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DESIGN, SYNTHESIS, AND ANTIMICROBIAL EVALUATION OF NOVEL ISATIN–MOXIFLOXACIN-BASED 1,2,3-TRIAZOLE DERIVATIVES

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ABSTRACT

The increasing emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis* has created an urgent need for the development of new antimycobacterial agents with improved efficacy. In the present study, a series of six novel isatin–moxifloxacin-based 1H-1,2,3-triazole derivatives (6A–6F) were designed and synthesized by combining the pharmacologically important moxifloxacin and isatin scaffolds through a 1,2,3-triazole linker. The synthetic strategy involved three major steps: propargylation of moxifloxacin, preparation of substituted N-(2-azidoethyl)isatin intermediates, and copper-promoted azide–alkyne cycloaddition to afford the target conjugates. The synthesized compounds were characterized by physicochemical and spectroscopic techniques, including melting point determination, thin-layer chromatography (TLC), infrared spectroscopy (IR), and ^1H nuclear magnetic resonance (^1H NMR). The final derivatives were obtained in yields ranging from 53–72%, with melting points of 143–176 °C. The compounds were freely soluble in alcohol, soluble in acetone and dimethyl sulfoxide, and insoluble in water and benzene. Antifungal activity was evaluated by the agar diffusion method against *Candida albicans* and *Aspergillus niger*. Compound 6C showed the highest antifungal activity among the synthesized derivatives. Antitubercular activity was evaluated against *M. tuberculosis* H37Rv and clinical isolates resistant to streptomycin, isoniazid, rifampicin and ethambutol using the luciferase reporter phage assay. At 100 µg/mL, all six

derivatives produced more than 50% reduction in relative light units against H37Rv, with compounds 6A and 6C showing more than 50% reduction against the resistant clinical isolates. The findings indicate that linking isatin and moxifloxacin through a 1,2,3-triazole scaffold provides promising compounds for further investigation as antimicrobial and antimycobacterial agents.

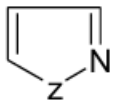
KEYWORDS: Isatin; Moxifloxacin; 1,2,3-Triazole; Antimicrobial activity; Antifungal activity; Antitubercular activity; *Mycobacterium tuberculosis*; MDR-TB; Medicinal chemistry.

1. INTRODUCTION

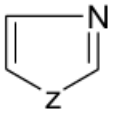
Tuberculosis (TB), caused predominantly by *Mycobacterium tuberculosis* (MTB), remains a major infectious disease and an important public-health challenge. The therapeutic management of TB has become increasingly complicated because of the emergence of drug-resistant strains, particularly multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB). MDR-TB is characterized by resistance to at least isoniazid and rifampicin, whereas XDR-TB additionally involves resistance to fluoroquinolones and other important second-line agents. The prolonged duration of conventional therapy and inadequate patient compliance further contribute to the development and transmission of drug-resistant disease. The dissertation emphasizes the continuing requirement for new antimycobacterial agents with improved efficacy and activity against resistant strains.

Imidazole, triazole, and thiazole antifungal

Azole antifungal drugs inhibit the enzyme lanosterol 14 α-demethylase; the enzyme necessary to convert lanosterol to ergosterol. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth. Imidazole: Bifonazole, Clotrimazole, Ketoconazole, Miconazole Triazoles: Albaconazole, Fluconazole, Itraconazole, Voriconazole Thiazoles: Abafungin Allylamines: Naftifine, Terbinafine Azoles: Azoles are five membered heterocyclic compounds with two or more heteroatom in which at least one is nitrogen. The neutral aromatic azoles system in general without exocyclic conjugation is:



Pyrazole	Z = NH
Isoxazole	Z = O
Isothiazole	Z = S



Imidazole	Z= NH
Oxazole	Z = O
Thiazole	Z= S

Properties of Azoles The carbon atoms of azole ring can be attacked by nucleophilic, electrophilic and/or free radicals. Some systems for examples the thiazole, imidazole and pyrazole nuclei show a high degree of aromatic sextet involved in a reaction such as isoxazole and oxazole nuclei are less aromatic and hence more prone to addition reaction.

Triazole

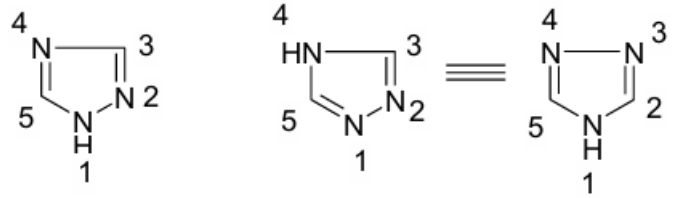
Five membered aromatic rings with three nitrogen atoms are called triazole. 1,2,4-triazole is also called as pyrrodiazole. The two possible combinations of the five atoms account for vicinal (v) and symmetrical(s) triazole. In chemical abstracts, v-triazole is also listed as 1H 1, 2, 3-triazole and 2H-1, 2, 3-triazole. The term osotriazoles refers to derivatives of 2H-1, 2, 3-triazole, particularly those prepared from osazones. s-triazole is also listed as 1H-1,2,4 triazole, 4H-1,2,4-triazole and 1,3,4–triazole. Each triazole exists in two dissimilar tautomeric forms. Replacement of the imino hydrogen atom by an alkyl or aryl group prevents tautomerism and thereby gives rise to the possibility of two 1-substituted osotriazoles. IR spectra shows stretching mode in the 1545—1335 cm-1 region (1545— 1535, 1470—1460, 1365—1335). The absorption maximum in the UV (measured in dioxane) is 205 nm (log s=0.2). The 1H NMR spectrum of 1,2,4- triazole (in HMPT) shows a CH-signal at δ = 8.17 and an NH-signal at δ =15.1. Triazole may be considered as a bio isostere of imidazole which is incorporated into the structures of many antimicrobial compounds.

The 1,2,4-triazole ring is a valuable heterocyclic pharmacophore and bioisosteric linker in medicinal chemistry. It can participate in hydrogen bonding and can influence the lipophilicity, polarity and hydrogen-bonding capacity of drug candidates. Previous studies cited in the dissertation have demonstrated the potential of triazole-containing compounds as antimicrobial and antitubercular agents.

Chemistry of 1,2,4 -Triazoles

1,2,4-Triazoles are cyclic hydrazidines with hydrogen atom (or substituent) on either

hydrazide nitrogen or amide nitrogen. Parent 1,2,4-triazole (1H-form) is in tauatomeric equilibrium with 1,3,4-triazole (4H-form). The interconversion of two tautomeric forms occurs rapidly and their separation is difficult, however 1,2,4-triazole tautomer is preferred over 1,3,4-triazole tautomer.



1H-1,2,4-triazole 4H-1,2,4-triazole 1H-1,3,4-triazlole

Stability of triazoles ring allows many of its derivatives to distil unchanged e.g. 1, 2, 3-triazole m.p. 204°C (740mm). Triazole halide explodes at temp above 260°C. 1-phenyl-4-methyl-5-hydroxy-1,2,3 triazole decomposes at 134°C and explodes at higher temp. Triazole carboxylic acids are unstable above 200°C. Many triazoles are water soluble and hygroscopic e.g. 4, 5- dimethyl 1,2,3- triazole forms at cryohydrate M.P. 97°C. The basicity is increases as alkyl groups are attached either to carbon or to nitrogen atoms of the ring. Acidity of 1,2,3-triazole is less than of tetrazole.

Mechanism of action of triazole

Triazoles act by inhibiting the fungal cytochrome P-450 enzyme lanosterol 14-demethylase and thus impair the biosynthesis of ergosterol for the cytoplasmic membrane and lead to the accumulation of 14-a-methylsterols. These methylsterols may disrupt the close packing of acyl chains of phospholipids, impairing the functions of certain membrane-bound enzyme systems such as ATPase and enzymes of the electron transport system and thus inhibiting growth of the fungi. The lower toxicity of triazoles compared to imidazole has been correlated with their lower affinity for mammalian Cytochrome P-450 and lesser propensity to inhibit mammalian sterol synthesis. However, because they are active against certain bacteria as well (which do not have ergosterol) other mechanism also appear to be involved.

Triazole inhibit fungal CytP-450 enzyme lanosterol 14-demethylase enzyme

↓

↓

Impair ergosterol biosynthesis

lead to the accumulation of 14-α-methylsterols

↓

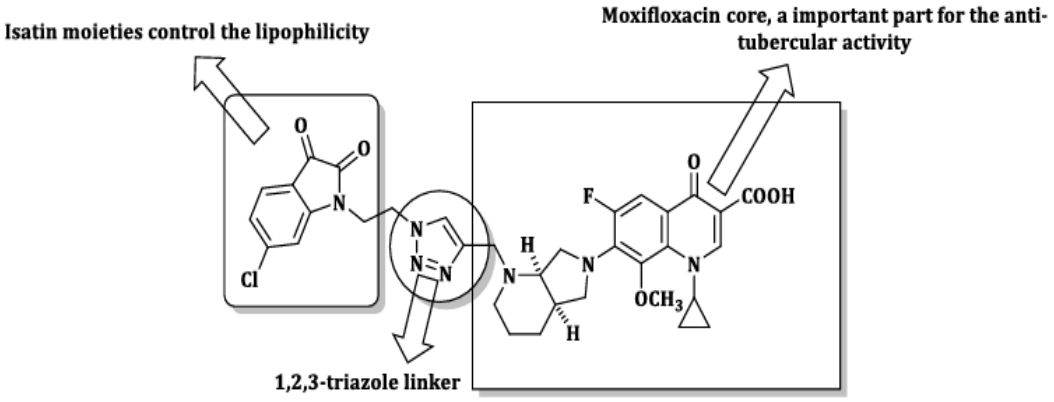
Disrupt the close packing of acyl chains of phospholipids

↓

↓

Impair the function of certain membrane-bound enzyme systems

Inhibit growth of the fungi



The rationale of the present work was therefore based on molecular hybridization of three pharmacologically relevant components: the fluoroquinolone moiety of moxifloxacin, the isatin nucleus and a 1,2,3-triazole linker. Based on this approach, six substituted isatin–moxifloxacin 1,2,3-triazole conjugates were designed, synthesized and evaluated for antimicrobial and antitubercular activity. The substituents introduced at the 6-position of the isatin nucleus were chlorine, bromine, nitro, methyl, methoxy and ethyl groups.

