Design, Synthesis, Characterization, and In Vitro Evaluation of Biquinoline-Isoniazid Hybrids as a New Class of Antimicrobial and Antimycobacterial Agents

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ABSTRACT :
The increasing prevalence of antimicrobial resistance and persistent challenges in tuberculosis treatment necessitate the development of novel therapeutic agents. In this study, a new series of biquinoline-isoniazid hybrids were designed, synthesized, and evaluated for their potential as antimicrobial and antimycobacterial agents. The molecular hybridization approach was employed to combine two pharmacophores—biquinoline, known for its broad-spectrum antimicrobial properties, and isoniazid, a frontline antitubercular drug—into a single molecular framework. The synthesized compounds were characterized using spectroscopic techniques such as FTIR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry to confirm their structural integrity. In vitro antimicrobial screening was performed against a panel of Gram-positive and Gram-negative bacteria as well as fungal strains, while antimycobacterial activity was assessed using **Mycobacterium tuberculosis** ***H37Rv. Several of the synthesized hybrids demonstrated significant antimicrobial potency, with minimum inhibitory concentrations (MICs) comparable to or better than standard drugs. Notably, certain derivatives exhibited enhanced antimycobacterial activity, suggesting a potential synergistic effect of the biquinoline and isoniazid moieties. These findings support the potential of biquinoline-isoniazid hybrids as promising leads for the development of next-generation antimicrobial and antitubercular agents.***

Keywords:Biquinoline-Isoniazid Hybrids,Antimicrobial, Antimycobacterial Agents.

I. INTRODUCTION

Heterocyclic compounds, also known as heterocycles or heterocyclics, are a diverse class of organic compounds that contain a cyclic structure with at least one atom other than carbon (called a heteroatom) as part of the ring. The most common heteroatoms are nitrogen, oxygen, and sulfur, though others such as phosphorus and selenium may also occur. These compounds represent one of the most important groups of organic molecules due to their wide distribution in nature, their structural diversity, and their broad spectrum of biological and industrial applications.

Heterocycles are classified on the basis of ring size, degree of saturation, number and type of heteroatoms, and aromaticity. They may be monocyclic, bicyclic, or polycyclic, and the electronic properties of the heteroatoms largely influence their stability and reactivity. Aromatic heterocycles, such as pyridine, furan, thiophene, and imidazole, are of particular significance due to their stability and ability to participate in electron delocalization.

