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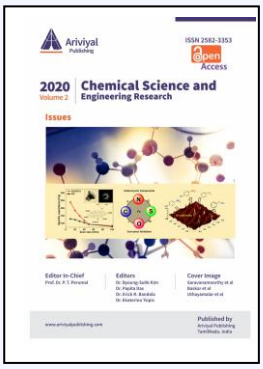

# Synthesis, Antimicrobial Evaluation and Molecular Docking Studies of Tetrazole Containing Hybrid Levofloxacin Derivatives

T. Elavarasan,*[a] D.Bhakiaraj,[a,b] R. Jagatheesan,[c] M. Mathavan,[d] R. Sampath Kumar[d] and M. Gopalakrishnan[a]

[a]*Synthetic Organic Chemistry Laboratory, Department of Chemistry, Annamalai University, Annamalainagar 608 002, Tamil nadu, India.*

[b]*Department of Chemistry, St.Joseph's College of Arts and Science, Cuddalore, Tamil nadu, India.*

[c]*Department of Chemistry, Vivekanandha College of Arts and Sciences for Women (Autonomous), Elayampalayam, Namakkal, Tamil nadu, India.*

[d]*Centre for Bioinformatics, School of Life Sciences, Pondicherry University, India.*

***Corresponding author E-mail address**: elavarasan@gmail.com (T. Elavarasan)

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**Abstract:** Synthesis of five-membered aromatic heterocyclic compounds and its derivatives is one of the most significant transformations in synthetic organic chemistry because of its much importance in pharmaceutical industry. Therefore, a greener and convenient experimental method is reported to synthesis some novel derivatives of tetrazole containing hybrid levofloxacin at ambient temperature using acetonitrile. This method results in excellent yields of tetrazole containing hybrid levofloxacin derivatives. Screening studies such as biological activities and spectral analysis are carried out and reported.

**Keywords:** Tetrazoles; Levofloxacin; Antibacterial; Fluroquinolone

## 1. Introduction

Tetrazole is one of the important five-membered aromatic heterocyclic compounds, with poly-nitrogen electron-rich planar structural features and bioactive functionality with numerous applications in medicinal chemistry.[1] This unique structure allows tetrazole derivatives to readily bind with various enzymes or receptors in organisms and thus displaying a broad spectrum of biological activities, which in turn have a considerable role in the pharmaceutical chemistry.[2,3] Studies in the field of medicinal chemistry related to tetrazoles are outnumbered only by investigations of imidazoles, but the number of papers and reviews devoted to tetrazoles is growing more quickly.[4] The World Health Organization declared that, the tetrazole ring as an important core structure in the methodology of the design of new drugs using the analogue based drug discovery (ABDD) method. So far nearly 20 biological activities of tetrazoles are studied and reported.[5-13]

The fluorine atom is an important element in drug design because of its electronic, lipophilic and steric parameters as high electronegativity, very low polarizability, hydrogen-bonding, solubility, relatively small size and steric effects. These important properties of fluorine can modulate both pharmacodynamic and pharmacokinetic effects of drugs with impact on biological activity.[14] A quinolone antibiotic in any member of a large group of broad-spectrum bactericides that share a bicyclic core structure related to the compound 4-quinolone.[15] Quinolones are one of the most essential synthetic drug classes used for the treatment of community or hospital acquired infectious diseases in view of their excellent antibacterial activity with minimum side effects (Fig. 1). Quinolones and fluoroquinolones are a relatively new class of synthetic antibiotics with potent bactericidal, broad-spectrum activity against many clinically important pathogens which are responsible for variety of infections including urinary tract infections (UTIs), respiratory tract infections (RTI), gastrointestinal infections, sexually transmitted diseases (STD) and skin infections. Structure–activity relationship (SAR) studies of quinolones revealed that modification at the C-7 position with an additional functional moiety was reported to be greatly influence their antibacterial potency, spectrum and safety.[16,17] Thus, several hybrid quinolone analogues derived by modification at C-7 position showed stronger antitumor[18]

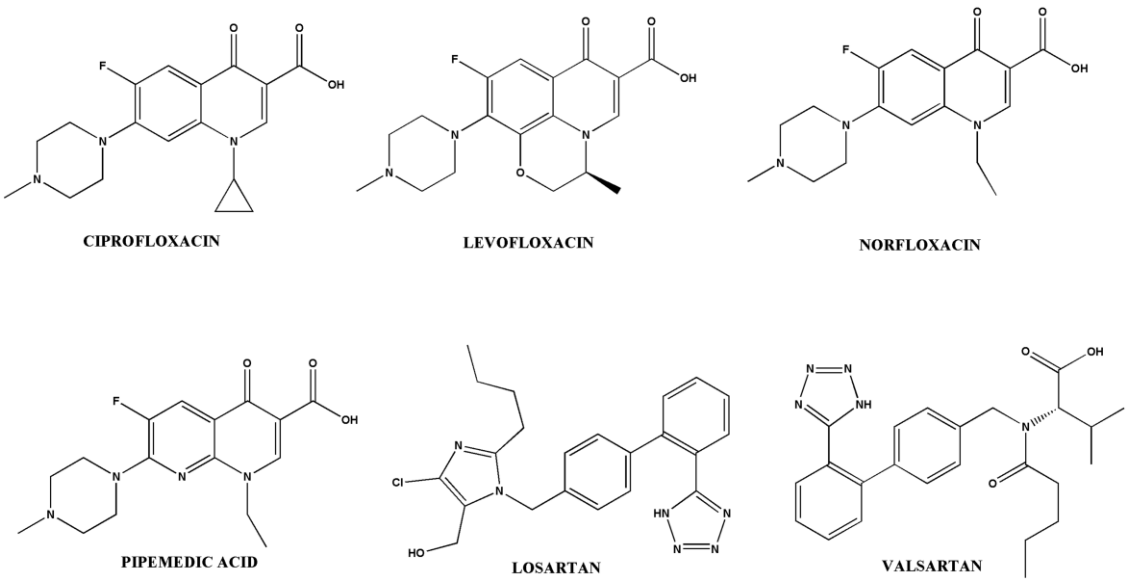



**Fig. 1.** Structure based quinolone and tetrazole containing antimicrobial drugs.

antibacterial[19] activity as well as increased lipophilicity compared with the parent quinolones. Above SAR studies concluded that modification of basic group of the C-7 position leads to enhancement of its antibacterial potency as well as antitumor potency.

