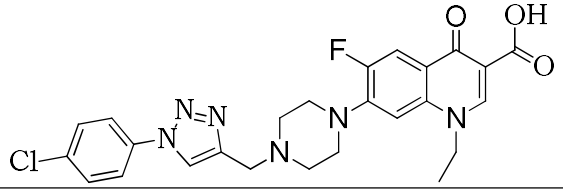

Scheme 1: Synthesis of Norfloxacin linked to Triazole Derivatives

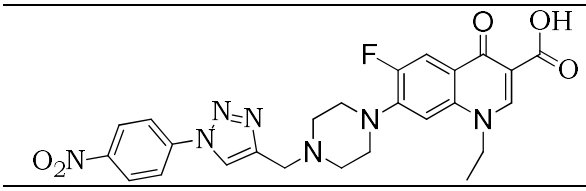
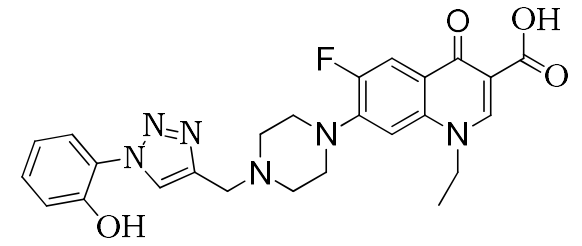
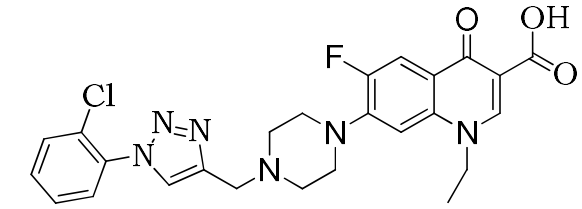
Reagents and conditions: (i) Propargyl bromide, $Et_3N$, dry THF, 0 °C, rt, 86% (ii) NaN3, Acetone, 6hr, rt, 87% $(iii)_A$ $CuSO_4.5H_2O$, Sodium ascorbate, t-BuOH:$H_2O$ (1:1), rt, 30 min, 60-75 %, MWI $(iii)_B$: $CuSO_4.5H_2O$, Sodium ascorbate, t-BuOH:$H_2O$ (1:1), 80-90 watts,6-16 min,68-75 %.

**Antibacterial Activity**

Our prepared triazoles 5a–I was screened for their antibacterial activity against representative Gram-positive microorganisms *S. pneumoniae, B. subtilis*, *S. aureus*, Gram-negative strains *E. coli*, *P. aeruginosa* by using Agarwal techniques and zone of inhibition (mm) along with CPF as shown in Table-2. From screening results revealed that the antibacterial efficiency of final quinolinone triazoles 5d, 5c, 5e and 5g shows a magnificent broad spectrum of activity against all Gram-(+ve) and Gram-(–ve) microorganisms.

Table 1: Physiochemical Properties of Novel Norfloxacin triazole Derivatives

| Entry | | Conventional Method hr/yield | | Microwave Irradiation min/yield | Log p/ Clog p |
|---|---|---|---|---|---|
|  | 5a | 7 | 10 min | 60/68 | ---/1.23 |
|  | 5b | 7 | 8 min | 75/72 | ---/1.23 |
|  | 5c | 7 | 18 min | 69/72 | 3.34/1.34 |
|  | 5d | 7 | 16 min | 78/69 | 3.33/2.91 |
|  | 5e | 7 | 10 min | 76/68 | 3.95/1.61 |
|  | 5f | 7 | 15 min | 78/69 | 4.02/2.00 |

| | | | | | |
|---|---|---|---|---|---|
|  | 5g | 7 | 6 min | 60/65 | ----/1.230 |
|  | 5h | 7 | 8 min | 72/75 | 3.07/0.88 |
|  | 5i | 7 | 10 min | 75/78 | 4.02/2.00 |

