Quinolones have been a valuable addition to the array of antimicrobial agents that are used to treat human infections. Quinolones, however, are not limited to clinical applications. They are also widely used to treat and prevent veterinary diseases in animals intended for human consumption and commercially farmed fish such as salmon and catfish. Enrofloxacin, [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-ethyl-1-piperazinyl)-3-quinoline carboxylic acid], another member of the fluoroquinolones family, is administrated to cattle, pigs, chicken, turkeys, sheep, rabbets, and dogs to control bacterial infections caused by sensitive organisms\textsuperscript{21}.

For the treatment of Bacterial infection Fluoroquinolones have immersed as frontline therapy, however resistance of these drugs develops very easily. In order to overcome drug resistance newer derivatives of Fluoroquinolones are needed. Combination of two biological active moieties in one molecule might results in an overall enhanced the biological activity. Limited work has been seen in the literature for condensation products of fluoroquinolones; considering all these facts, in the present work we have synthesized several new derivatives of substituted Mannich bases by incorporating the various 1,2,4-Thiadiazoles with different fluoroquinolones and studied their antimicrobial activities as well as antifungal activity using the schemes given below.
Scheme 1

Step 1:- Preparation of 1,2,4-Thiadiazoles

\[
\text{R} = \text{H, CH}_3, \text{C}_2\text{H}_5, \text{OC}_2\text{H}_5, \text{etc.}
\]

Some more Fluoroquinolones will be taken

Step 2:-

R = H, CH₃, C₂H₅, OC₂H₅, etc.
General procedure for preparation of Mannich bases of fluoroquinolones: (Scheme 1)

**Step 1:** Aryl amine (0.5 mol) was taken in a 250-ml beaker containing 100 ml distilled water. Concentrated HCl (25 ml) was added and contents were warmed to dissolve the amine. Ammonium thiocynate (0.5 mol) was added to the amine solution and the mixture was heated. The mixture was poured on crushed ice, the precipitate thus obtained was filtered off by suction and recrystallised from ethanol. Spectral analysis, m.p. and elemental analysis confirm the formation of the corresponding thiourea(1a-1g).

3-amino-4-aryl-5-imino-1,2,4-thiadiazoline (2a-2g) were synthesized by warming corresponding aryl thiourea (1a-1g, 0.5 mol) in a conical flask and dissolving in warm 10 ml of HCl. Hydrogen peroxide (60-70 ml, 20 vol.) was added drop wise from the separating funnel with continuous stirring, the mixture was kept aside for 2 h. The oxidized mixture was diluted with water and separated sulphur was then removed by filtration. The filtrate on neutralization with dilute ammonia, gave precipitate which was collected by filtration and recrystallised from ethanol.

**Step 2:** The final compounds (cip 3a-3g) were synthesized warming (2a-2g, 0.5 mol) in a conical flask and dissolving in ethanol. Formaldehyde (5 mol) in glacial acetic acid was then added to this solution. Ciprofloxacin (0.5 mol) in ethanol was added with continuous stirring. The reaction mixture was stirred for 4 hrs. Precipitate obtained, was collected by filtration and recrystallised from ethanol. The purity of the final compound was confirmed by thin layer chromatography using silica gel glass plates and a solvent system of benzene, ethanol (9:1). The spots were developed in iodine chamber and visualized under ultra violet lamp.
Scheme 2

**Step 1:**

\[ \text{ArNH}_2 + \text{NH}_4\text{SCN} \rightarrow \text{NHCNH}_2 \text{Ar} \]

**Step 2:**

\[ \text{ArH}_2\text{C} - \text{C-OH} + \text{SOCl}_2 \rightarrow \text{ArH}_2\text{C} - \text{C-Cl} \]

**Step 3:**

\[ \text{RArNHCNH}_2 + \text{ArCH}_2\text{CCl} \rightarrow \text{RArNHCCH}_2\text{Ar} \]

Here:

\[ \text{Ar} = \text{Phenyl, Substituted Phenyl, etc.} \]
\[ \text{R} = \text{Methyl, Ethyl, etc.} \]
General procedure for preparation of Arylacetamides: (Scheme 2)

Step 1: 2-aminobenzothiazole (I) was synthesized by heating aniline 0.3 mol, and concentrated hydrochloric acid (25ml). 0.4 mol of saturated solution of ammonium thiocynate in water (30gm in 60ml water) was added slowly in above solution. The mixture was boiled until the solution got turbid. The solution was poured in ice water. The precipitate was filtered and recrystallized from ethanol. 0.1 mol of phenylthiourea in glacial acetic acid (75ml) was brominated by using bromine solution in glacial acetic acid (5%) till the orange yellow color appeared. The slurry was poured in cold water and make alkaline with 50% aq. Ammonium solution. The precipitate was filtered and washed with water, dried and recrystallized with ethanol. Spectral analysis, m.p. and elemental analysis confirm the formation of the corresponding 2-aminobenzothiazoles.