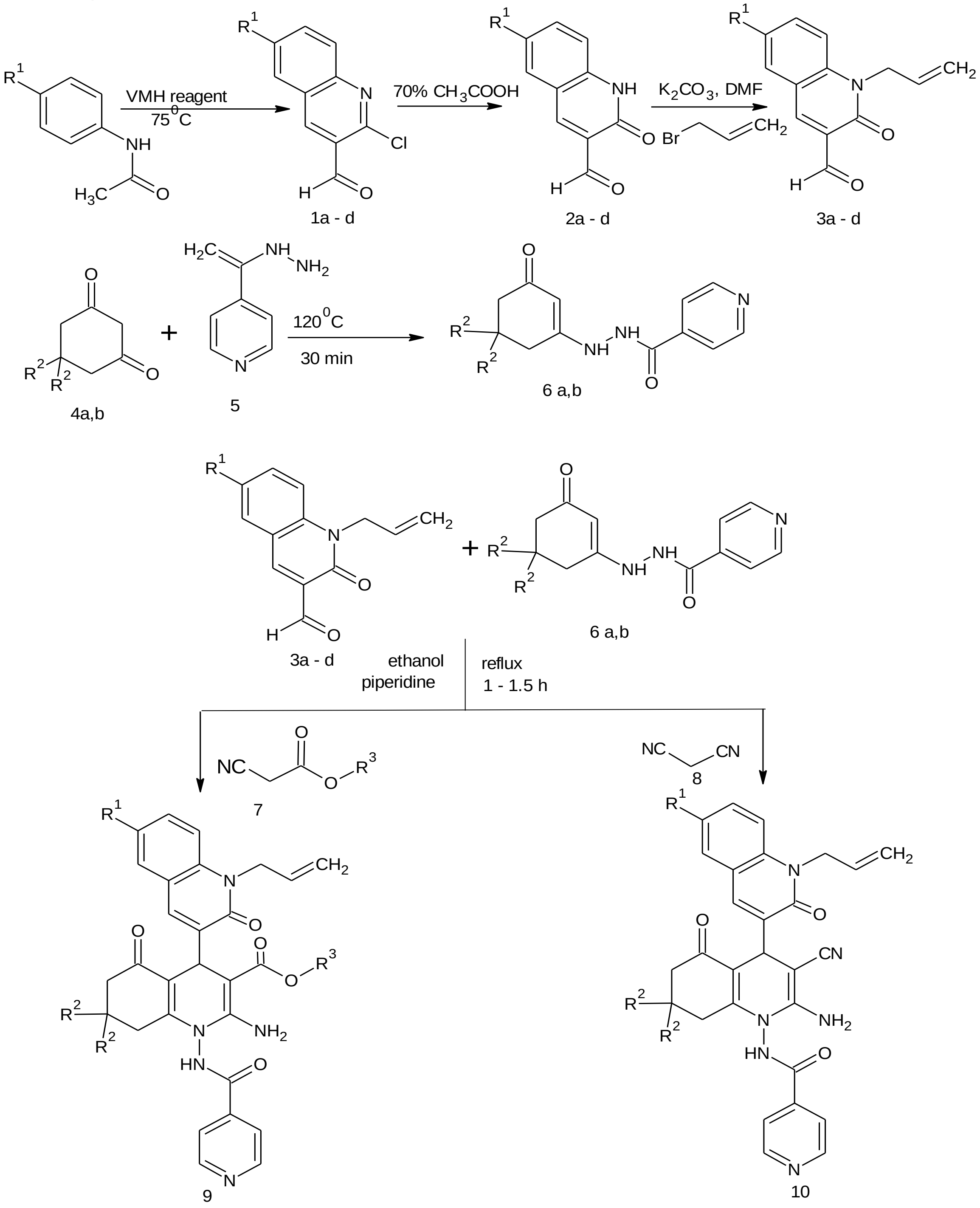
In nature, heterocyclic frameworks form the structural backbone of numerous biomolecules, including nucleic acids (purines and pyrimidines), vitamins (e.g., thiamine, riboflavin), alkaloids, and antibiotics. In synthetic chemistry, heterocycles play a crucial role in the design of pharmaceuticals, agrochemicals, dyes, polymers, and advanced materials. In fact, more than half of all known drugs contain at least one heterocyclic ring, highlighting their importance in medicinal chemistry.

II. EXPERIMENTAL WORK

All the reagents were obtained commercially and used with further purification. Solvents used were of analytical grade.Meltingpoints were taken in open capillaries and are uncorrected.Thin-layer chromatography (TLC, on aluminium plates coated with silica gel 60 F254, 0.25 mm thickness, Merck) was used for monitoring the progress of all reactions, purity and homogeneity of the synthesized compounds.UV radiation and/or iodine vapours were used as the visualizing agents.Elemental analysis (% C, H, N) was carried out by Perkin-Elmer 2400 series-II elemental analyzer and all compounds are within ±0.4% of the theory specified.The IR spectra were recorded on a FTIR MB 3000 spectrophotometer (Perkin Elmer Spectrophotometers) using (400-4500 cm-1) and only the characteristic peaks are reported in cm^{-1}.^{1}H NMR and 13C NMR (APT) spectra were recorded in DMSO-

*d*6 solvent on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) at 400 MHz and 100 MHz respectively using TMS as an internal standard. Chemical shifts are reported in parts per million (ppm) Mass spectra were scanned on a Shimadzu LCMS 8030 spectrometer. Mode of ionization employed was ESI (electrospray ionization).

Present Working Scheme



General procedure for synthesis of titled compounds

Synthesis of 6-substituted-2-chloroquinoline-3-carbaldehydes (1a-d)

Dimethylformamide (19.2 ml, 0.250 mol) was charged in a three-necked roundbottomflask equipped with a thermometer, a drying tube and mechanical stirrer and cooled to 0 ºC. To it phosphorous oxychloride (64.4 ml, 0.70 mol) was added drop wise with stirring at 0-10 ºC. To the solution, 4-substituted acetanilide (0.10 mol) was added and the mixture was heated under reflux for 6 hr at 75 oC. The reaction mass then cooled to room temperature and poured in crushed ice (300 gm) with mechanical stirring. The product isolated was filtered and washed with water till neutral. The solid was crystallized from ethyl acetate to give light yellow powder

Synthesis of 6-substituted-2-oxo-1,2-dihydroquinoline-3-carbaldehydes (2a-d)

6-substituted-2-chloroquinoline-3-carbaldehydes **1a-d** (0.05 mol) and 70% acetic acid (100 mL) were charged in a round bottom flask equipped with condenser. The suspension was slowly heated under reflux for 5-6 hr. After the completion of reaction (checked by TLC), the reaction mixture was cooled. The precipitated solid product was filtered and washed well with water and dried. The crude product was purified by leaching in (10:10 v/v) mixture of methanol and chloroform to obtain pure solid sample.