1.2 AIM AND OBJECTIVES

The primary aim of the study was to design and synthesize novel isatin–moxifloxacin-based 1,2,3-triazole derivatives and evaluate their antimicrobial potential.

The specific objectives were:

1. To design a series of substituted isatin–moxifloxacin 1,2,3-triazole hybrids.
2. To synthesize the target compounds using a multistep synthetic approach.
3. To purify and characterize the synthesized derivatives using physicochemical and spectroscopic techniques.
4. To determine the melting point, TLC profile and qualitative solubility of the synthesized compounds.
5. To evaluate antifungal activity against *Candida albicans* and *Aspergillus niger*.

6. To investigate antitubercular activity against *M. tuberculosis* H37Rv and resistant clinical isolates.
7. To identify promising derivatives for further antimycobacterial investigation.

2.1 MATERIAL AND METHODS

2.1.1. REAGENT AND SOLVENT

The drugs Moxifloxacin, Isoniazid and Rifampicin was procured from Taj Pharmaceutical Pvt. Ltd, Mumbai. The 3-bromopropyne, Potassium carbonate and Dimethyl formamide was purchased from CDH, New Delhi. The 6-chloroindoline-2,3-dione, 6-bromoindoline-2,3-dione, 6-nitroindoline-2,3-dione, 6-methyloindoline-2,3-dione, 6-methoxyindoline-2,3-dione, 6-ethylindoline-2,3-dione and Sodium azide was purchased from Sigma Aldrich, Delhi.

2.1.2. SYNTHESIS

Synthesis Scheme-I

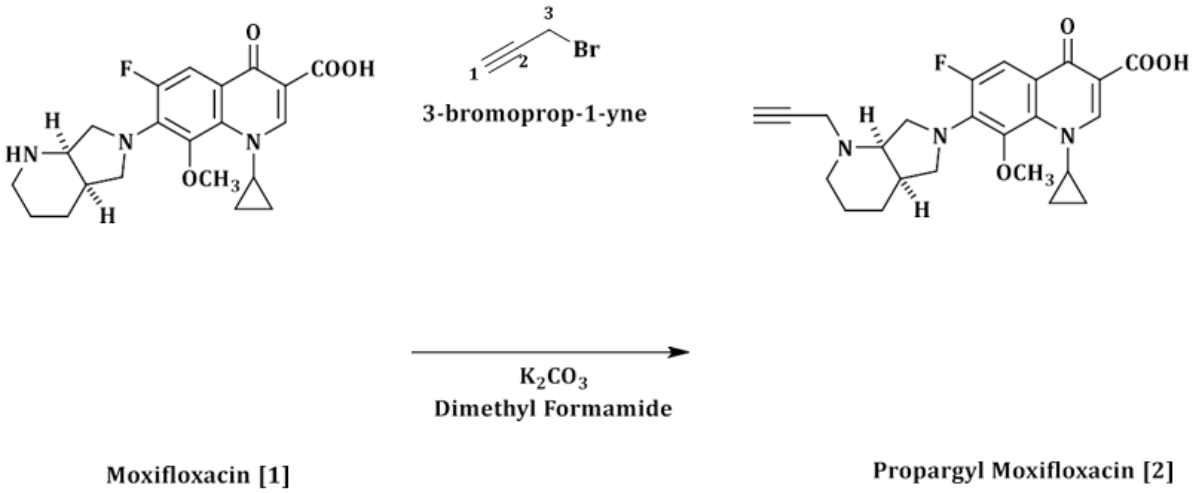


Table 4.1: List of the chemicals and their quantity used in Synthesis Scheme-I.

S. No.	Chemical	Mol. wt. (g/mol)	Quantity taken (g)
1.	Moxifloxacin	401.18	4.01
2.	3-bromopropyne	118.60	1.18
3.	Potassium carbonate	138.20	1.38
4.	Dimethyl Formamide	73.10	0.73

2.1.3. Procedure

The moxifloxacin (0.01 M) was reacted with 3-bromopropyne (0.01 M) in the presence of potassium carbonate solution in water and Dimethyl formamide (DMF). The solution was refluxed for 45 minutes at 60˚C. The resultant was poured to ice cold water to precipitate the resultant product and filter out the product. The synthesized compound was further washed

several times with distilled water.[56] The synthesized compounds propargyl moxifloxacin (Yield 72%) was recrystallized from ethanol and water (7: 3%v/v). The List of chemical and quantity used in synthesis scheme-I was represented in Table 1.

Synthesis Scheme-II

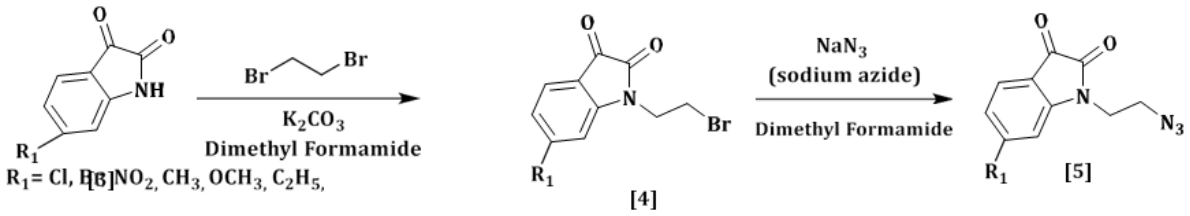


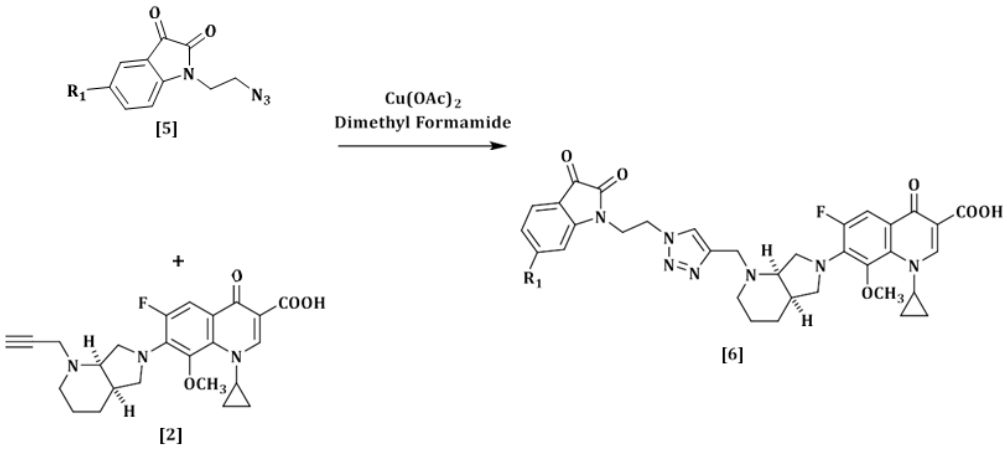
Table 4.1: List of the chemicals and their quantity used in Synthesis Scheme-II.

S. No.	Chemical	Mol. wt. (g/mol)	Quantity taken (g)
1.	6-chloroindoline-2,3-dione	181.58	1.81
2.	6-bromoindoline-2,3-dione	226.03	2.26
3.	6-nitroindoline-2,3-dione	192.13	1.92
4.	6-methyloindoline-2,3-dione	147.18	1.47
5.	6-methoxyindoline-2,3-dione	177.17	1.77
6.	6-ethylindoline-2,3-dione	175.19	1.75
7.	Potassium carbonate	138.20	1.38
8.	Dimethyl Formamide	73.10	0.73
9.	Sodium azide	65.01	0.65

Procedure

The substituted indoline-2,3-dione derivatives [Compound 3, yield 62%] was reacted with the 1,2-dibromo methane in the presence of potassium carbonate solution and dimethyl formamide to form 1-(2-bromoethyl)-6-substituted indoline-2,3-dione [Compound 4, yield 55%]. The reaction was carried out at 60˚C.[57] The result product was filter and washed with cold water. The compounds 4, was further reacted with sodium azide and dimethyl formamide at 60˚C to form compound 5, 1-(2-azidoethyl)-6-substitutedindoline-2,3-dione, yield 78%) was filtered out and washed with cold water. The List of chemical and quantity used in synthesis scheme-II was represented in Table 2

Synthesis Scheme-III

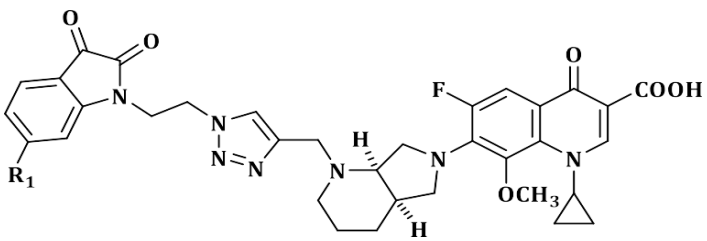


Procedure

To a mixture of N-(2-azidoethyl)-isatin (1-(2-azidoethyl)-6-substitutedindoline-2,3-dione) (0.01 M; 5) and propargyl moxifloxacin (0.01 M; 2) in Dimethyl formamide (50 ml), Cu(OAc)2 (10 mg) was added under nitrogen atmosphere. The 1,2,3-triazole derivatives 6A to 6F (yield: 53-72%) via Cu-promoted azide–alkyne cycloaddition reaction in the presence of Cu(OAc)2 in dimethyl formamide (DMF). The mixture was allowed to react for 48 hrs. at room temperature and after removal of the solvent, the residual product[6] was purified by silica gel column chromatography eluted with DCM: MeOH (10: 1). The List of chemical and quantity used in synthesis scheme-III was represented in Table 3.