Step 2: Aryl acetyl chloride (II) was synthesized by heating aryl acetic acid 0.3 mol, and thionyl chloride 0.3 mol in benzene (50ml). The mixture was boiled until the solution got dried. The resultant mixture was triturated with ether in open air, until it gave yellow coloured Aryl acetyl chloride (II).

Step 3: Arylacetamide (III) was synthesized by dissolving 2-aminobenzothiazole (I) 0.1 mol and Aryl acetyl chloride (II) 0.1 mol in benzene (30ml). The reaction mixture was kept in a cool place for a day. The purity of the Arylacetamide (III) was confirmed by thin layer chromatography using silica gel glass plates and a solvent system of ethyl acetate, hexane at different concentrations (9:1, 1:1, 1:9). The spots were developed in iodine chamber and visualized under ultra violet lamp.
Scheme 3

\[ \text{CONHNH}_2 + \text{KOH} + \text{CS}_2 \xrightarrow{\text{Ethanol}} \text{CONHNHC} = \text{SK} \]

\[ \text{Hydrazine hydrate} \]

\[ \text{Diethyl ether} \]

\[ \text{Fluroquinolones} \]

\[ 3a-3e \]

\[ \text{2} \]
General procedure for preparation of Schiff Bases: (Scheme 3)

Synthesis of 4-amino-3–(4-pyridyl)-5-mercapto-4H-1,2,4-triazol (2)
Isonicotinic acid hydrazide 13.7 g (0.1 mol) was dissolved in 200 mL absolute alcohol containing potassium hydroxide 11.2 g (0.1 mol) at room temperature 12.5 mL carbondisulfide was added in parts and was stirred for 16 hours at room temperature. 100 mL of diethyl ether was added and stirred for further 3 h. 10.3 g (0.1 mol, 99%) hydrazine hydrate was added gradually to the potassium dithiocarbazinate salt dissolved in 100 mL water with stirring and was refluxed for 8 h during which hydrogen sulphide gas evolved and the colour of the reaction mixture changed to deep green. It was then cooled and acidified with hydrochloric acid to pH 1. The yellow colored solid was isolated by filtration and recrystallised from ethanol to give compound (2).

General procedure for Synthesis of 1-cyclopropyl-6-fluoro-4-(3-mercapto-5-(pyridin-4-yl)-1,2,4-triazol-4-ylimino)-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (3a-3e)
Few drops of glacial acetic acid were added to a solution of 0.01 mol of compound 2 (4-amino-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazol) in dimethyl formamide (20 mL) and 0.01 mol of various fluroquinolones derivatives was added and refluxed for 9 hours. The reaction mixture was cooled and the precipitate precipitate obtained was filtered, dried in vacuum and recrystallised from ethanol to give 1-cyclopropyl-6-fluoro-4-(3-mercapto-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-ylimino)-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (3a-3e).
All the synthesized compounds from Scheme 1, 2 & 3 were tested in-vitro along with parent drug. Ciprofloxacin, gatifloxacin and norfloxacin were used as reference drug for antibacterial activity whereas greseofulvin was used as a reference drug for antifungal activity.

Twofold serial dilutions of the compounds and reference were prepared in Mueller-Hinton agar (Hi-media, Mumbai). Drugs (10.0 mg) were dissolved in DMSO (1 ml) and the solution was diluted with water (9 ml). Further progressive double dilution with melted Mueller-Hinton agar was performed to obtain the required concentrations of 100, 50, 25, 12.5 and 6.25, μg./mL. The bacteria inocula were prepared by suspending overnight colonies from Mueller-Hinton agar media in 0.85% saline. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to approximately 0.5 McFarland standards (1.5 ×108 CFU/ml). The suspensions were then diluted in 0.85% saline to give 107 CFU/ml. Petri dishes were spot inoculated with 1 μl of each prepared bacterial suspension (104 CFU/spot) and incubated at 35-37°C for 18 hrs. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.