Levofloxacin is used for the treatment of urinary tract infections, abdominal infections and pneumonia. As of 2007, the Infectious Disease Society of America (IDSA) and the American Thoracic Society (ATS) recommended levofloxacin and other respiratory fluoroquinolones as first line treatment for community acquired pneumonia when co-morbidities such as heart, lung, or liver disease are present or when in-patient treatment is required.[20] The American Urological Association (AUA) recommends levofloxacin as a first-line treatment to prevent bacterial prostatitis when the prostate is biopsied[21] and as of 2004 it was recommended to treat bacterial prostatitis by the NIH research network studying the condition.[22] The fluroquinolone derivatives have good antibacterial activity and tetrazole derivatives have good antifungal activity.

In view of the above considerations and as an extension of our continuous research work,[23] the quinolone was combined with tetrazoles to form a hybrid structure possessing improved biological activity. Hence, the present work describes the synthesis of a new series of tetrazole containing hybrid levofloxacin derivatives (7a–7g) and their biological screening studies.

## 2. Experimental Section

*2.1. General Experimental Procedure*

Seven novel derivatives of tetrazoles, containing hybrid levofloxacin (7a-7g) were synthesized by mixing 1-(1-aryl-1H-tetrazol-5-yl)-2-(piperazin-1-yl)ethanone and 10-chloro-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (6) in acetoniltrile at room temperature (Scheme-1). Precipitation occurred after quenching the reaction with addition of ice water. The precipitates were filtered, washed with water, and dried under vacuum. $^{1}$H, $^{13}$C NMR and mass spectrometry was used to deduce

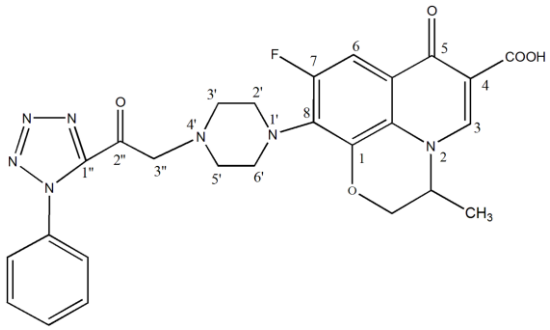


**Fig. 2.** Structure of the compound **7a.**

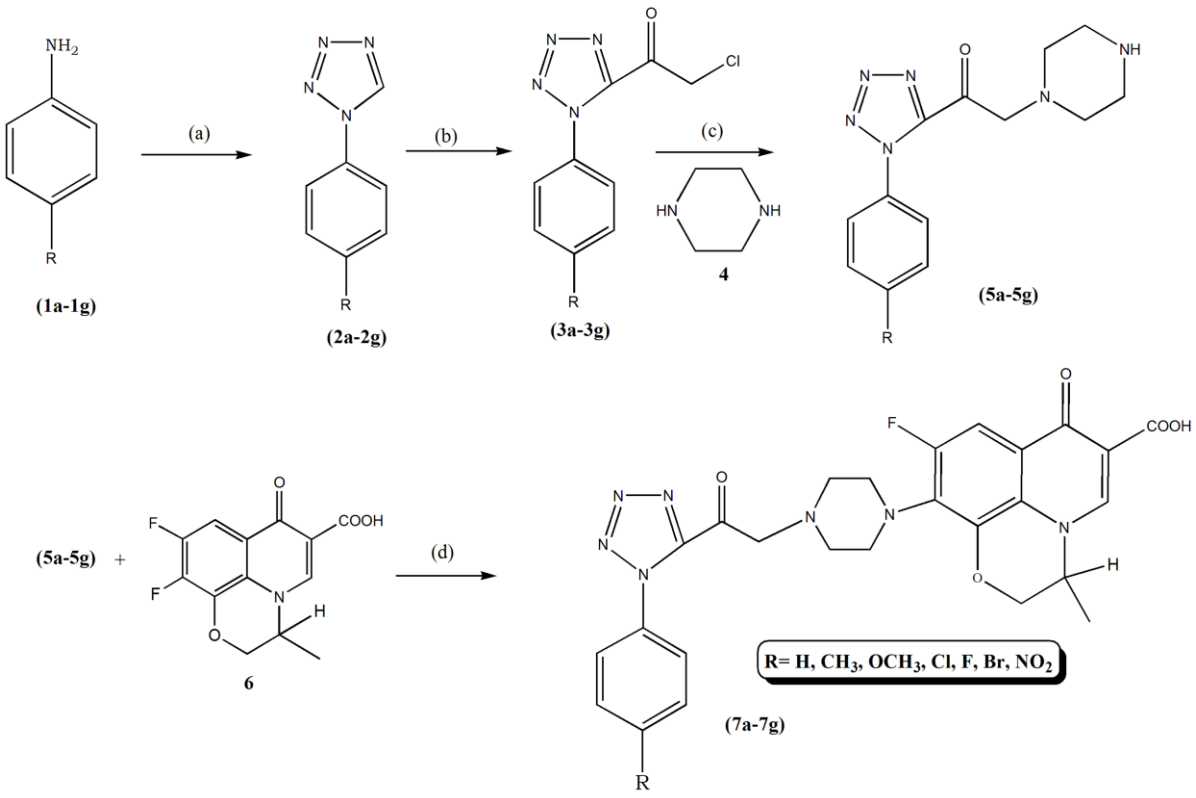
the structures of the desired products. All the synthesized compounds showed satisfactory NMR data. Synthesis procedure and spectral conformations (FT-IR and NMR) of 2a-2g, 3a-3g and 5a-5g are provided in the Supporting Information.