Table 4.3: List of the chemicals and their quantity used in Synthesis Scheme-III.

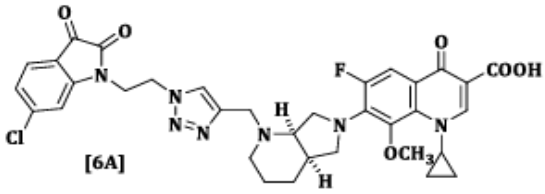
S. No.	Chemical	Mol. wt. (g/mol)	Quantity taken (g)
1.	Propargyl Moxifloxacin	439.49	4.39
2.	1-(2-azidoethyl)-6-chloroindoline-2,3-dione	250.64	2.50
3.	1-(2-azidoethyl)-6-bromoindoline-2,3-dione	295.10	2.95
4.	1-(2-azidoethyl)-6-nitroindoline-2,3-dione	261.20	2.61
5.	1-(2-azidoethyl)-6-methylindoline-2,3-dione	230.23	2.30
6.	1-(2-azidoethyl)-6-methoxyindoline-2,3-dione	246.23	2.46
7.	1-(2-azidoethyl)-6-ethylindoline-2,3-dione	244.25	2.44



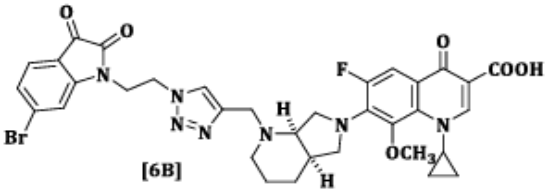
R1= Cl, Br, NO2, CH3, OCH3, C2H5

2.1.4. List of the synthesized compounds with chemical name

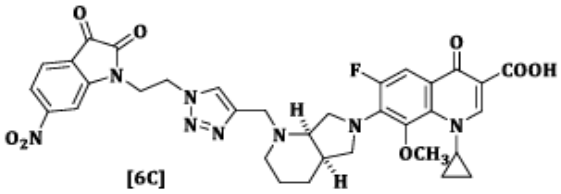
1. **6A:** 7-((4aR,7aR)-1-((1-(2-(6-chloro-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl) -1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
2. **6B:** 7-((4aR,7aR)-1-((1-(2-(6-bromo-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
3. **6C:** 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-((1-(2-(6-nitro-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
4. **6D:** 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-((1-(2-(6-methyl-2,3-dioxo indolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
5. **6E:** 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-((1-(2-(6-methoxy-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
6. **6F:** 1-cyclopropyl-7-((4aR,7aR)-1-((1-(2-(6-ethyl-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



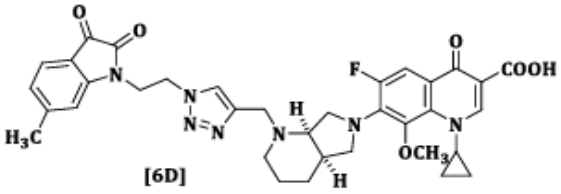
[6A]



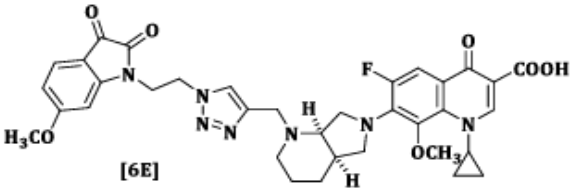
[6B]



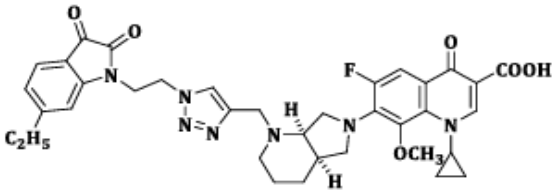
[6C]



[6D]



[6E]



[6F]

3.1. Structure Characterization

(a) Instrument and equipment

- **Melting point:** Melting points of the synthesized compounds were determined by open glass capillary method and were uncorrected.
- **Solubility:** The solubility studies of the synthesized compounds were performed in various solvents at room temperature qualitatively.
- **TLC:** Thin layer chromatography was performed on precoated silica gel-G glass plates using DCM and methanol, in ratio 10: 1 as mobile phase to ascertain the purity of the synthesized compounds. Iodine was used as detecting agent.

- **IR:** The infrared spectra of the compounds were recorded on Bruker alpha software.
- **^{1}H NMR:** ^{1}H NMR spectral analysis of the synthesized compounds was recorded on Bruker Avance III 500 MHz (AV 500) in deuterated DMSO using tetramethylsilane (TMS) as internal standard.

3.2. Physicochemical Characterization

Table 3.4: Physicochemical properties of the synthesized compounds.

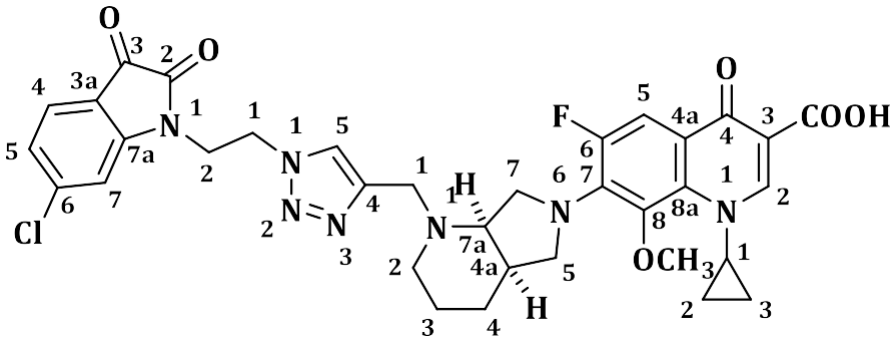
Comp.	% yield	Physical appearance		Melting point (˚C)	Rf value
		Color	State		
6A	72	Light yellow solid	Solid	170-172°C	0.62
6B	53	Yellow	Solid	143-145°C	0.58
6C	60	Deep Yellow	Solid	160-162°C	0.55
6D	65	Light Yellow	Solid	174-176°C	0.64
6E	68	Yellow	Solid	156-158°C	0.52
6F	58	Yellow	Solid	160-162°C	0.33

Table 3.5: Solubility studies of the synthesized compounds.

Compound	Water	Alcohol	Acetone	Glacial Acetic Acid	Benzene	Dimethyl Sulfoxide
6A	-	+++	+	++	-	++
6B	-	+++	+	++	-	++
6C	-	+++	+	++	-	++
6D	-	+++	+	++	-	++
6E	-	+++	+	++	-	++
6F	-	+++	+	++	-	++

-Insoluble; + = Slightly soluble; ++ = soluble; +++ = Freely soluble

3.3. Spectroscopic analysis of Compounds by IR and ^{1}HNMR Compound 6A



IUPAC name: 7-((4aR,7aR)-1-((1-(2-(6-chloro-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

Chemical Formula: $C_{34}H_{33}ClFN_7O_6$

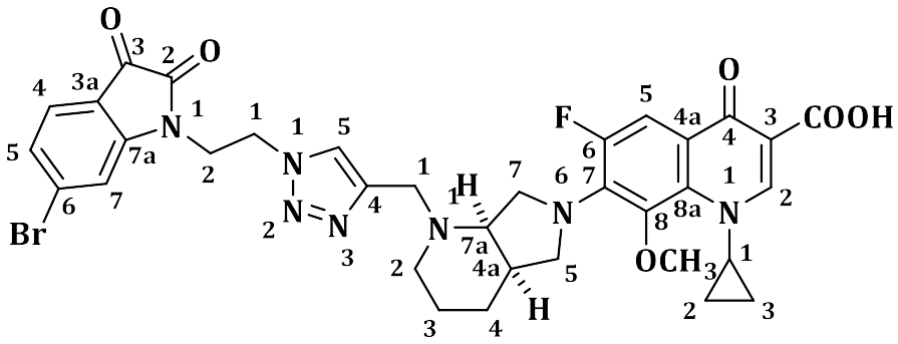
Molecular Weight: 690.13; m/z: 689.22 (100.0%)

Elemental Analysis: C (59.17%); H (4.82%); N (14.21%); O (13.91%)

Log P: 3.04

^{1}HNMR (400 MHz; DMSO-d6, ppm): 2.65–2.70 (m, 1H), 2.77–2.79 (m, 1H), 3.55–3.81 (m, 8H), 4.12–4.15 (m, 3H), 4.25 (s, 3H, NOCH3), 4.66–4.68 (m, 2H), 6.88 (d, 1H, Ar–H), 7.12 (d, 1H, Ar-H), 7.58–7.60 (m, 2H, Ar–H), 7.88 (1H, s, triazole-H), 8.64 (1H, s, C2–H), 15.20 (1H, brs, COOH).