They are generally exhibited moderate to good potency inhibiting the growth of Gram-(+ve) organisms such as *Staphylococcus aureus Streptococcus pneumoniae,* zone of inhibition 33, 30 mm values respectively. Comparison of results for 5d, 5c, and 5e follows the trend of 5d > 5c > 5e against all the strains tested suggesting that introduction of these functional groups 2,5-dimethoxy, 4-methyl and 4-methoxy attached to the benzene ring is advisable. In addition, the compounds 5e, 5f showed very good antibacterial activity against *E.coli*, then the reference CPF with zone of inhibition 29, 28 and 31 mm values respectively. Moreover, the most active analogs 5i, 5c and 5d were found to be more potent antibacterial activity response against Gram-(-) strain *P. Aeruginosa* with 28, 20 mm zone of inhibition. Furthermore, the activity of synthesized triazoles against Gram-(–ve) microorganisms was in the order; 2-chloro > 4-methoxy > 2,5-dimethoxy on benzene ring attached to triazole core. From screening results, target compounds are generally good active when the compounds have electron releasing groups like –OMe, $–CH_3$, –Cl etc.

Table-2: Antibacterial Sensitivity of Final 1,2,3-triazole linked to Norfloxacin

| Entry | Diameter of Zone of Inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | *S.pneumoniae* | *B. subtilis* | *S. aureus* | *E. coli* | *P. aeruginosa* |
| 5a | 13 ± 0.4 | 14 ± 0.2 | 18 ± 0.2 | 21 ± 0.4 | 18 ± 0.3 |
| 5b | 12 ± 0.3 | 16 ± 0.4 | 24 ± 0.1 | 19 ± 0.4 | 17 ± 0.3 |
| 5c | 21 ± 0.5 | 24 ± 0.1 | 26 ± 0.1 | NI | 28 ± 0.5 |
| 5d | NI | 18 ± 0.3 | 33 ± 0.2 | 16 ± 0.1 | 20 ± 0.5 |
| 5e | 19 ± 0.4 | 17 ± 0.3 | 11 ± 0.1 | 29 ± 0.4 | 19 ± 0.1 |
| 5f | 21 ± 0.2 | 28 ± 0.5 | 10 ± 0.2 | 28 ± 0.2 | NI |
| 5g | 25 ± 0.3 | 12 ± 0.3 | 18 ± 0.2 | 11 ± 0.1 | 10 ± 0.3 |
| 5h | 30 ± 0.4 | NI | 11 ± 0.1 | 15 ± 0.2 | 12 ± 0.4 |
| 5i | 14 ± 0.2 | 25 ± 0.4 | 10 ± 0.2 | 22 ± 0.4 | 28 ± 0.1 |
| CPF | 34 ± 0.2 | 30 ± 0.1 | 33 ± 0.2 | 31 ± 0.1 | 35 ± 0.4 |

**Molecular Docking Studies**

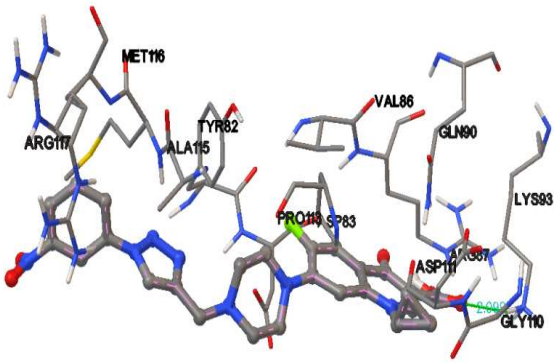
Molecular modelling is one of the most useful methods for calculations between protein and ligand interactions and Autodock4.2 is an efficient method for the prediction of potential ligands. The 3-dimensional crystal structure of FQs with topoisomerase–IV from *S. pneumoniae* and 4KPE retrieved from protein data bank and docking score, amino acids interactions of every ligand was calculated in terms of Kcal/mol (Table-2, Fig.-1.). The docking scores and hydrogen bond interactions have been done for all the

final triazole compounds used in the present study and are denoted as thick green color lines. In Fig.-1 having, the right side, left side, or above of the molecule against human DNA Topoisomerase–IV of 4KPE present in a blue or light green helical shape.