9-fluoro-3,7-dihydro-3-methyl-7-oxo-10-(4-(2-oxo-2-(1-phenyl-1H-tetrazol-5-yl)ethyl)piperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylicacid (**7a**)

White solid; mp: 122-126 °C; IR (KBr): 1553, 1702, 1746, 2815-2989, 3045, 3426 cm$^{-1}$; $^{1}$H NMR (400 MHz, DMSO-d$_6$) δ: 1.45 (s, 3H), 2.77 (s, 4H) 3.19 (s, 4H), 4.47-4.50 (d, 2H, J=12 Hz), 4.67-4.69 (d, 2H, J= 8Hz), 5.00-5.01 (d, 1H), 7.06 -7.51 (m, 5H), 7.79-7.83 (t, 1H, J= 12 Hz), 8.14 (s, 1H), 10.40 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d$_6$): δ17.75, 44.53, 52.99, 54.94, 60.69, 68.84, 103.43, 147.01, 165.63,172.45, 176.34; MS (m/z): 533.18 (M$^{+}$). Anal.Calcd.for $C_{26}H_{24}FN_7O_5$ (%): C, 58.53; H, 4.53; N, 18.38; Found (%): C, 58.40; H, 4.48; N, 18.27.

9-fluoro-3,7-dihydro-3-methyl-7-oxo-10-(4-(2-oxo-2-(1-p-tolyl-1H-tetrazol-5-yl)ethyl)piperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij] quinoline-6-carboxylic acid (**7b**)

White solid; mp: 250-254 °C; IR (KBr): 1552, 1718, 1688, 2853-2963, 3120, 3415 cm$^{-1}$; $^{1}$H NMR (400 MHz, DMSO-d$_6$) δ: 1.47 (s, 3H),2.25 (s, 3H) 2.47 (s, 4H) 2.78 (s, 4H), 3.17 (s, 2H), 4.69-4.70 (d, 2H), 4.99-5.00 (d, 1H), 7.38 -7.40 (d, 2H, J = 8 Hz),7.78 -7.80 (d, 2H, J = 8 Hz),8.0 (s, 1H), 10.30 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d$_6$): δ17.73, 21.32, 46.40, 49.46, 54.96, 62.00, 68.64, 103.42, 119.60-129.31,




**Scheme 1.** Synthetic route for the novel hybrid compounds containing tetrazole and levofloxacin moiety (7a-7e)

147.00, 165.60, 172.42, 176.40, MS (m/z): 547.2 ($M^+$). Anal.Calcd.for $C_{27}H_{26}FN_7O_5$ (%): C, 59.23; H, 4.79; N, 17.91; Found (%): C, 59.79; H, 4.68; N, 17.83.

9-fluoro-3,7-dihydro-10-(4-(2-(1-(4-methoxyphenyl)-1H-tetrazol-5-yl)-2-oxoethyl)piperazin-1-yl)-3-methyl-7-oxo-2H-[1,4] oxazino[2,3,4-ij]quinoline-6-carboxylic acid (**7c**)

Pale yellow solid; mp: 268-272°C; IR (KBr): 1552, 1776, 1720, 2860-2952, 3036, 3430 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ: 1.36 (s, 3H),2.39 (s, 4H) 2.68 (s, 4H), 3.20 (s, 2H), 3.61 (s, 3H), 4.48-4.49 (s, 1H), 6.77-6.79 (d, 2H, J = 8 Hz), 7.88 -7.90 (d, 2H, J = 8 Hz), 7.65-7.70 (t, 1H), 8.23 (s. 1H), 10.13 (s, 1H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): δ17.72, 45.15, 53.33, 54.85, 55.13, 61.24, 68.84, 103.40, 114.01-121.34, 146.86, 165.71, 172.39, 176.19; MS (m/z): 563.19($M^+$). Anal.Calcd.for $C_{27}H_{26}FN_7O_6$ (%): C, 57.55; H, 4.65; N, 17.40; Found (%): C, 57.46; H, 4.58; N, 17.32

10-(4-(2-(1-(4-chlorophenyl)-1H-tetrazol-5-yl)-2-oxoethyl)piperazin-1-yl)-9-fluoro-3,7-di -hydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (**7d**)

White solid; mp: 224-226 °C; IR (KBr): 1558, 1633, 1715, 2860-2952, 3096, 3488 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ: 1.97 (s, 3H), 2.43 (s, 4H) 2.60 (s, 4H), 3.89 (s, 2H), 4.49 (s, 2H), 5.05 (s, 1H), 7.67-7.69 (d, 2H, J = 8 Hz), 7.88 -7.90 (d, 2H, J = 8 Hz), 7.26-7.28 (d, 1H), 7.37-7.39 (d. 1H), 10.05 (s, 1H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): δ18.13, 44.53, 52.99, 54.13, 61.69, 65.25, 118.3-129.70, 142.25, 165.25, 173.70, 176.13; MS (m/z): 567.14 ($M^+$). Anal.Calcd.for $C_{26}H_{23}FN_7O_5$ (%): C, 54.98; H, 4.08; N, 17.26; Found (%): C, 54.89; H, 3.98; N, 17.11.