Compound 6B



IUPAC NAME: 7-((4aR,7aR)-1-((1-(2-(6-bromo-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

Chemical Formula: $C_{34}H_{33}BrFN_7O_6$

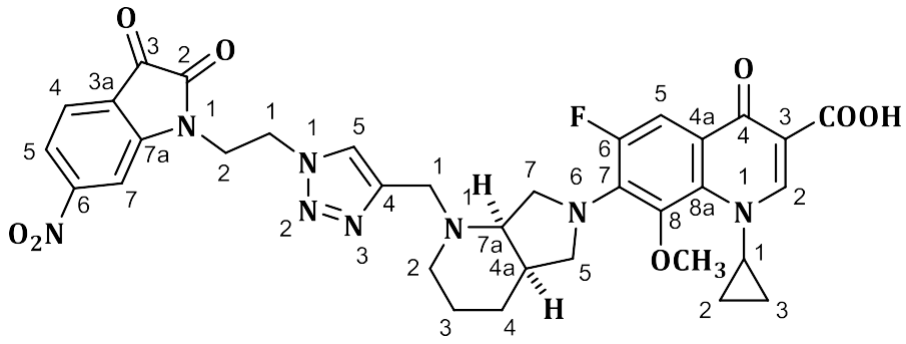
Molecular Weight: 734.58**; m/z: 733.17 (100.0%)**

Elemental Analysis: C (55.59%); H (4.53%); N (13.35%); O (13.07%)

Log P: 3.31

^{1}HNMR (400 MHz; DMSO-d6, ppm): δ 0.96–1.62 (m, 9H), 2.03–2.09 (m, 1H), 2.38–2.40 (m, 1H), 2.56–2.58 (m, 1H), 2.76–2.79 (m, 1H), 3.56–3.88 (m, 11H), 4.11–4.13 (m, 3H), 4.60–4.62 (m, 2H), 6.80 (d, 1H, Ar–H), 7.10 (d, 1H, Ar–H), 7.61–7.62 (m, 1H, Ar–H), 7.69 (d, 1H, Ar–H), 7.87 (1H, s, triazole-H), 8.66 (1H, s, C2-H), 13.52 (1H, brs, NOH), 15.29 (1H, brs, COOH).

COMPOUND 6C



IUPAC NAME: 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-((1-(2-(6-nitro-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

Chemical Formula: $C_{34}H_{33}FN_8O_8$

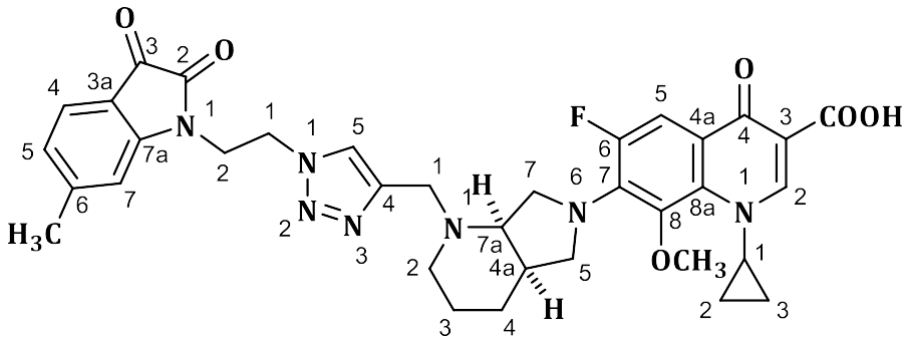
Molecular Weight: 700.68; m/z: 700.24 (100.0%)

Elemental Analysis: C (58.28%); H (4.75%); N (15.99%); O (18.27%)

Log P: 3.97

^{1}HNMR (400 MHz; DMSO-d6, ppm): δ 1.00–1.57 (m, 9H, 2.07–2.09 (m, 1H), 2.38–2.39 (m, 1H), 2.64–2.66 (m, 1H), 2.72–2.76 (m, 1H), 3.58–3.83 (m, 8H), 4.10–4.13 (m, 3H), 4.60–4.62 (m, 2H), 6.90 (s, 1H, Ar–H), 7.27 (s, 1H, Ar–H), 7.54 (d, 1H, Ar–H), 7.88 (1H, s, triazole-H), 8.64 (1H, s, C2–H), 8.68, 9.01 (s, 1H, CONH2), 12.17 (s, 1H, NNHCO), 15.21 (1H, brs, COOH).

COMPOUND 6D



IUPAC NAME: 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-((1-(2-(6-methyl-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

Chemical Formula: $C_{35}H_{36}FN_7O_6$

Molecular Weight: 669.71; m/z: 669.27 (100.0%)

Elemental Analysis: C (62.77%); H (5.42%); N (14.64%); O (14.33%)

Log P: 2.97

^{1}HNMR (400 MHz; DMSO-d6, ppm): δ1.03–1.55 (m, 9H), 2.04–2.06 (m, 1H), 2.36–2.39 (m, 17H), 2.51 (s, 3H, CH3); 2.66–2.68 (m, 1H), 2.75–2.78 (m, 1H), 3.55–3.81 (m, 11H), 4.11–4.14 (m, 3H), 4.61–4.62 (m, 2H), 6.88 (s, 1H, Ar–H), 7.34 (s, 1H, Ar–H), 7.65 (d, 1H, Ar–H), 7.83 (1H, s, triazole-H), 8.67 (1H, s, C2–H), 8.69, 9.00 (s, 1H, CONH2), 12.16 (s, 1H, NNHCO), 15.26 (1H, brs, COOH).

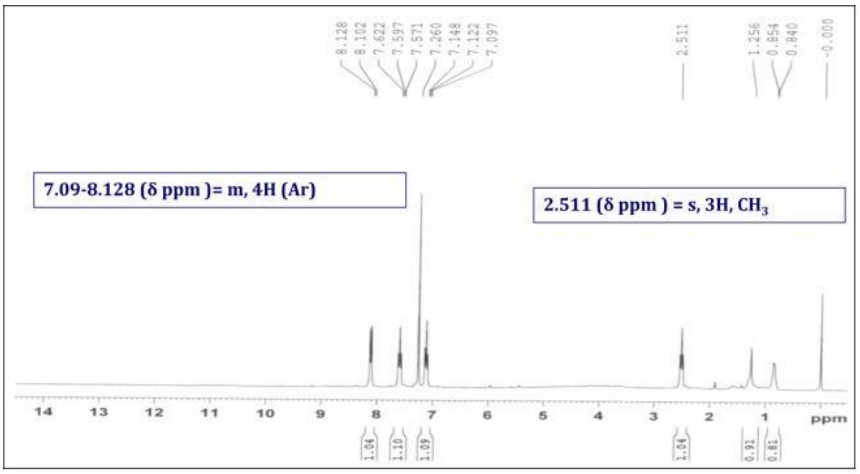
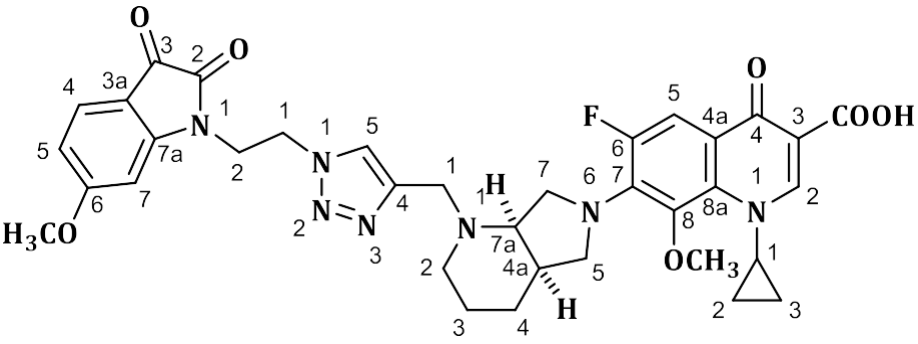


Figure 4.1: IR spectrum of compound 6D.

Compound 6E



IUPAC NAME: 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-((1-(2-(6-methoxy-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

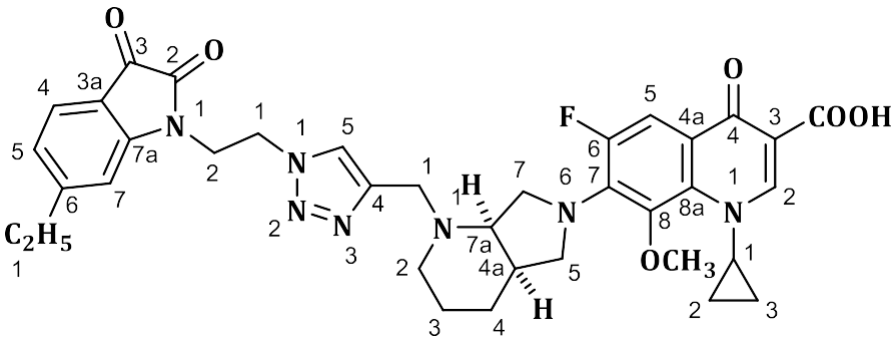
Chemical Formula: C35H36FN7O7

Molecular Weight: 685.71; **m/z: 685.27 (100.0%)**

Elemental Analysis: C (61.31%); H (5.29%); N (14.30%); O (16.33%)

[1]HNMR (400 MHz; DMSO-d6, ppm): δ 1.01–1.64 (m, 9H), 2.11–2.13 (m, 1H), 2.36–2.38 (m, 1H), 2.65–2.74 (m, 1H), 2.83–2.85 (m, 1H), 3.59–3.85 (m, 11H), 4.14–4.17 (m, 3H), 4.24 (s, 3H, NOCH3), 4.65–4.67

Compound 6F



IUPAC NAME: 1-cyclopropyl-7-((4aR,7aR)-1-((1-(2-(6-ethyl-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

Chemical Formula: C36H38FN7O6

Molecular Weight: 683.74; m/z: 683.29 (100.0%)

Elemental Analysis: C (63.24%); H (5.60%); N (14.34%); O (14.04%)

[1]HNMR (400 MHz; DMSO-d6, ppm): δ 0.95–1.57 (m, 9H), 2.06–2.08 (m, 1H), 2.31– 2.34 (m, 1H), 2.58–2.60 (m, 1H), 2.82–2.84 (m, 1H), 3.54–3.79 (m, 8H), 4.09–4.12 (m, 3H), 4.59–4.61 (m, 2H), 6.93 (d, 1H, Ar–H), 7.42–7.47 (m, 2H, Ar–H), 7.61 (d, 1H, Ar-H), 7.98 (1H, s, triazole-H), 8.65 (1H, s, C2-H), 15.26 (1H, brs, COOH).