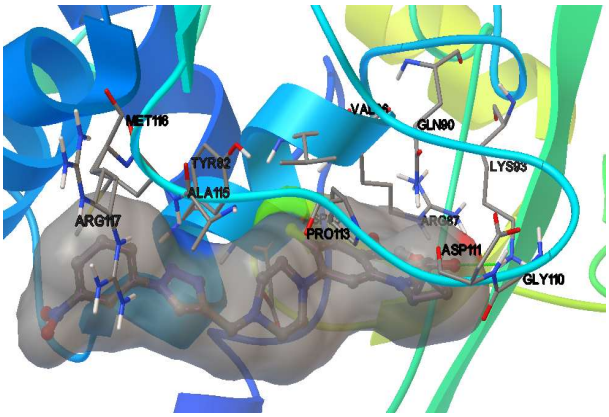
Out of 9 compounds 5 compounds are interacting with amino acid Met116and three compounds are interacting with amino acid Lys93. From docking results compounds 5g, 5f exhibited the highest hydrogen bonding interactions withaminoacids Arg28 (2.23 A˚) and Ala115 (2.15 A˚) values respectively (Fig.-1). Compounds 5h, 5a, 5g exhibit the highest binding energy of ΔG= -8.12, -7.61, -7.26 kcal/mol, showing amino acids interactions with Val40, Asp83, Lys93, Tyr23, Arg28. Compound 5b, 5c, 5d shows very good dissociation constant (KI = 19.21, 25.98, 20.13 µM with amino acid interactions viz., Met116, His76, Ser80. Target compounds 5e, 5f, 5c and 5b having good binding interactions ΔG = -6.82, -6.70, -6.26 and -6.22 kcal/mol values respectively**.** From docking studies, all the compounds show excellent binding energy and good interactions with topoisomerase-IV from *S. pneumoniae*.

Table 2: Docking Studies of Target Compounds with Topoisomerase –IV of 4KPE

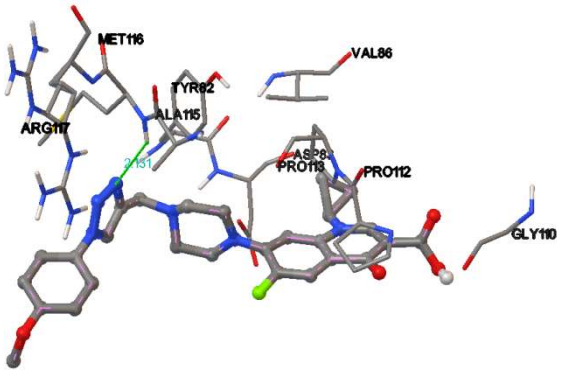
| Entry | Ligand Interacting Amino Acids | Binding Energy (ΔG) | Dissociation Constant (KI) |
|---|---|---|---|
| 5a | Lys93 | -7.61 | 2.65 |
| 5b | Met116 | -6.22 | 19.21 |
| 5c | Met116 | -6.26 | 25.98 |
| 5d | His76, Ser80 | -5.04 | 20.13 |
| 5e | Met116, Lys93 | -6.82 | 10.05 |
| 5f | Met116, Lys93 | -6.70 | 12.30 |
| 5g | Tyr23, Arg28 | -7.26 | 4.75 |
| 5h | Val40, Asp83 | -8.12 | 1.11 |
| 5i | Met116 | -5.17 | 16.1 |
| CPF | Met116, Lys93 | -6.75 | 15.24 |