9-fluoro-10-(4-(2-(1-(4-fluorophenyl)-1H-tetrazol-5-yl)-2-oxoethyl)piperazin-1-yl)-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (**7e**)

White solid; mp: 236-238 °C; IR (KBr): 1557,1728, 2853-2963, 3090, 3415 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ: 1.35 (s, 3H), 2.44 (s, 4H) 2.64 (s, 4H), 3.09 (s, 2H), 4.50-4.63 (d, 2H), 5.89 (s, 1H), 7.44 (s, 2H), 7.73 (s, 2H), 7.78 (s, 1H), 8.01 (d. 1H), 10.37 (s, 1H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): δ 15.27,45.44, 53.77, 61.39, 69.82, 112.29, 119.59-131.68, 146.92, 164.76, 172.52, 177.71; MS (m/z): 565.53 ($M^+$). Anal.Calcd.for $C_{26}H_{23}F_2N_7O_5$ (%): C, 56.62; H, 4.20; N, 17.78; Found (%): C, 56.52; H, 4.11; N, 17.70.

10-(4-(2-(1-(4-bromophenyl)-1H-tetrazol-5-yl)-2-oxoethyl)piperazin-1-yl)-9-fluoro-3,7-dihydro-3-methyl-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (**7f**)

Colourless solid; mp: 244-246 °C; IR (KBr): 1557,1702, 1746, 2860-2952, 3090, 3448 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ: 1.45-1.47 (d, 3H), 2.75 (s, 8H) 3.07-3.12 (d, 2H), 4.67-4.69 (d, 2H, J= 8 Hz), 5.00-5.02 (d, 1H), 7.36 (s, 2H), 7.49 (s, 2H), 7.80-7.83 (t, 1H), 8.24 (s. 1H), 10.43 (s, 1H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): δ 17.74, 42.88, 48.83, 54.98, 59.83, 68.85, 103.42, 121.36-131.68, 147.04, 165.57, 172.15, 176.43; MS (m/z): 611.09 ($M^+$). Anal.Calcd.for $C_{26}H_{23}BrFN_7O_5$ (%): C, 50.99; H, 3.79; N, 16.01; Found (%): C, 50.88; H, 3.69; N, 15.93.

**Table 1:** Antibacterial activity of tetrazole containing hybrid levofloxacin derivatives (7a-7g) against *Bacillus subtilis*

| Compound Name | Area of inhibition zone (mm) | | | | |
|---|---|---|---|---|---|
| | *C | 50 µl | 100 µl | 150 µl | 200 µl |
| 7a | 10 | - | 10 | 10 | 10 |
| 7b | 10 | - | 10 | 11 | 11 |
| 7c | 10 | 10 | 11 | 11 | 12 |
| 7d | 10 | 12 | 13 | 14 | 16 |
| 7e | 10 | 13 | 15 | 18 | 20 |
| 7f | 10 | - | 10 | 11 | 12 |
| 7g | 10 | 12 | 13 | 15 | 18 |

9-fluoro-3,7-dihydro-3-methyl-10-(4-(2-(1-(4-nitrophenyl)-1H-tetrazol-5-yl)-2-oxoethyl) piperazin-1-yl)-7-oxo-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (**7g**)

Yellow solid; mp: 244-246 °C; IR (KBr): 1561,1649, 1714, 2857-2945, 3079, 3443 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ: 1.82 (d, 3H), 2.33 (s, 4H), 2.49 (s, 4H), 4.66-4.69 (d, 4H), 4.99-5.01 (d, 1H), 7.40-7.42 (s, 2H, J=8 Hz), 7.81-7.83 (d, 2H, J=8 Hz), 8.24-8.26 (d, 1H, J=8 Hz), 8.26-8.27 (s, 1H),10.67 (s, 1H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): δ20.18, 43.88, 48.33, 54.98, 58.53, 66.85, 115.71, 122.67-131.64, 142.86, 168.00, 168.34, 187.48; MS (m/z): 578.17 ($M^{+}$). Anal.Calcd.for $C_{26}H_{23}FN_8O_7$ (%): C, 53.98; H, 4.01; N, 19.37; Found (%): C, 53.89; H, 3.93; N, 18.42.

## 3. Results and Discussions

The synthesized compound 9-fluoro-3,7-dihydro-3-methyl-7-oxo-10-(4-(2-oxo-2-(1-phenyl-1H-tetrazol-5-yl)ethyl)–piperazin-1-yl)-2H [1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (7a) Fig. 2, take as a reference compound for the discuss of their analysis of IR, $^{1}H$, $^{13}C$ and HSQC NMR and Mass spectral values.

In the IR spectrum of compound 7a, the absorptions observed in the range of 3229-3045 $cm^{-1}$ are due to aromatic C-H stretching frequency. A strong absorption observed at 1702 $cm^{-1}$ is due to C=O stretching frequency. The absorption observed at 1746 $cm^{-1}$ is due to C=O stretching frequency of carboxylic acid group. The absorptions observed in the range of 2939-2815 $cm^{-1}$ are due to aliphatic C-H stretching frequency. A strong absorption observed at 1553$cm^{-1}$ is due to C=N stretching frequency. The absorption observed in the range of 1479 $cm^{-1}$ is due to C-N stretching frequency. The presence of C=O stretching frequency of carboxylic acid group indicates the formation of the quinoline carboxylic acid group. The mass spectrum of compound 7a shows the molecular ion peak at m/z 534 (M+H+.) by the addition of one proton which is consistent with the proposed molecular mass (533) of the compound.