Synthesis of 1-allyl-6-substituted-2-oxo-1,2-dihydroquinoline-3-carbaldehydes (3a-d)

A 100 mL round bottomed flask, fitted with a guard-tube, was charged with a mixture of 6-substituted-2-oxo-1,2-dihydroquinoline-3-carbaldehyde **1a-d** (0.025 mol), allyl bromide (0.0275 mol) and anhydrous potassium carbonate (0.0375 mol) in dry dimethylformamide (25 mL). The mixture was allowed to stir at rt for 3-5 hr and the progress of the reaction was monitored by TLC. After the completion of reaction (as evidenced by TLC), the reaction mixture was poured into chilled water (250 mL) with continuous stirring. The solid mass separated was collected by filtration, washed well with water, dried and crystallized from ethyl acetate to get pure solid product.

Synthesis of *N′*-(3-oxocyclohex-1-enyl) isonicotinohydrazides (6a, b)

In a round bottom flask, equimolar (3 mmol) mixture of 1,3- cyclohexanedione/dimedone **4a, b**and isoniazid **11** heated at 120 oC till the completion of the reaction as confirmed by the TLC (30 min.). The crude products obtained were purified by recrystallization with ethanol : water (1:4) mixture and dried. The products **6a, b**was obtained quantitatively with an excellent purity.

Synthesis of title compounds (9a-b) and (10a)

A 100 mL round bottomed flask, fitted with a reflux condenser, was charged with a mixture of 1-allyl-2-oxo-1,2-dihydroquinoline-3-carbaldehydes **3a-d** (1 mmol), *N′*- (3-oxocyclohex-1-enyl) isonicotinohydrazides **6a, b** (1 mmol), methyl cyanoacetate **7a**/ethyl cyanoacetate **7b**/ isopropyl cyanoacetate **8b**/ malononitrile **8a** (1 mmol) and a catalytic amount of piperidine (0.2 mmol) in ethanol (10 mL). The reaction mixture was heated under reflux for 1-1.5 h. and the progress of the reaction was monitored by TLC. After the completion of reaction (as evidenced by TLC), the reaction mixture was cooled to room temperature and stirred magnetically for further 20 min., the solid mass separated was collected by filtration, washed well with ethanol (15 mL) and purified by leaching in equal volume ratio of chloroform and methanol (15 mL) to obtain pure solid sample

Table 1: Various biquinolone – isoniazid hybrid compound

Compound	R^1	R^2	R^3	Molecular formula
9a	CH_3	CH_3	C_2H_6	$C_{34}H_{37}N_5O_5$
9b	OCH_3	H	CH_3	$C_{30}H_{29}N_5O_6$
9c	H	H	CH_3	C29H27N5O5
10a	H	H	---	$C_{28}H_{24}N_6O_3$
10b	H	CH_3	---	C30H28N6O3

Table 2: Physical character of titled compound

Compound	Molecular formula	Molecular weight	% Yield	Melting point	Colour (nature)
9a	$C_{34}H_{37}N_5O_5$	595.69	75 %	234-236 °C	White (powder)
9b	$C_{30}H_{29}N_5O_6$	555.58	81 %	242-244 °C	Light yellow (crystal)
9c	C29H27N5O5	525.56	85 %	210-212 °C	White (powder
10a	$C_{28}H_{24}N_6O_3$	525.56	85 %	210-212 °C	White (small needle crystal)
10b	C30H28N6O3	520.58	81 %	228-230 °C	White (crystal)