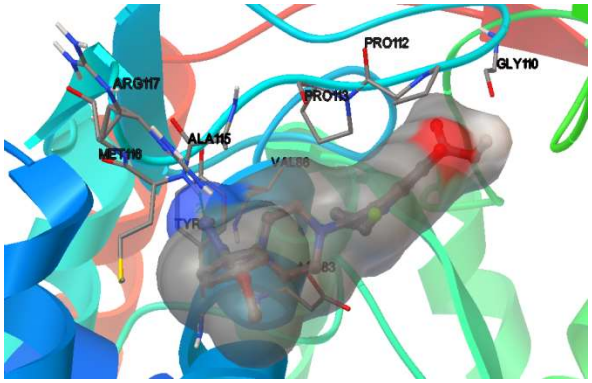

5a-The Side Chain Binding Mode Interaction



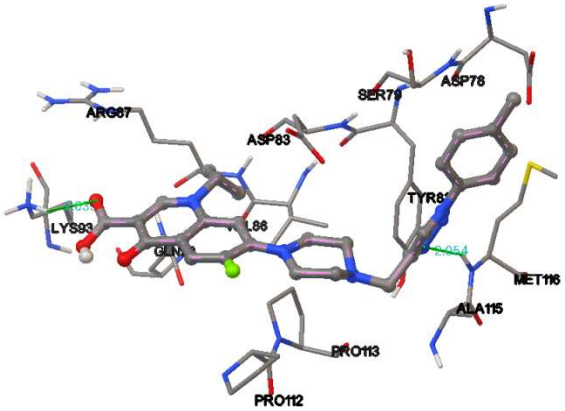

5a-The Surface Flexibility Binding Mode Interaction



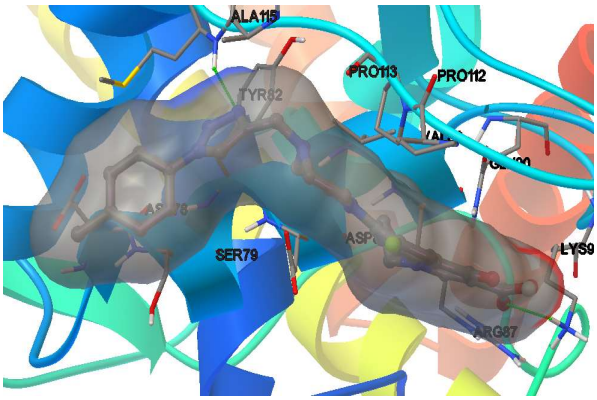

5c-The Side Chain Binding Mode Interaction



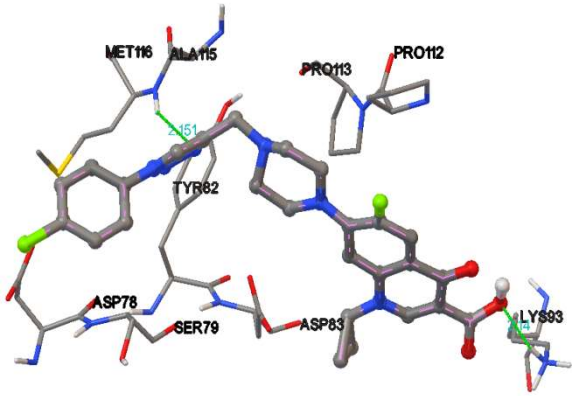

5c-The Surface Flexibility Binding Mode Interaction



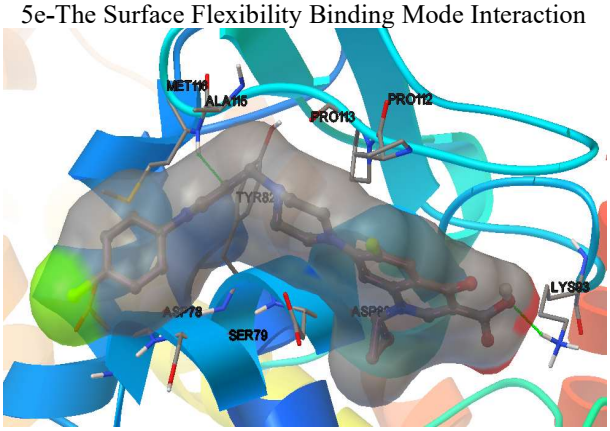

5e-The Side Chain Binding Mode Interaction



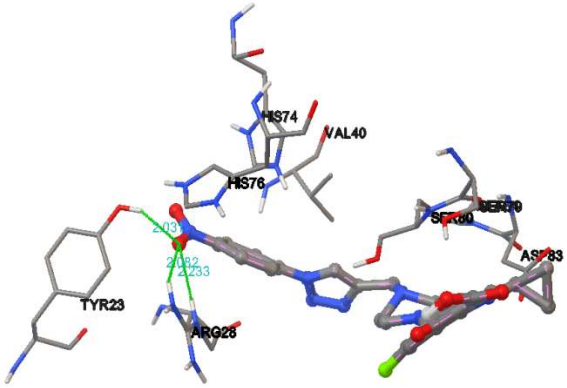

5e-The Surface Flexibility Binding Mode Interaction



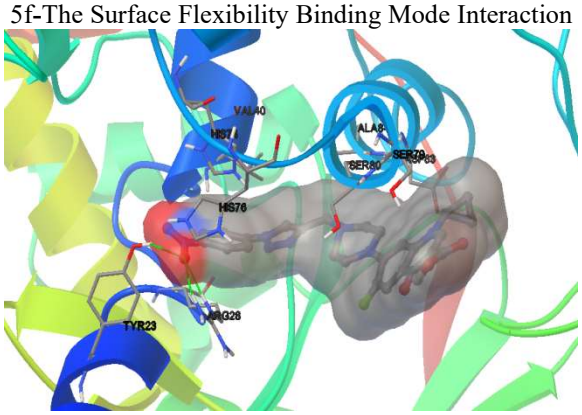

5f-The Side Chain Binding Mode Interaction



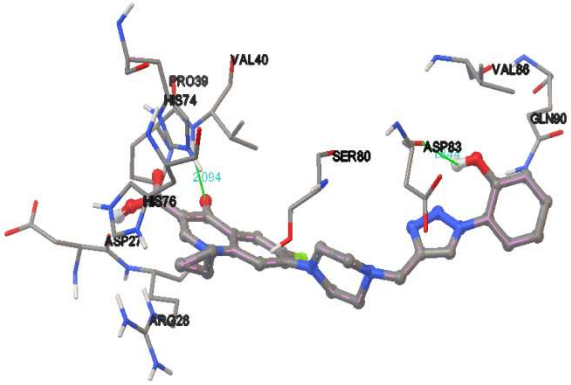

5f-The Surface Flexibility Binding Mode Interaction



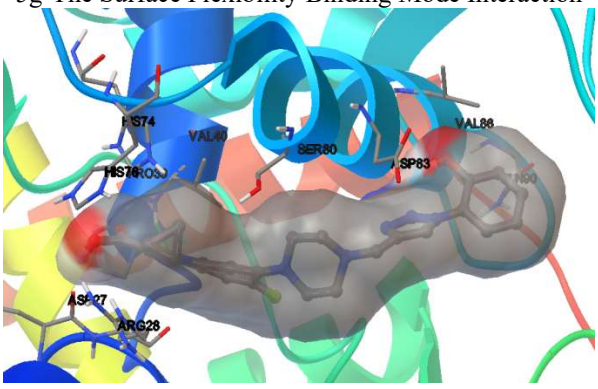

5g-The Side Chain Binding Mode Interaction




5g-The Surface Flexibility Binding Mode Interaction




5h-The Side Chain Binding Mode Interaction




5h-The Surface Flexibility Binding Mode Interaction

Fig.-1: Docking Pose Interactions of Target Compounds with the Active Site of Topoisomerase-IV of 4KPE

**Molinspiration Calculations**

As most of the designed compounds of the present study showed higher antimicrobial activity and some of them showed comparable antibacterial properties compared to oral bioavailability was considered to play an important role in the development of bioactive molecules as therapeutic agents. Therefore, a computational study for prediction of ADME properties of the molecules was performed by determination of lipophilicity, topological polar surface area (TPSA), absorption (% ABS) and simple molecular descriptors used by Lipinski in formulating his ‘‘Rule of Five’’. All the calculations [number of hydrogen bond acceptors, number of hydrogen bond donors, number of rotatable bonds] were performed by using Molinspiration online toolkit (http://www.molinspiration.com) (Table-3). Zhao and co-authors,[26] have been calculated the percentage of absorption (% ABS) by using the formula 109-0.345 X TPSA and TPSA calculations by using the fragment-based method.[27] Whereas polar surface area, lipophilicity are the important properties of a molecule in transport across biological membranes and too high TPSA values give to poor bioavailability and absorption of a drug.