In the $^{1}H$-NMR spectrum of compound, 9-fluoro-3,7-dihydro-3-methyl-7-oxo-10-(4-(2-oxo-2-(1-phenyl-1H-tetrazol-5 yl)ethyl)piperazin-1-yl)-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid 7a, the methyl protons H3 appeared at 1.46-1.47 ppm. The piperazine H3' and H5' protons are appeared as a sharp singlet at 2.77 ppm. The methylene H3'' protons are appeared at 3.19 ppm. The methylene proton H2 and methine proton H3 are appeared at 4.47-4.50 and 5.00-5.01 ppm respectively. The piperazine H2' and H6' protons are merged with DMSO solvent peak at 2.50 ppm. The aromatic protons are appeared in the range of 7.07-7.51 ppm. The H8 proton appeared as a doublet at 7.81-7.83 ppm and the H5 proton appeared at 8.14 ppm. The hydroxyl proton of the carboxylic acid group appeared at 10.44 ppm.

In the $^{13}C$-NMR spectrum of compound (7a), the methyl group signal of C3 carbon appeared at 17.75 ppm. The piperazine group methylene carbons C2', C6' are appeared at 44.53 ppm. Methylene carbons C2 and C3'' are appeared at 68.84 and 60.69 ppm respectively. The methine carbons C3, C5 and C8 are appeared at 54.94, 103.43 and 147.01 ppm respectively. The aromatic carbons are appeared at 119.58-128.94 ppm. Aromatic ring ipso carbon is appeared at 137.46ppm. The tetrazole ipso carbon appeared at 150.24 ppm. The carbonyl group signals of C2'' and C7 carbons appeared at 176.34 and 172.45 ppm respectively. The carbonyl carbon of carboxylic acid group attached at C6 position appeared at 165.63 ppm.

For the further confirmation of the structures, compound 7e, considered as a representative compound and HSQC spectrum was recorded. In the HSQC spectrum of Compound 7e, it is seen that the carbon signals at 176.34, 172.45 and 165.63 ppm have no correlation with any of the proton signals and hence they due to the carbonyl groups at the C2'', C7 and C6 carbons respectively. The proton signalsat 1.46-1.47 ppm have correlation with the carbon signal at 17.75 ppm which reveals that, the carbon signal at 17.75 ppm is due to the methyl carbon attached at C3 position and the proton signals at 1.46-1.47 ppm are due to the methyl protons at H3. The proton signals at 2.71 ppm have correlation with the carbons signal at 44.53 ppm which reveals that, the carbon signal at 44.53 ppm is due to the C2' and C6' carbon of the piperazine ring and the proton signals at 2.71 ppm are due to the H2' and H6'protons attached at the methylene protons of the piperazine ring. The carbon signal at 52.99 ppm which is merged with DMSO solvent signals, correlates with the proton signal at 2.50 ppm, this observation clearly shows that, the carbon signal at 52.99 ppm due to C3' and C5' carbons of the piperazine ring. The carbon resonance at 60.69 ppm correlates with the proton signal at 3.19 ppm and this correlation confirms that the carbon signal at 60.69 ppm is due to C3'' carbon and the proton signal at 3.19 ppm is due to H3''proton of the ethanone moiety. The proton signals at 7.06-7.51 ppm correlates with the carbon signals at 119.58-128.94 ppm which reveals that the carbon resonance at 119.58-128.94 ppm is due to the aromatic carbons of the phenyl ring. The carbon resonance at 103.42-103.62 ppm correlates with the proton signal at 7.79-7.83 ppm and this correlation confirms that the carbon signal at 103.42-103.62 ppm is due to the methane carbon attached at C5 and the proton signal at 7.79-7.83 ppm is due to the H5 proton. The proton signal at 8.14 ppm have correlation with the carbon signal at 147.01 ppm, which reveals that the carbon signal at 147.01 ppm is due to the C8 carbon and the proton signals at 8.14

ppm are due to the methane protons H8. The $^{13}C$ resonances at 150.24 ppm have no correlation with any of the protons, and from this observation it is concluded that the carbon signal at 150.24 ppm is due to the ipso carbon of the tetrazole ring. From the above spectral analysis, the structure of the representative parent compound is confirmed.

*3.1. Antimicrobial studies*

The pharmacological importance of various heterocyclic compounds paved the way toward active research in synthetic and medicinal chemistry. In such away, all the synthesized compounds were screened for their antimicrobial activities against various microbial strains. Compounds with variety of substitutions were synthesized and screened for activity to analyze and improve the activity. The synthesized compounds (7a-7g) were screened for their antimicrobial activities. All the synthesized compounds were screened for their antibacterial activities by disc diffusion method. For the bacterial strain, *Bacillius subtilis* is used and for the fungal strai, *candida albicans* is used to test the activities. All the newly synthesized compounds show good antimicrobial activity against both *Bacillus subtilis* and *candida albicans*.

*3.2. Antibacterial assay*

The antibacterial activity of the compounds was carried out by determining the zone of inhibition using disc diffusion method. Sterile Muller Hinton agar plates were prepared. Then 0.1 ml of test organism was taken from the stock (broth) and swabbed on the agar medium in aseptic condition. The filter paper disc of 2 mm diameter (Whatman's No.1 Filter paper) were prepared and sterilized. The synthesized compound extracts (7a-7g) to be tested were prepared with various concentration viz., 50 µl/ml, 100 µl/ml, 150 µl/ml and 200 µl/ml and were added to each disc of holding capacity of 10 microlitres. The sterile impregnated disc with synthesized compound extracts (7a-7g) were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Positive control disc of Streptomycin were prepared and placed on the agar surface. All the plates were incubated at 37°C for 24 hours.[24] After incubation, the area of inhibition zoneJETBT1 0 01.s17( )] TJETBT1 0 0 1 36 615926 Tm[(te)4(ds)3( u5(re)-5(s)5(.)] TJETBT1 0 0 1