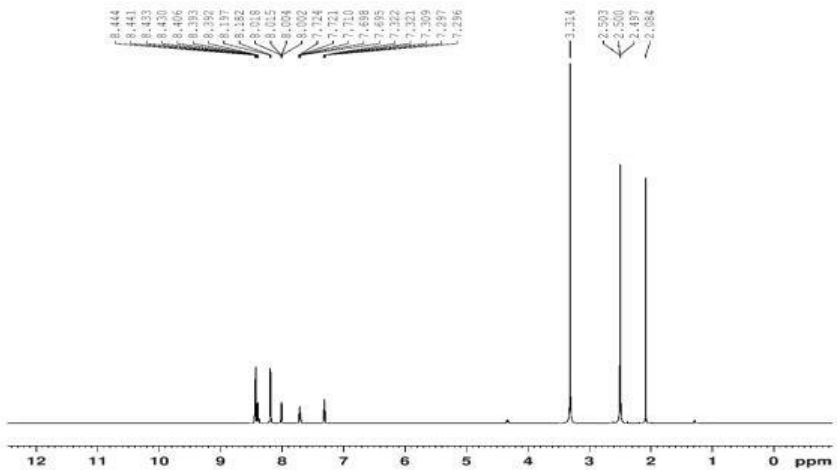


Figure 4.2: IR spectrum of compound 6F

3.4. DISCUSSION

The six new derivatives of 1,2,3-triazole derivatives has been synthesized and characterized by Proton Nuclear Resonance Spectrophotometer (^{1}HNMR). ^{1}HNMR spectrum of the synthesized compounds has shown the peaks at ppm δ 0.95–1.57 (m, 9H), 2.06–2.08 (m, 1H), 2.31–2.34 (m, 1H), 2.58–2.60 (m, 1H), 2.82–2.84 (m, 1H), 3.54–3.79 (m, 8H), 4.09–4.12 (m, 3H), 4.59–4.61 (m, 2H), 6.93 (d, 1H, Ar–H), 7.42–7.47 (m, 2H, Ar–H), 7.61 (d, 1H, Ar-H), 7.98 (1H, s, triazole-H), 8.65 (1H, s, C2-H), 15.26 (1H, brs, COOH). The melting point was in the ranges of 140 ˚C to 176˚C of Thin layer chromatography was performed for every individual process and has shown the Retention factor less than 0.7.

These all the characterized parameter depicted that compound were synthesized as per expected and confirmed by ^{1}HNMR analysis.

4.1 ANTIMICROBIAL EVALUATION

Microbiology is the study of microscopic organisms such as bacteria, viruses, fungi and protozoa. This discipline includes fundamental research on the biochemistry, physiology, cell biology, ecology, evolution and clinical aspects of microorganisms, including the host response to these agents. Bacterial resistance to existing drugs is a growing problem in the world. Considerable researches have been performed on the synthesis of new quinazolinone derivatives with potent antimicrobial activity. These derivatives possess antibacterial activities, especially against the gram-positive strains, and fungi through their interaction with the cell wall and DNA structure. The term chemotherapeutic agents were initially restricted to antibiotics, but now since microbial metabolites have been isolated for their antimicrobial activity. Hence both synthetic and microbiologically produced drugs need to be included together. However, it would be more meaningful to use the term Anti-microbial agent (AMA) to designate synthetic as well as naturally obtained drugs that attenuate microorganisms.

The antimicrobial drugs occupy uniqueness in the history of medicine. During the entire proceeding history of medicine, less than a handful of drugs had been submitted to systemic laboratory investigation. The exponential development in the antibacterial field is the inevitable result of the momentum created by sulfonamides and penicillin.[96] The term microbe is sometimes applied only to the unicellular microphytes. However, in its broader sense it includes multicellular microphytes and microbes as well therefore antimicrobial studies include antibacterial, antifungal, antiprotozoal and antimycotic studies. After the

development of desired new drug molecules with different structure an antimicrobial screening program is necessary to uncover the interesting activity of the compounds. The inhibition of the microbial growth under standardized condition may be utilized for demonstrating the therapeutic efficacy of the synthesized compounds.

Microorganisms inhabit various sites of the human body, including the skin, nose, mouth and digestive gut. Drugs which are helpful in combating the infectious disease caused by microbes are known as antimicrobial agent. Therefore, it was thought worthwhile to explore out the possibility of the compounds of the present series to be effective antimicrobial agent.

4.1.1 Antimicrobial Evaluation

The different methods were available for the evaluation of the antimicrobial activity but agar diffusion methods is widely accepted for the determination of Zone inhibition of fungal strains. The Zone inhibition is determined by agar diffusion method**.**

4.1.2 Agar diffusion method

This method gives the extent of growth of the microorganism, inoculated into a solid nutrient agar bed by the antimicrobial substance. The test substance is kept in a cup made of agar bed, diffuses into it and inhibits the growth of microorganism. The diameter of the Zone inhibition is measured in comparison with suitable drug substance, is considered as potency of that substance. The diameter of Zone inhibition is directly proportional to the concentration of the drug substances added into the cup, thickness of the agar bed, diffusion coefficient of the antimicrobial substance into the agar cup, sensitivity of the microorganism to the test substance and the temperature. The appropriate media is sterilized and cooled to 42°C, incubated with the test organism, mixed uniformly and poured into petri plates and cooled. Bores are made into it; specified test solution is added and left at room temperature for 30 min. Incubate at 37°C for 24 hrs. The Zone inhibition is measured in mm.

4.1.3. Antifungal screening of the Synthesized Compounds

For antifungal screening the following fungal species were used (Table 5.1).

Table 4.1: List of Fungal Strains.

S. No.	Fungal strain	MTCC* No.	Incubation Temperature (^{O}C)	Incubation period
1.	*Aspergillus Niger*	281	25	5 Day
2.	*Candida albicans*	227	25	2 Day

*Procured from IMTECH Chandigarh.

- ***Aspergillus Niger*** **(MTCC-281)**

It belongs to the class deuterromycota. *Aspergillus Niger* is a mould that is rarely reported as a cause of pneumonia]. Less thermotolerant, ideal temperature for growth is 30-34°C, making germination difficult in human body temperature of at least 37°C**.**

- ***Candida albicans*** **(MTCC-227)**

It is pathogenic yeast, which belongs to the same class as *Aspergillus niger*. It is responsible for the disease known as candidiasis that can affect skin, mucous membranes and nails.

4.2 EXPERIMENTAL

4.2.1 Preparation of Solution of Standard Drug

A stock solution of Fluconazole (1 mg/ml) was prepared in DMF and further diluted as reported for antibacterial studies.

4.2.2 Preparation of solution of the synthesized compounds

The solutions were prepared in the same way as mentioned under antibacterial screening. All those compounds screened earlier for antibacterial activity were also tested for their antifungal activity. The fungi employed for the screening were *Aspergillus Niger* and *Candida albicans*. Fluconazole (2-(2,4-difluorophenyl)-1,3-di(1H-1,2,4-triazol-1-yl)propan-2-ol) (Figure 5.1) was employed as standard to compare the results. The test organisms were sub-cultured using Potato-DextroseAgar (PDA) medium.

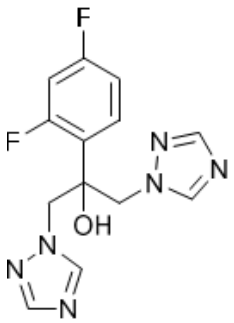


Figure 4.1: Chemical structure of Fluconazole.

The tubes containing sterilized medium were inoculated with test fungi and kept at room temperature for obtaining growth. After that, they were stored at 4ºC in a refrigerator. The activity of the derivatives was performed by Agar diffusion-based cup plate method at different concentration level. Fluconazole was used as standard drug at concentration remains

same. Fluconazole solution prepared at a concentration of 1000 g/ml in sterilized distilled water.

4.3 ANTITUBERCULAR ACTIVITY

In present work antitubercular activity was determined by Luciferase Reporter Phage (LRP) assay method.

4.3.1 LUCIFERASE REPORTER PHAGE (LRP) ASSAY

Luciferase reporter mycobacteriophages have the potential of greatly reducing the time needed for *M. tuberculosis* analysis in a simple and relatively inexpensive assay. The basis of assay is the construction of recombinant bacteriophage that carry a so called "reporter gene", whose activity can easily be monitored. Genes encoding a Luciferase enzyme such as that from the firefly, *Pnotinus pyralis* are ideal reporters, since the activity of gene product can be easily monitored by action of a substrate (luciferin) and measurement of emitted light in the presence of ATP. The availability of sensitive light detection systems makes possible the detection of small amounts of luciferase enzyme and consequently small numbers of bacterial cells. It is this potential for assaying small numbers of cells that is the basis of the phage assay, since it eliminates the need for extensive bacterial growth; moreover, light-producing organism are rarely found in clinical specimens and the phage particle contains only the luciferase gene and not the enzyme and thus gives no light in the absence of the host.