Table-3: Predicted ADME, Lipinski Parameters and Molecular Properties of the Synthesized Compounds 6a-j

| Entry | %ABS | TPSA | n-ROTB | n-ON Acceptors | n-OHNH Donors | milog P | n Violations | Volume | Drug Likeness Score |
|---|---|---|---|---|---|---|---|---|---|
| 5a | 60.01 | 142.32 | 7 | 12 | 1 | 0.88 | 2 | 439.62 | 1.32 |
| 5b | 60.01 | 142.32 | 7 | 12 | 1 | 0.85 | 2 | 439.62 | 1.46 |
| 5c | 72.52 | 105.73 | 7 | 10 | 1 | 0.79 | 1 | 441.83 | 1.35 |
| 5d | 69.33 | 114.97 | 8 | 11 | 1 | 0.98 | 2 | 467.38 | 0.92 |
| 5e | 75.70 | 96.50 | 6 | 9 | 1 | 1.18 | 0 | 432.85 | 1.38 |
| 5f | 75.70 | 96.50 | 6 | 9 | 1 | 1.41 | 1 | 429.82 | 1.73 |
| 5g | 60.01 | 142.32 | 7 | 12 | 1 | 0.69 | 2 | 439.62 | 1.29 |
| 5h | 58.72 | 116.73 | 6 | 10 | 2 | 0.25 | 0 | 424.31 | 1.64 |
| 5i | 75.70 | 96.50 | 6 | 9 | 1 | 1.57 | 1 | 429.82 | 1.76 |
| CPF | 83.12 | 74.57 | 3 | 6 | 2 | -0.70 | 0 | 285.46 | 0.84 |

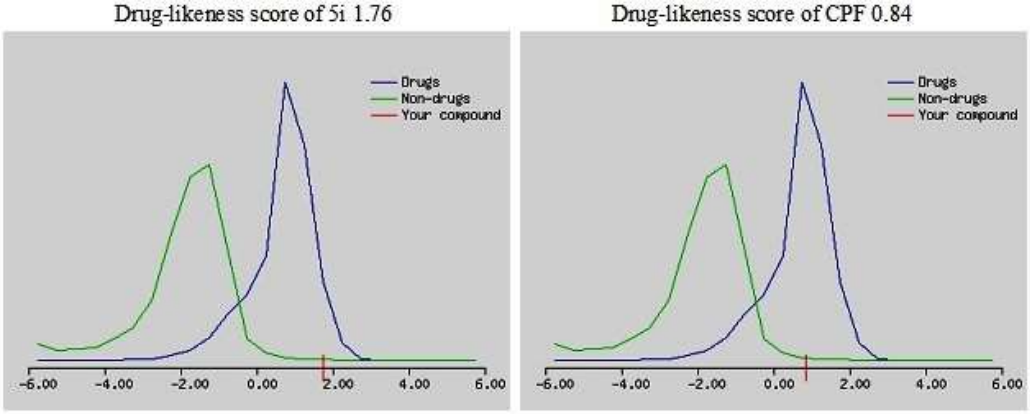



Fig.-2: Maximum Drug Like-ness Scores of 5i Compared to CPF

## CONCLUSION

A new series of norfloxacin–1H–1,2,3–triazole hybrids 5a-i were synthesized and characterized by using $^{1}$H NMR, $^{13}$C NMR, Mass spectral techniques. A new series of triazole derivatives were screened for their antibacterial activity against five bacterial strains, targets 5d, 5c, 5e and 5g, exhibited an excellent broad spectrum of activity against all Gram–(+) strains and Gram–(–) strains with an excellent zone of inhibition (mm). A computational study for the prediction of interactions like cation-Π, hydrogen bonding, Π-Π, Vanderwall forces, between our target compounds with Topoisomerase –IV of 4KPE. Targets 5g and 5f show highest hydrogen bonding 2.23 A˚, 2.15 A˚ and binding energy ΔG= –7.26, –6.70 kcal/mol with amino acids interactions Arg28 and Ala115. The designed product 5a-i shows suitable drug-like properties

and is expected to present a good bioavailability profile. Finally, we concluded that this call of 1,2,3–triazole derivatives linked to fluoro quinolones is an interesting profile for further experimental research, especially in the area of antibacterial exploration.

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