**Table 3:** Molecular docking results of the target molecules with crystal structure of YmaH from *Bacillus subtilis* (PDB ID: **3HSB**)

| Compounds | Docking score | Glide Emodel score | Hydrogen Bond interactions | Pi-Pi stacking | Hydrophobic interactions |
|---|---|---|---|---|---|
| **7a** | -5.179 | -73.984 | A:Ser-60, F:Asn-27, F:Lys-50 | A:Phe-24, F:Phe-29 | A:Ala-63, A:Pro-64, F:Leu-25, F:Leu-31 |
| **7b** | -6.843 | -81.233 | A:Ser-60, F:Asn-27 | A:Phe-24, F:Phe-29 | A:Val-21, A:Leu-25, A:Ala-63, A:Pro-64, F:Leu-25, F:Leu-31 |
| **7c** | -7.019 | -69.242 | A:Thr-22, A:Ser-60, F:Asn-27 | A:Phe-24 | A:Tyr-20, A:Val-21, A:Ala-63, A:Pro-64, F:Leu-25, F:Phe-29, F:Leu-31 |
| **7d** | -6.280 | -67.953 | A:Ser-60, F:Asn-27 | A:Phe-24, F:Phe-29 | A:Ala-63, A:Pro-64, F:Leu-25, F:Leu-31 |
| **7e** | -4.560 | -59.152 | A:Arg-32, A:Gln-52 | B:Phe-24 | A:Tyr-20, A:Leu-25, A:Phe-29, A:Leu-31, B:Ala-63 |
| **7f** | -7.289 | -76.674 | A:Ser-60, F:Asn-27 | A:Phe-24, F:Phe-29 | A:Val-21, A:Leu-25, A:Ala-63, A:Pro-64, F:Leu-25, F:Leu-31 |
| **7g** | -4.560 | -59.152 | A:Arg-32, A:Gln-52 | B:Phe-24 | A:Tyr-20, A:Leu-25, A:Phe-29, A:Leu-31, B:Ala-63 |

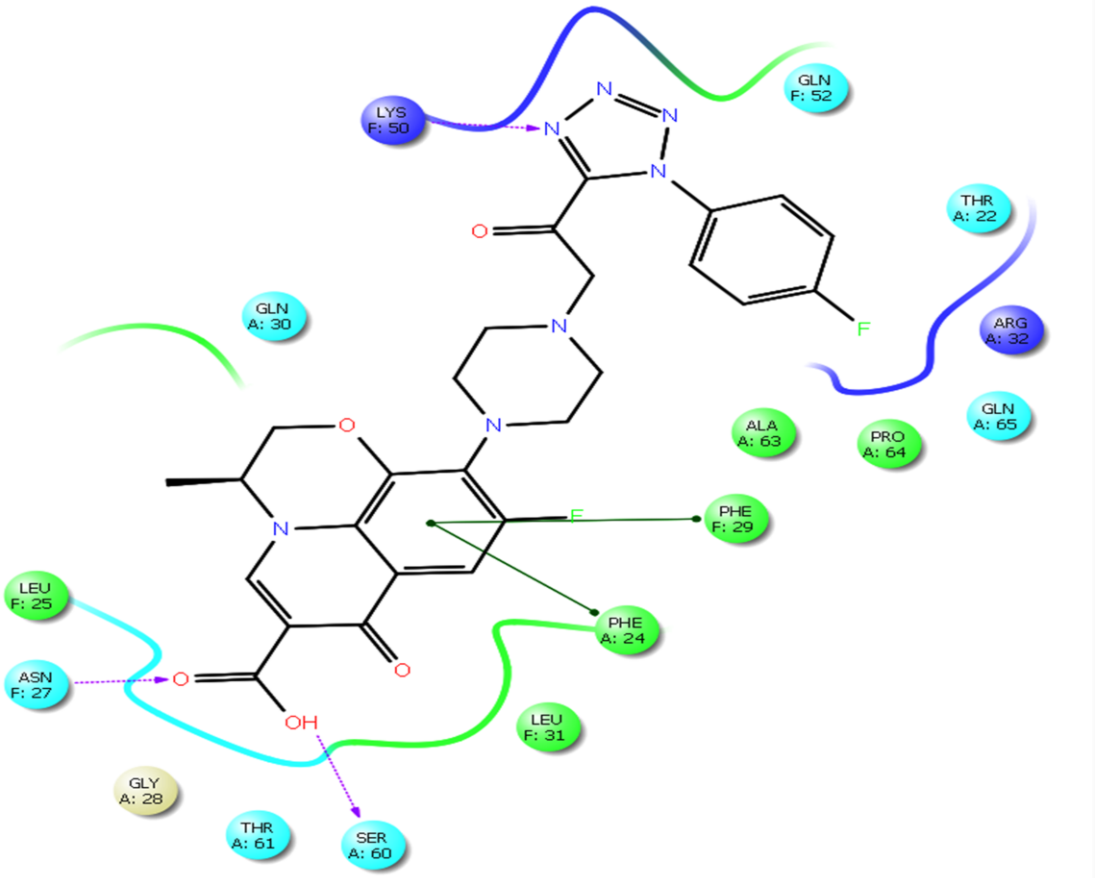



**Fig. 3.** Docking model of the compound 7e with crystal structure of YmaH (3B60)

the observed antimicrobial activity of the seven newly synthesized compounds.