LPR assay is based on the observation that luciferase reporter phages will make *M. tuberculosis* cells glow. Light production requires that the phage DNA be injected and replicated and that the luciferase gee be expressed and has sufficient ATP present within the cell. Anything that interferes with any of these such as contact with drugs will prohibit light production. Compound screening can be performed by dividing the *M. tuberculosis* cells into aliquots and incubating each aliquot with a different compound. If the strain is compound susceptible the cell will become sick and no light will be produced. A compound is considered to be an anti-mycobacterial agent if 50% reduction is observed when compared to the control using a luminometer.[58]

4.3.2 EXPERIMENTAL

Antitubercular activity of all the synthesized compounds were carried out in Microcare lab, Surat (Gujarat) by using following procedure, Stock solutions (1000 □g/mL) were prepared using DMSO (10%) and stored in sterile dark bottles for subsequent experiment. Two

working concentrations (50 and 100 □g/L) were prepared with 7H9 (liquid growth medium) and sterilized by filtration through 0.45□ membrane filter.

4.3.2.1 Test microorganism: *Mycobacterium tuberculosis*

Drug sensitive reference strain (H37Rv) and one clinical isolate of *M. tuberculosis* (MDR), grown and maintained on Lowenstein Jensen (L-J) medium was used for the study.

4.3.2.2 Preparation of culture suspension

A thick suspension (equivalent to McFarland No: 2 turbidity standard) of *M. tuberculosis* was prepared in G7H9 in the following way: A representative amount of growth of bacteria was picked from LJ medium and transferred to sterile screw capped tube (Bijou bottle) containing six to eight glass beads (3mm diameter) and 1mL of G7H9 broth. The suspension was homogenized using a Vortex mixture for 15-20 seconds and adjusted to required turbidity using 7H9 broth. The culture was left to stand for a few minutes to allow large clumps to settle.

4.3.2.3 Preparation of assay mixture

For each sample, two controls (compound free), two tests at concentration 50 and 100µg/mL of compound in 7H9 and a reference drug control (Rifampicin 2µg/mL) were included. From the prepared culture suspension, 100 µL suspension of *M. tuberculosis* was added to 400 µL of the respective media vials and incubated at 37°C for 72 hrs.

4.3.2.4 LRP assay

40 µL of 0.1M $CaCl_2$ and 50 µL of the high titer phage, phAE129 (Luciferase reporter phage construct propagated in *M. smegmatis* mc^2155 to obtain a high titer in 10^{-9} PFU/mL by harvesting the same from lacey plates) were added to all the vials and incubated at 37°C for 4 hrs. After incubation 100 µL was mixed with an equal amount of working D-luciferin solution in a luminometer star tube. Light production was measured at 10 seconds integration in a luminometer; Monolight 2010 and expressed as relative light units (RLU). Reduction in the light output in the compound-containing medium compared to control medium was measured and percentage reduction in RLU was calculated. Antimycobacterial activity is indicated by fifty percent reduction in relative light units (RLU) in the presence of compound in comparison with compound free control.

$$\text{RLU Reduction (\%)} = \frac{\text{Control RLU} - \text{Sample RLU}}{\text{Control RLU}} \times 100$$

4.3.2.5 Standard drug

Rifampicin, Isoniazid and moxifloxacin were used as standard for LRP assay to determine the antitubercular potential of the synthesized compounds. Rifampicin is typically used to treat *Mycobacterium* infection including tuberculosis and Hansen's disease. It can be used to treat BCG-oma, which follows as an uncommon complication of BCG vaccination for tuberculosis. With multidrug therapy used as the standard treatment of Hansen's disease, Rifampicin is always used in combination with Dapsone and Clofazimine to avoid eliciting drug resistance. Rifampicin inhibits bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent RNA polymerase. Isoniazid (INH) was working on the mechanism of mycolic acid inhibition.

4.4 RESULT AND DISCUSSION

4.4.1 Antifungal activity

The antifungal activity result stated that all synthesized compounds (6A-6F) of isatin-moxifloxacin based 1,2,3-triazole derivatives have shown good to moderate activity against tested organisms and was shown in Table 5.1.

Table 5.1: Antifungal activity of synthesized 1,2,3-triazoles derivatives.

COMPOUND	Zone inhibition (mm)			
	C. Albicans		*A. Niger*	
Concentration	**50**	**100**	**50**	**100**
6A	14.75±0.53	27.65±0.43	23.34±0.28	28.25±0.32
6B	8.32±0.33	12.72±0.34	14.52±0.26	17.64±0.32
6C	16.72±0.34	29.32±0.26	19.62±0.23	29.72±0.18
6D	10.45±0.28	22.20±0.22	22.23±0.43	24.35±0.25
6E	9.22±0.65	25.25±0.34	18.42±0.46	21.55±0.12
6F	9.64±0.88	15.32±0.44	16.65±0.16	20.52±0.12
DMSO (Control)	-	-	-	-
Fluconazole	25.30 ±0.32	30.25±0.26	24.25±0.32	29.20±0.23

The order of the activity: 6C > 6A > 6E > 6D > 6F > 6B

Compounds 6A (27.65±0.43), 6B (12.72±0.34), 6C (29.32±0.26), 6D (22.20±0.22), 6E (25.25±0.34) and 6F (15.32±0.44) has shown good activity as compared to standard drug against C. Albicans (Fungi strains) at 100 µg concentration. Compounds 6A (28.25±0.32),

6B (17.64±0.32), 6C (29.72±0.18), 6D (24.35±0.25), 6E (21.55±0.12) and 6F (20.52±0.12) has shown good activity as compared to standard drug against C. Niger (Fungi strains) at 100 µg concentration.

However, further studies on activity and long term toxicity are to be carried out before any conclusion are drawn, as these categories of drug are known to have potential antimicrobial activity. Testing on different models can further substantiate the antimicrobial activity of the synthesized analogues. The antifungal activities of the 1,2,3-triazole derivatives are mostly widely used and some of them are in clinical practice as antimicrobial agents. In particular 1,2,3-triazole derivatives in recent years are extensively studied for the development of newer antimicrobial agents.

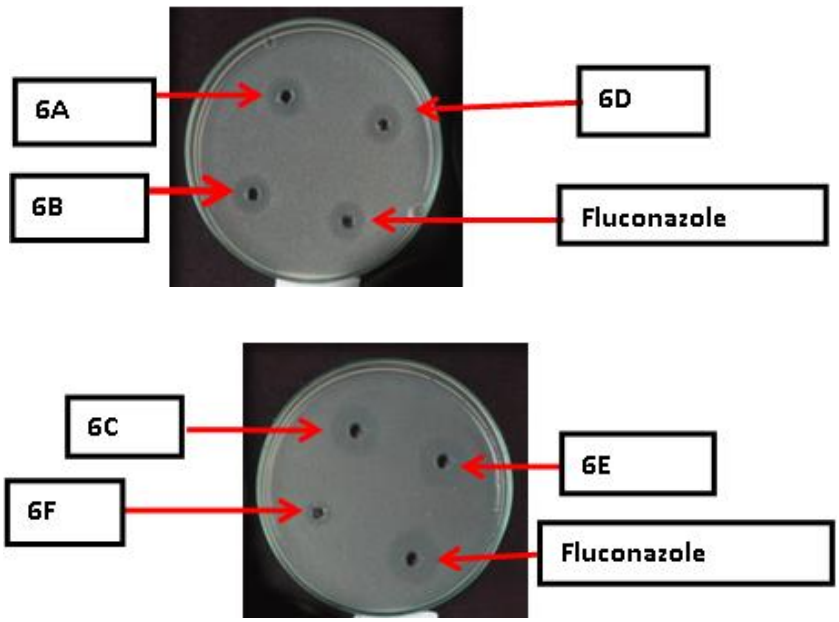
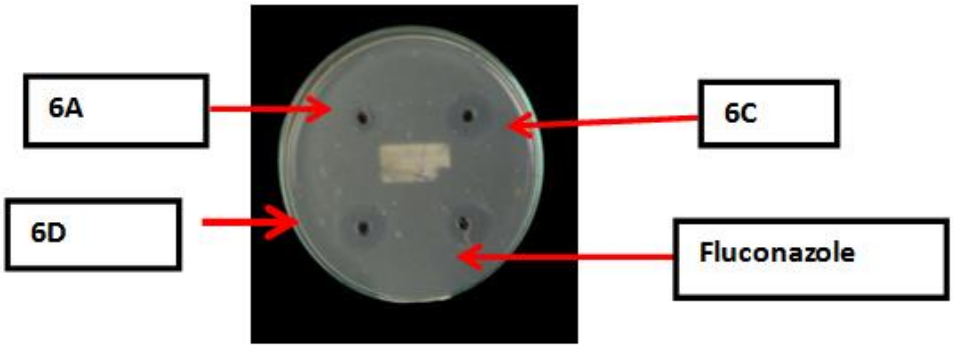


Figure 5.2: Zone inhibition against *C. Albicans.*



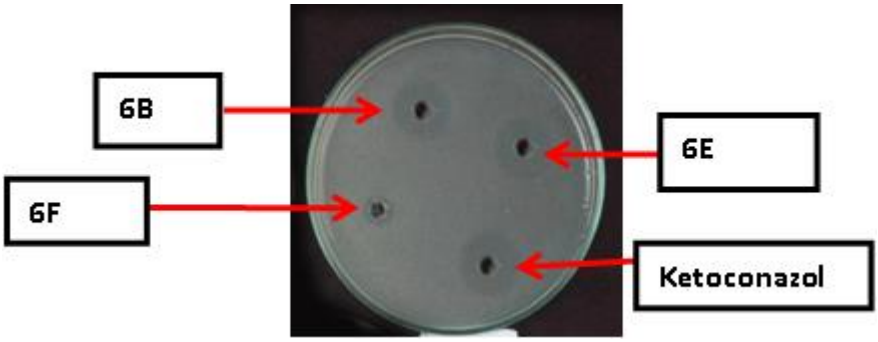


Figure 5.3: Zone inhibition against *A. Niger.*

4.5 Antitubercular activity

All the newly synthesized compounds were assayed *in-vitro* for antitubercular activity against *M. tuberculosis* H37Rv and clinical isolates: S, H, R, and E resistant *M. tuberculosis*. In case of antimycobacterial activity % reduction in relative light units (RLU) was calculated using luciferase reporter phages (LRP) assay at two different concentrations (50 and 100 µg/ml) using Rifampicin as a reference standard. The observed percentage reduction in RLU is tabulated in Table-5.2. Compound is considered to be an antimycobacterial if 50% reduction in the relative light units (RLU) is observed when compared to the control using a luminometer.