Table 3: Elemental analysis of titled compounds

Compound	Molecular formula	Elemental Analysis (%)					
		Calculated			Found		
		C	H	N	C	H	N
9a	$C_{34}H_{37}N_5O_5$	68.55	6.26	11.76	68.61	6.41	11.85
9b	$C_{30}H_{29}N_5O_6$	64.85	5.26	12.61	64.78	5.10	12.62
9c	C29H27N5O5	66.27	5.18	13.33	66.11	5.41	13.65
10a	$C_{28}H_{24}N_6O_3$	68.28	4.91	17.06	68.25	5.07	16.75
10b	C30H28N6O3	69.22	5.42	16.14	69.04	5.65	15.96

SPECTRAL ANALYSIS

Compound 9a: Isopropyl 1-allyl-2'-amino-1'-(isonicotinamido)-6,7',7'-trimethyl-2,5'-dioxo-1,1',2,4',5',6',7',8'- octahydro-3,4'-biquinoline-3'-carboxylate

IR (KBr, *v*max, cm^{-1}): 3324 and 3250 (asym. and sym. str. of -NH2), 2942 (Ar C-H str.), 1649 and 1688 (C=O str.)

^{1}H NMR (400 MHz, DMSO-*d6*): δ0.78 (d, 3H, *J*=6.4 Hz, HC-(CH3)2), 0.93 (s, 6H, 2×CH3), 1.23 (d, 3H, *J*=6.4 Hz, HC-(CH3)2), 1.85-2.19 (m, 4H, 2×CH2), 2.37 (s, 3H, CH3), 4.62 (d, 2H, *J*=13.6 Hz, N-CH2), 4.76 (d, 1H, *J*=17.6 Hz, N-CH2CH=CH-*trans*), 4.85 (s, 1H, CH), 5.00 (d, 1H, *J* = 9.2 Hz, N-CH2CH=CH-*cis*), 5.03 (m, 1H, HC- (CH3)2), 5.79 (m, 1H, CH=CH2), 7.23 8.89 (m, 10H, Ar-H+NH2), 11.83 (s, 1H, NH)

^{13}C NMR (APT) (100 MHz, DMSO-*d6*): δ20.64, 22.38, (HC-(CH3)2), 22.81, 25.63, 30.58 (3×CH3), 31.93 (CH2), 35.82 (CH), 37.79 (C(CH3)2), 43.93 (allylic N-CH2), 49.93 (CH2-CO), 65.20 (HC-(CH3)2), 74.19 (C-COOCH(CH3)2), 109.85, 114.85, 116.11, 120.47, 120.95, 122.36, 122.55, 128.38, 130.97, 131.16, 133.09, 133.20, 136.55, 138.82, 150.71, 150.78, 153.56, 154.69 (18C, Ar-C+allylic C=C), 160.17, 165.65, 168.95, 195.06 (C=O)

MS: $[M]^+$595.4

Compound 9b: Methyl 1-allyl-2'-amino-1'-(isonicotinamido)-6- methoxy-2,5'-dioxo 1,1',2,4',5',6',7',8'-octahydro-3,4'- biquinoline-3'-carboxylate

IR (KBr, *v*max, cm^{-1}): 3020 (ArC-H str.), 1720, 1666, 1613(C=O str.)

^{1}H NMR (400 MHz, DMSO-*d6*): δ1.54-2.69 (m, 6H, 3×CH2), 3.49 (s, 3H, COOCH3), 3.80 (s, 3H, OCH3), 4.71 (d, 2H, J = 12.4 Hz, N-CH2), 4.84 (d, 1H, J = 17.6 Hz, N-CH2CH=CH-trans), 4.93 (s, 1H, CH), 5.07 (d, 1H, J = 10.4 Hz, N- CH2CH=CH-cis), 5.83 (m, 1H, CH=CH2), 7.10-8.86 (m, 10H, Ar-H+NH2), 11.75 (s, 1H, NH)

^{13}C NMR (APT) (100 MHz, DMSO-*d6*): δ20.95, 24.68 (2×CH2), 35.13 (CH), 36.54 (CH2-CO), 44.13 (allylic N-CH2), 50.58 (COOCH3), 55.90 (OCH3), 74.08 (CCOOCH3), 110.89, 111.12, 116.16, 116.43, 118.43, 121.48, 122.21, 122.48, 132.98, 133.61, 134.48, 137.07, 138.84, 150.86, 150.88, 154.38, 154.61, 155.20 (18C, Ar-C+allylic C=C), 159.84, 165.79, 169.70, 195.15 (C=O)

MS: $[M]^+$ 555.4

Compound 9c: Methyl 1-allyl-2'-amino-1'-(isonicotinamido)-6,7',7'-trimethyl-2,5'-dioxo-1,1',2,4',5',6',7',8'-octahydro-3,4'-biquinoline-3'-carboxylate.