Protein Preparation Wizard of Schrodinger Maestro software version 9.2; (Schrodinger LLC, New York, NY, 2011) was used to prepare mLST8 protein which assigned proper bond orders, added hydrogens, deleted water molecules beyond 5 Å and minimized the structure using Optimized Potentials for Liquid Simulations (OPLS2005) force field. Lig Prep version 2.5 (Schrodinger LLC, New York, NY) tool was used to prepare the ligands which generated all possible states with correct geometries at target pH of 7.0 and Epik version 2.2 (Schrodinger, LLC, New York, NY) was used to generate ionized and tautomeric states of each ligand. The molecular docking tool, Glide (v5.7, Schrodinger, LLC, New York, NY, 2011) was used to generate a grid around the binding site residues of mLST8 with a scaling factor of 1.0 Å and partial charge cut off set to 0.25. The binding site residues of mLST8protein were predicted using CASTp server (http://sts.bioe.uic.edu/castp/calculation.php), which measured the volume and area of all the available surface pockets and works based on the alpha shape and pocket algorithm.[25-26] The prepared protein and ligands were then processed for performing molecular docking using Glide Extra Precision (XP) module[27-28] to identify most favourable interactions. The top scoring ligands were identified on the basis of G score, which is a cumulative score of various interaction energies as shown in Equation (1),

G Score = 0:065×vdW+0:130 ×Cou l+ Lipo + Hbond + Metal + BuryP + RotB + Site (1)

**Table 4:** Molecular docking results of the target molecules with *Dihydrofolate Reductase* from *Candida albicans* (PDB ID: **1AI9**).

| Compounds | Docking score | Glide Emodel score | Hydrogen Bond interactions | Pi-Pi stacking | Hydrophobic interactions |
|---|---|---|---|---|---|
| **7a** | -6.444 | -74.442 | Arg-72 | Phe-36 | Ile-9, Val-10, Ala-11,, Ile-19, Met-25, Ile-33, Ile-62, Pro-63, Phe-66, Leu-69, Pro-70, Ile-112, |
| **7b** | -6.990 | -84.136 | Arg-72 | - | Ile-9, Val-10, Ala-11,Ile-19, Met-25, Ile-33, Phe-36, Ile-62, Pro-63, Phe-66, Leu-69, Pro-70, Ile-112, Ala-115, Tyr-119 |
| **7c** | -6.453 | -80.616 | Arg-72 | Phe-36 | Val-10, Ala-11,Ile-19,Tyr-21, Met-25, Ile-33, Ile-62, Leu-69,Pro-70, Ile-112, Ala-115, Tyr-118 |
| **7d** | -6.404 | -81.357 | Ala-11, Arg-72 | -- | Val-10, Ala-11,Ile-19,Met-25, Ile-33, Phe-36, Ile-62, Pro-63, Phe-66, Leu-69, Pro-70,Ile-112, Ala-115, Tyr-118 |
| **7e** | -6.569 | -83.282 | Arg-72 | -- | Ile-9, Val-10, Ala-11,Ile-19, Met-25, Trp-27,Ile-33, Phe-36,Ile-62, Pro-63, Phe-66, Leu-69, Pro-70,Ile-112, Ala-115, Tyr-118 |
| **7f** | -6.902 | -79.467 | Ala-115, Arg-72 | Phe-36 | Val-10, Ala-11, Ile-19,Met-25, Trp-27, Ile-33, Ile-62,Leu-69,Pro-70,Ile-112, Tyr-118 |
| **7g** | -5.879 | -80.308 | Ala-11, Arg-72, Ala-115 | Phe-36 | Val-10, Ala-11,Ile-19, Met-25,Ile-33,Ile-62,Leu-69, Pro-70, Ile-112,Tyr-118 |