Table 5.2: The Percentage of reduction in relative light units (RLU).

Code	Percentage of Reduction in RLU			
	Mycobacterium tuberculosis H37Rv		Clinical isolate: S, H, R & E resistant *M. tuberculosis* (MDR)	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
6A	24.07	77.06	38.07	57.82
6B	32.65	52.23	33.52	29.65
6C	20.12	78.23	12.67	53.56
6D	24.45	65.67	36.76	32.56
6E	18.52	68.89	21.09	31.67
6F	12.65	52.06	27.65	19.89
Rifampicin (2 µg/mL)	75.32		34.44	
Isoniazid (2 µg/mL)	72.35		42.65	
Moxifloxacin (2 µg/mL)	68.32		52.36	

*Where S- Streptomycin, H- Isoniazid, R- Rifampicin, E- Ethambutol and MDR- Multi Drug Resistant.

The percentage of reduction in relative light units (RLU) for *M. tuberculosis* H37Rv at 50 µg/ml ranged from 12.65 to 32.65 and % reduction in relative light units (RLU) for *M. tuberculosis* H37Rv at 100 µg/ml ranges from 52.06 to 78.23. Critical observation of the

experimental results revealed that at 100 µg/mL, all the designed compounds has exhibited more than 50 % reduction in relative light units (RLU) against *M. tuberculosis* H37Rv that show the potent activity of compounds.

The percentage reduction in relative light units (RLU) for clinical isolates: S, H, R, and E resistant *M. tuberculosis* at 50µg/ml ranges from 12.67 to 38.07 and percentages of reduction in relative light units (RLU) for clinical isolates: S, H, R, and E resistant *M. tuberculosis* H37Rv at 100µg/ml ranges from 19.89 to 47.82. In case of clinical isolates: S, H, R, and E resistant *M. tuberculosis,* more than 50% reduction in RLU was seen only with compound 6A and 6C at 100 µg/mL concentration. Though the compounds showed same small level of reduction in RLU but the reduction is insignificant. Higher potency of 6A and 6C compound suggests that it is more active than rifampicin. One of the most significant inferences of this study is that heterocyclic drugs, not the antibiotics, are always best and first line of drugs. The compounds 6A and 6C was active against both the micro-bacterium strains.

5.1. SUMMARY AND CONCLUSION

The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating disease. Most of the activity in this discipline is directed to the new natural or synthetic organic compounds, organic compounds have a major place in therapy and they being with increasingly specific pharmacological activities are clearly the dominant force. Thousands of organic chemicals are prepared annually throughout the world, and many of them enter into pharmacological screening to determine if they have useful biological activity. Among these organic chemicals, heterocyclic nucleus containing compounds occupy a major portion.

The thesis work is broadly classified into two section

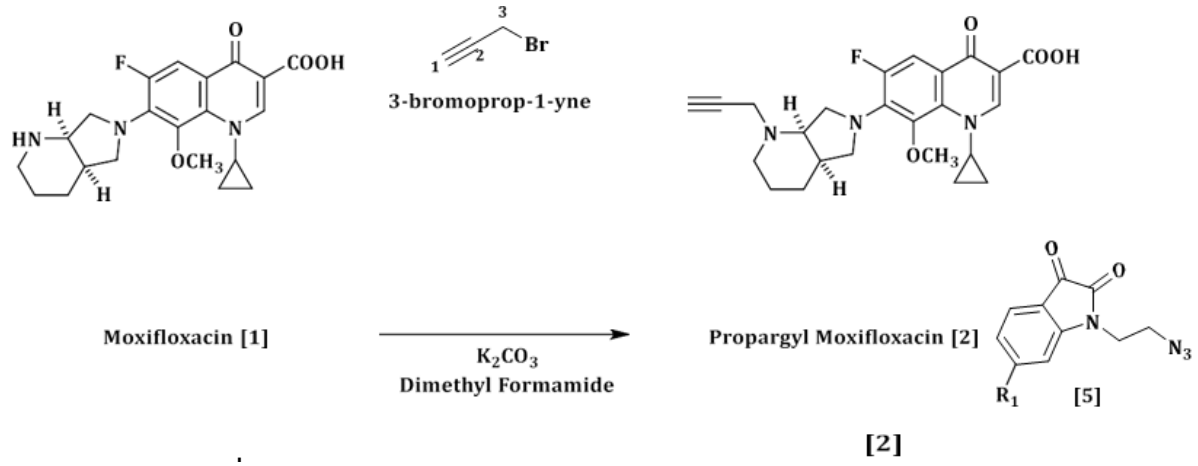
5.1.1 Section -1

In this section, detailed methods and procedures for synthesis of various 1,2,3-triazole derivatives along with their purification and physicochemical parameters had given. Literature is flooded with reference to show the efficacy of substituted 1,2,3-triazole with N-(2-azidoethyl)-isatin (1-(2-azidoethyl)-6-substitutedindoline-2,3-dione) and propargyl moxifloxacin was used to synthesized the compound, 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-((1-(2-(6-nitro-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl) octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid as potent medicinal agents for various pharmacological activities like antibacterial, antifungal

and antitubercular etc.

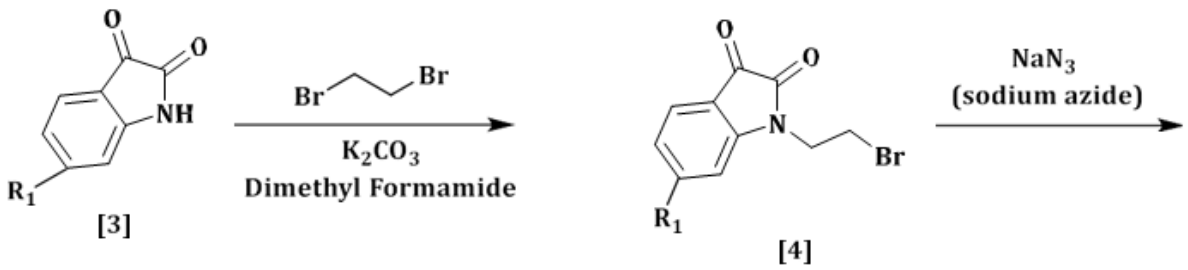
5.1.2 SCHEME FOR SYNTHESIS

Synthesis Scheme-I



[2]

Synthesis Scheme-II



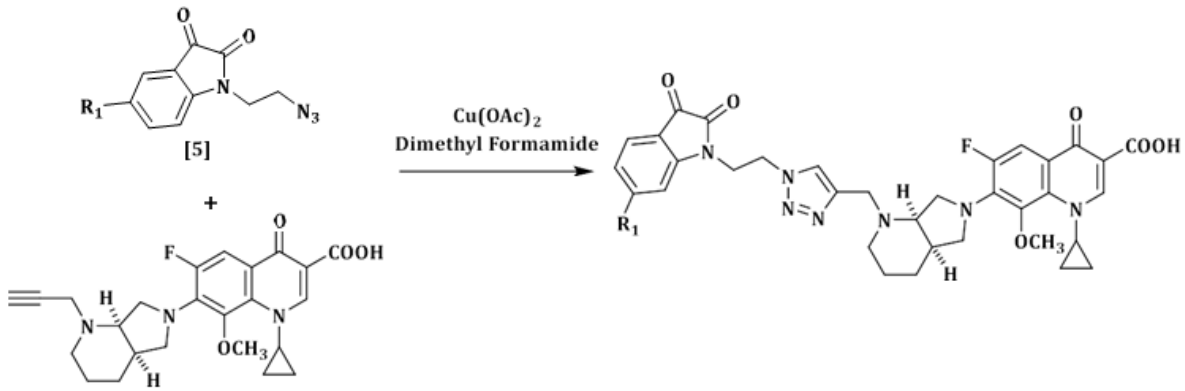
[3]