FTIR spectrum of compound 9c

IR (KBr, *v*max, cm^{-1}): 2916.04 (ArC-H str.), 1689.00, 1649.88, 1553.68(C=O str.)

^{1} H NMR spectrum of compound 9c

^{1}H NMR (400 MHz, DMSO-*d6*): δ1.78-2.74 (m, 6H, 3×CH2), 4.72 (d, 2H, *J* = 13.6 Hz, N-CH2), 5.01 (d, 1H, *J* = 18.0 Hz, N-CH2CH=CH-*trans*), 5.13 (s, 1H, CH), 5.15 (d, 1H, *J* = 9.6 Hz, N-CH2CH=CH-*cis*), 5.92 (m, 1H, CH=CH2), 6.36-8.85 (m, 11H, Ar-H+NH2), 11.42 (s, 1H, NH)

^{13}C NMR (APT) (100 MHz, DMSO-*d6*): δ21.15, 24.70 (2×CH2), 29.19 (CH), 36.44 (CH2-CO), 44.46 (allylic N-CH2), 58.39 (C-CN), 110.73, 113.78, 115.19, 117.01, 120.94, 122.28, 122.44, 128.64, 129.20, 130.30, 133.08, 133.24, 135.27, 135.43, 138.29, 138.80, 150.78, 152.86 154.76 (19C, Ar-C+allylic C=C), 160.67, 165.99, 195.05 (C=O)

MS: $[M]^+$525.56

Compound 10a: *N*-(1-allyl-2'-amino-3'-cyano-2,5'-dioxo- 1,2,5',6',7',8'-hexahydro-3,4' biquinolin-1'(4'*H*)-yl)isonicotinamide

IR (KBr, *v*max, cm^{-1}): 3324.17 and 3260.61 (asym. and sym. str. of -NH2), 2916.95 (Ar C-H str.), 2179.65 (C≡N str.), 1688.08and 1649.69 (C=O str.)

^{1} H NMR spectrum of compound 10a

^{1}H NMR (400 MHz, DMSO-*d6*): δ1.78-2.74 (m, 6H, 3×CH2), 4.72 (d, 2H, *J* = 13.6 Hz, N-CH2), 5.01 (d, 1H, *J* = 18.0 Hz, N-CH2CH=CH-*trans*), 5.13 (s, 1H, CH), 5.15 (d, 1H, *J* = 9.6 Hz, N-CH2CH=CH-*cis*), 5.92 (m, 1H, CH=CH2), 6.36-8.85 (m, 11H, Ar-H+NH2), 11.42 (s, 1H, NH)

^{13}C NMR spectrum of compound 10a

13C NMR (APT) (100 MHz, DMSO-d6):δ21.15, 24.70 (2×CH2), 29.19 (CH), 36.44 (CH2-CO),44.46 (allylic N-CH2), 58.39 (C-CN), 110.73, 113.78, 115.19, 117.01, 120.94, 122.28, 122.44, 128.64, 129.20, 130.30, 133.08, 133.24, 135.27, 135.43, 138.29, 138.80, 150.78, 152.86 154.76 (19C, Ar-C+allylic C=C), 160.67, 165.99, 195.05 (C=O)

MS: $[M]^+$525.56

ANTIMICROBIAL ACTIVITY

In vitro antimicrobial activity of the compounds **9a-b**and **10a**were assessedagainst three representatives of Gram-positive bacteria *viz. Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium tetani*; three Gram-negative bacteria *viz*. *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and two fungi *viz*. *Candida albicans*, *Trichophyton rubrum* by the broth microdilution MIC (Minimum Inhibitory Concentration) method as recommended by National

Committee for Clinical Laboratory Standards (NCCLS).

Ampicillin, chloramphenicol, ciprofloxacin and norfloxacin were used as standard antibacterial drugs, whereas griseofulvin and nystatin were used as standardantifungal drugs.Antimicrobial activity data **Table 4.**

Procedure

We have used the **Broth Dilution Method** for antimicrobial study recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [78]

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism i.e. aim of broth dilution methods is to determine the lowest concentration of the assayed antimicrobial agent (MIC) that, under defined test conditions, inhibits the visible growth of the pathogen being investigated. MIC values are used to determine susceptibilities of pathogen to drugs and also to evaluate the activity of new antimicrobial agents.