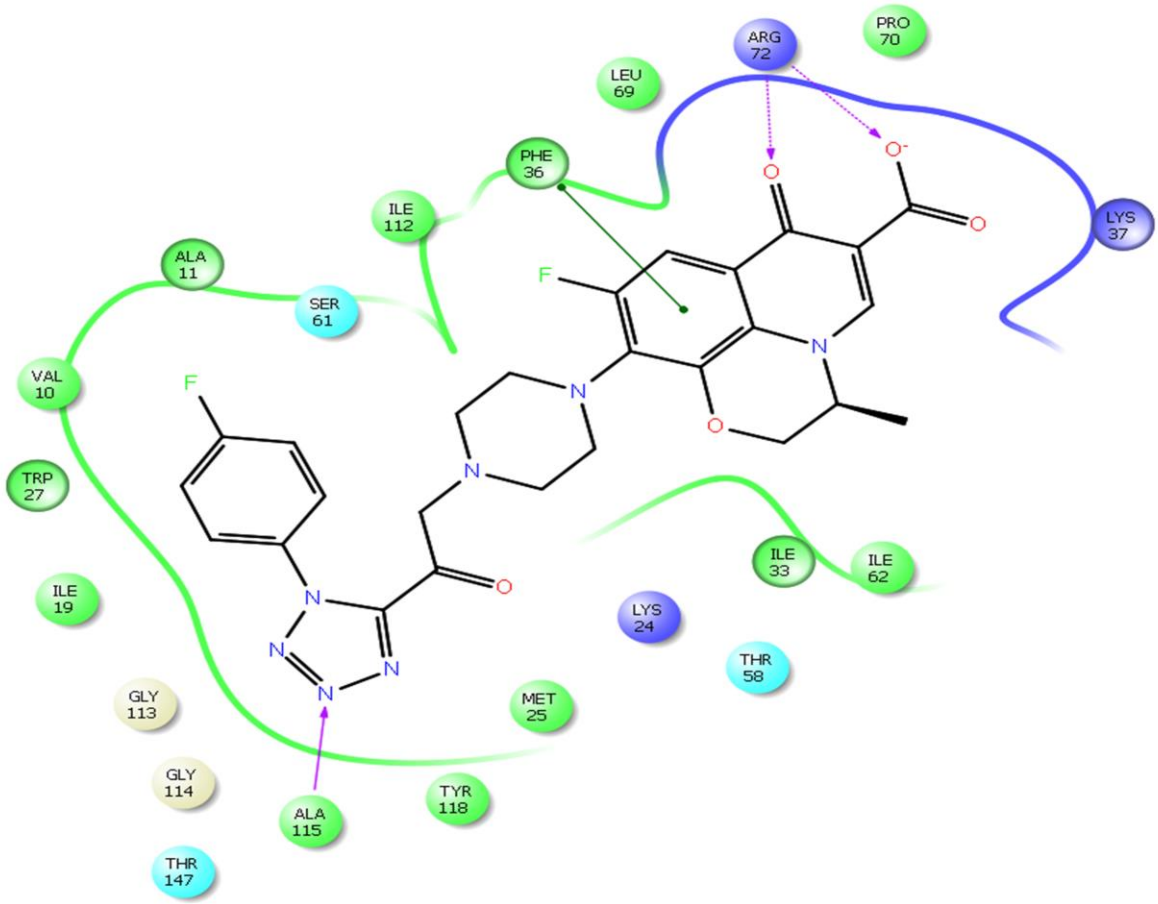



**Fig. 4.** Docking model of the compound 7e with DihydrofolateReductase (1AI9)

where vdW is van der Waals forces, Coul is Coulomb forces, Lipo is Lipophilic contact term, Hbond is Hydrogen bond, Metal is Metal binding term, BuryP is penalty for buried polar groups, RotB is penalty for freezing rotatable bonds and Site represents the polar interactions at the binding site. The intermolecular interactions were analyzed between the protein– ligand complexes using Discovery Studio Visualizer version3.1 (Accelrys, San Diego, CA)[29] and LigPlotþversion1.4 (European Bioinformatics Institute, London, England)[30] whereas visualization of protein–ligand complexes was accomplished using UCSF Chimera Tool version 1.11.2 (Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, CA).[31] Further, the approximate binding free energies between protein– ligand complexes were computed by Molecular Mechanics-Generalized Born model and Solvent Accessibility(MM-GBSA) method, using PRIME module of Schrodinger Prime version 3.0 (LLC, New York, NY, 2011).[32] The binding free energy was calculated using Equation (2),

$DG_{bind} = DE_{MM} + DG_{GB} + DG_{SA}$ (2)

Where $DG_{bind}$ is the free energy of protein–ligand complex, DEMM is the difference of energy calculated using OPLS2005 force field, $DG_{GB}$ is the difference of the polar desolvation energy calculated by the Generalized Born (GB) continuum model, and $DG_{SA}$ is the difference of the non-polar desolvation energy by the solvent accessible surface area (SASA).

Aromatic carbonyl functional groups of all the synthesized compounds (7a-7e) was found to be close to $Zn^{2+}$ atom in the active site, and established the hydrogen bond, which is the major interactions of the ligands. From the literature collections,[33,34] suitable protein for docking for *b. subtilis* and *c. albicans* is 3HSB and 1AI9 respectively. To understand the interaction of bacterial protein receptor with synthesized molecules (7a-7e) the crystal structure of YmaH from *B. subtilis* was downloaded from protein data bank (http://dx.doi.org/10.2210/pdb3HSB/pdb) and studied with the glide program. All the docking and the glide E model score for the synthesized compounds are shown in Table 3.

The use of docking and glide E model scores for ranking the different derivatives within a series is always not dependable. The molecular docking, antibacterial and antifungal study results show that, the glide scores and MIC values of the synthesized compounds do not have any correlation. The glide scores are mainly used to identify the active and inactive compounds. The docking results reveal that all the synthesized compounds inside 3HSB protein is showed good binding energy toward the target protein ranging from -7.28 to -4.56 kcal/mol. The docking results revealed that compounds 7b, 7c and 7e showed maximum docking score -6.84, -7.01, and -7.28 kcal/mol respectively are due to dipole-dipole and hydrogen bond interaction with amino acids of targeted protein. Therefore, it can be inferred that tetrazole containing hybrid levofloxacin derivatives 7c and 7e could be taken up further evaluation towards novel drug design against *Bacillus subtilis*.
The docking model of the compound 7e Crystal structure of YmaH is shown in Fig. 3.

The docking results reveal that all the synthesized compounds (7a-7e) showed binding energy toward the target protein 1AI9 for *c. albicans* (http://dx.doi.org/10.2210/pdb1AI9/pdb) and gets results ranging from -6.99 to -5.58 kcal/mol are shown in Table 4. Among the seven tetrazole containing hybrid levofloxacin derivatives the fluoro and methoxy substituted compounds have good docking score against targeted protein 1AI9. The docking results all the newly synthesized compounds (7a-7e) showed in Table 4. The flouro compound 7e hydrogen bond interactions with Ala 115 and Arg 72, hydrobhobic interactions with 11 proteins. The docking model for the compound 7e with *Dihydrofolate Reductase* (1AI9) is shown in Fig. 4. The above results show that, the docking studies have widened the scope of developing a new class of antimicrobial agents of tetrazole containing hybrid levofloxacin derivatives.

## 4. Conclusions

A series of 9-fluoro-3,7-dihydro-3-methyl-7-oxo-10-(4-(2-oxo-2-(1-aryl-1H-tetrazol-5-yl)ethyl)piperazin-1-yl)-2H-[1,4]oxazino[2,3,4ij]quinoline-6-carboxylicacid (7a-7e) derivatives were newly synthesized. Spectral analysis, antibacterial and antifungal activities are done for all the synthesized compounds. The synthesized compounds 7d, 7g and 7e are showing good antimicrobial activity against *Bacillus subtilis* and *candida albicans*. Docking studies results also support the experimental findings, that the compounds 7d, 7e and 7g are having potent antimicrobial activities.

## Supporting Information

Full experimental detail, $^{1}H$ and $^{13}C$ NMR spectra, IR and Supplemental data for this article can be accessed on the publisher's website.

## Conflicts of Interest

The authors declare no conflict of interest.

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