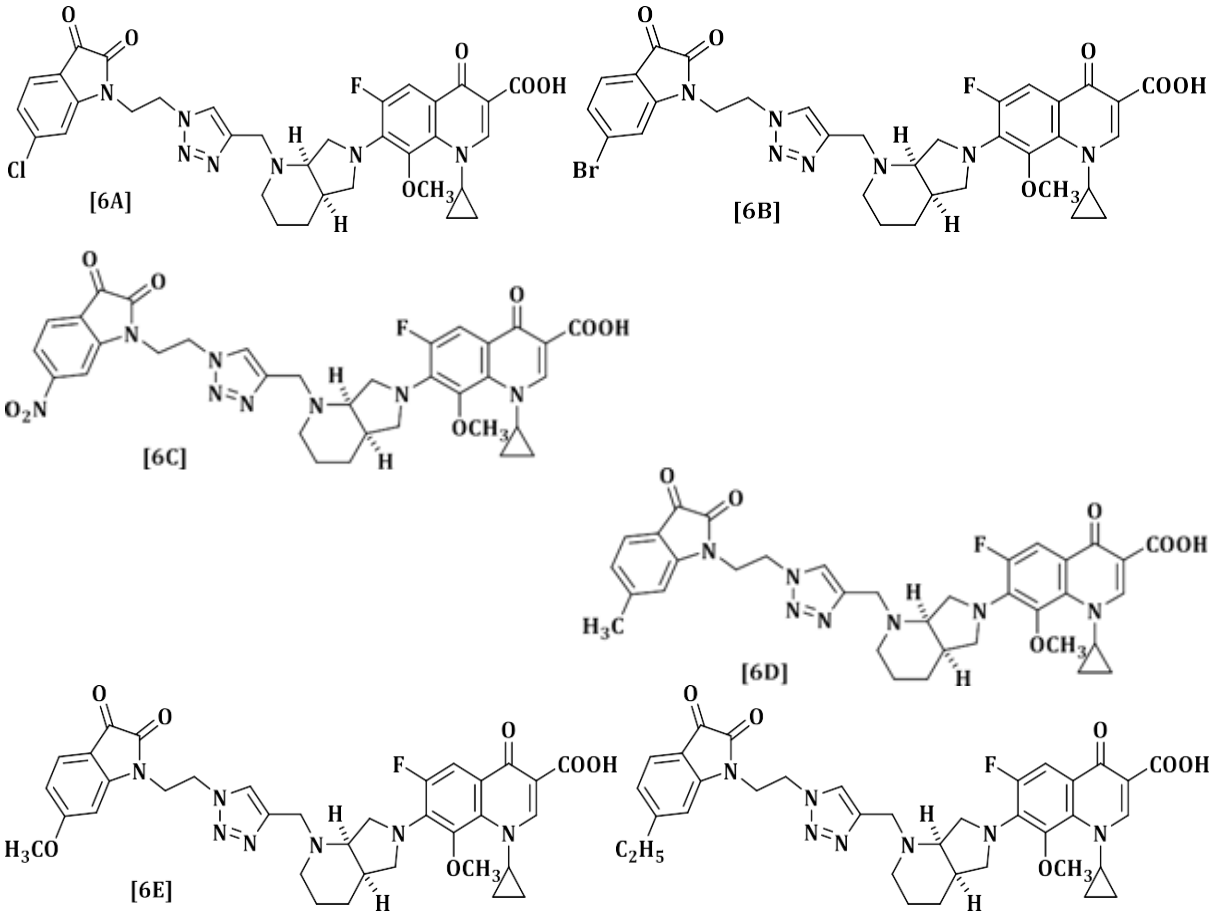
[4]

R_1= Cl, Br, NO_2, CH_3, OCH_3, C_2H_5,

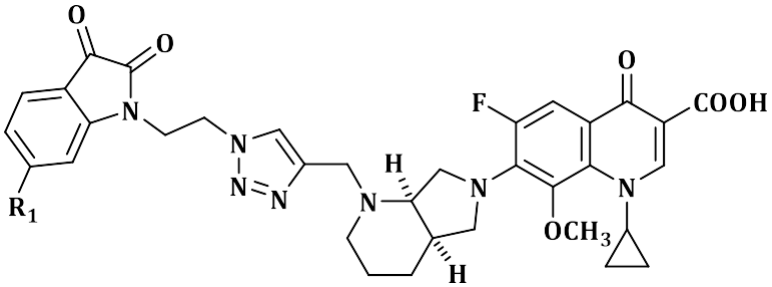
[6]

Synthesis Scheme-III





1.1 List of Final Synthesized compounds



R1= Cl, Br, NO2, CH3, OCH3, C2H5

1.2 List of the synthesized compounds with chemical name

1. **6A:** 7-((4aR,7aR)-1-((1-(2-(6-chloro-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
2. **6B:** 7-((4aR,7aR)-1-((1-(2-(6-bromo-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl) methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
3. **6C:** 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-((1-(2-(6-nitro-2,3-dioxoindolin-

1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

4. **6D:** 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-((1-(2-(6-methyl-2,3-dioxo indolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
5. **6E:** 1-cyclopropyl-6-fluoro-8-methoxy-7-((4aR,7aR)-1-((1-(2-(6-methoxy-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
6. **6F:** 1-cyclopropyl-7-((4aR,7aR)-1-((1-(2-(6-ethyl-2,3-dioxoindolin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

All the compounds were synthesized with good yields and purity. The compounds were characterized by subjecting to various spectral studies such as IR and ^{1}H NMR etc.

5.2 PHYSICO-CHEMICAL CHARACTERIZATION

Physicochemical properties of the synthesized compounds

Comp.	% yield	Physical appearance		Melting point (˚C)	Rf value
		Color	State		
6A	72	Light yellow solid	Solid	170-172°C	0.62
6B	53	Yellow	Solid	143-145°C	0.58
6C	60	Deep Yellow	Solid	160-162°C	0.55
6D	65	Light Yellow	Solid	174-176°C	0.64
6E	68	Yellow	Solid	156-158°C	0.52
6F	58	Yellow	Solid	160-162°C	0.33

Solubility studies of the synthesized compounds

Compound	Water	Alcohol	Acetone	Glacial Acetic Acid	Benzene	Dimethyl Sulfoxide
6A	-	+++	+	++	-	++
6B	-	+++	+	++	-	++
6C	-	+++	+	++	-	++
6D	-	+++	+	++	-	++
6E	-	+++	+	++	-	++
6F	-	+++	+	++	-	++

- Insoluble; + = Slightly soluble; ++ = soluble; +++ = Freely soluble

5.3 DISCUSSION

The six new derivatives of 1,2,3-triazole derivatives has been synthesized and characterized by Proton Nuclear Resonance Spectrophotometer (^{1}HNMR). ^{1}HNMR spectrum of the

synthesized compounds has shown the peaks at ppm δ 0.95–1.57 (m, 9H), 2.06–2.08 (m, 1H), 2.31–2.34 (m, 1H), 2.58–2.60 (m, 1H), 2.82–2.84 (m, 1H), 3.54–3.79 (m, 8H), 4.09–4.12 (m, 3H), 4.59–4.61 (m, 2H), 6.93 (d, 1H, Ar–H), 7.42–7.47 (m, 2H, Ar–H), 7.61 (d, 1H, Ar-H), 7.98 (1H, s, triazole-H), 8.65 (1H, s, C2-H), 15.26 (1H, brs, COOH). The melting point was in the ranges of 140 ˚C to 176˚C of Thin layer chromatography was performed for every individual process and has shown the Retention factor less than 0.7. These all the characterized parameter depicted that compound were synthesized as per expected and confirmed by ^{1}HNMR analysis.

Section – 2

This section discussed the pharmacological evaluation results of newly synthesized compounds. All the synthesized compounds were characterized and screening for their antimicrobial activity and antitubercular activity. The synthesized compounds (6A-6F) were screened for antifungal activity by Agar diffusion method. All these synthesized compounds were screened for the following microbiological activities

[A] Antifungal activity: Compounds 6A (27.65±0.43), 6B (12.72±0.34), 6C (29.32±0.26), 6D (22.20±0.22), 6E (25.25±0.34) and 6F (15.32±0.44) has shown good activity as compared to standard drug against C. Albicans (Fungi strains) at 100 µg concentration. Compounds 6A (28.25±0.32), 6B (17.64±0.32), 6C (29.72±0.18), 6D (24.35±0.25), 6E (21.55±0.12) and 6F (20.52±0.12) has shown good activity as compared to standard drug against C. Niger (Fungi strains) at 100 µg concentration.

[B] Antitubercular activity: The percentage of reduction in relative light units (RLU) for *M. tuberculosis* H37Rv at 50 µg/ml ranged from 12.65 to 32.65 and % reduction in relative light units (RLU) for *M. tuberculosis* H37Rv at 100 µg/ml ranges from 52.06 to 78.23. Critical observation of the experimental results revealed that at 100 µg/mL, all the designed compounds has exhibited more than 50 % reduction in relative light units (RLU) against *M. tuberculosis* H37Rv that show the potent activity of compounds.

The percentage reduction in relative light units (RLU) for clinical isolates: S, H, R, and E resistant *M. tuberculosis* at 50 µg/ml ranges from 12.67 to 38.07 and percentages of reduction in relative light units (RLU) for clinical isolates: S, H, R, and E resistant *M. tuberculosis* H37Rv at 100 µg/ml ranges from 19.89 to 47.82. In case of clinical isolates: S, H, R, and E resistant *M. tuberculosis*, more than 50% reduction in RLU was seen only with compound 6A and 6C at 100 µg/mL concentration. The six new derivatives of isatin-Moxifloxacin based

1,2,3-triazoles derivatives has been prepared and evaluated for their antifungal activity against two fungal strains by using Agar diffusion method. The Fluconazole was used as standard to compare the antifungal potential of the synthesized compounds. In summary, a set of novel isatin–Moxifloxacin based 1H-1,2,3-triazole derivatives have gain greater lipophilicity compared to moxifloxacin alone. The compounds were designed, synthesized and evaluated for their in-vitro antimycobacterial activities against MTB H37Rv and MDR-MTB strains. All synthesized compounds exhibited excellent activities against the MTB H37Rv and MDR-MTB strains. The SAR of isatin–Moxifloxacin based 1H-1,2,3-triazole derivatives was enriched, and the results warrant further development of the anti-TB properties of this kind of conjugates

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