This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. The tube dilution test is the standard method for determining levels of resistance to an antibiotic.

Following is the typical procedure to carry out Broth Dilution Method what we followed.

- The *in vitro* antimicrobial activity of the synthesized compounds and standarddrugs were assessed against representative panel of Gram-positive bacteria, Gramnegativebacteria and fungi.
- Inoculum size for test strain was adjusted to 108 CFUmL-1 (Colony Forming Unitper milliliter) by comparing the turbidity (turbidimetric method).
- Mueller Hinton Broth was used as nutrient medium to grow and dilute thecompound suspension for the test bacteria and Sabouraud Dextrose Broth used forfungal nutrition.
- Ampicillin wasused as standard antibacterial drugs, whereas griseofulvin was used as standard antifungal drugs.
- DMSO was used as diluents / vehicle to get desired concentration of synthesizedcompounds and standard drugs to test upon standard microbial strains.
- Serial dilutions were prepared in primary and secondary screening. Eachsynthesized compound and standard drugs were diluted obtaining 2000 µgmL-1concentration, as a stock solution. In primary screening 1000, 500 and 250 µgmL-1concentrations of the synthesized drugs were taken. The active synthesizedcompounds found in this primary screening were further diluted to obtain 200,100, 62.5, 50, 25, 12.5 and 6.250 µgmL-1 concentrations for secondary screeningto test in a second set of dilution against all microorganisms.
- The control tube containing no antibiotic is immediately sub cultured (beforeincubation) by spreading a loopful evenly over a quarter of the plate on a mediumsuitable for the growth of the test organism. The tubes are then put for incubationat 37 °C for 24 hr for bacteria and 48 hr for fungi. The highest dilution (lowestconcentration) showing at least 99 % inhibition or preventing appearance ofturbidity is considered as Minimal Inhibitory Concentration (µgmL−1) i.e. theamount of growth from the control tube before incubation (which represents theoriginal inoculum) is compared. A set of tubes containing only seeded broth andthe solvent controls were maintained under identical conditions so as to make surethat the solvent had no influence on strain growth. The result of this is muchaffected by size of the inoculum. The

test mixture should contain 108 CFUmL^{-1}organisms. The protocols were summarized and compared with standard drugs asthe Minimal Inhibitory Concentration (µgmL^{-1}).

Table 4. Antimicrobial activity (MIC, µg/mL) of compounds

Compound	**Gram-positive bacteria**			**Gram-negative bacteria**			**Fungi**	
	S.aureus	*B.subtilis*	*C.tetani*	*E. coli*	*S. typhi*	*P,aeruginosa*	*C. albicans*	*T.rubrum*
9a	500	500	500	125	200	200	500	1000
9b	**200**	250	125	100	125	125	1000	>1000
10a	210	500	260	100	125	500	500	>1000
Ampicillin	250	250	250	100	100	-----	-----	------
Griseofulvin	-----	-----	-----	------	------	------	500	100

ANTIMYCOBACTERIAL ACTIVITY

In vitro antituberculosis activity of all the newly synthesized compounds **9a-b** and **10a** was determined against *M. tuberculosis* H37Rv strain by usingLowenstein-Jensen slope method (conventional method) andthe observed results are presented in **Table 5**in the form of % inhibition, relative tothat of standard antitubercular drug rifampicin.

We have used the **Minimal Inhibition Concentration** reported by Rattan[79]to evaluate the anti-tuberculosis activity. It is one of the non-automated *in vitro* bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in bottle.

Procedure (Lowenstein-Jensen slope method)

Methods used for primary and secondary screening: Each synthesized drug was diluted obtaining 2000 µgmL^{-1}concentration, as a stock solution.

- **Primary screening:** In primary screening 6.25 µgmL-1 concentration of thesynthesized drugs were taken. The active synthesized drugs found in this primaryscreening were further tested in a second set of dilution against allmicroorganisms.
- **Secondary screening:** The drugs found active in primary screening weresimilarly diluted to obtain 500 µgmL^{-1}, 250 µgmL$^{-1,}$ 200 µgmL^{-1}, 125 µgmL^{-1}, 100µgmL^{-1}, 50 µgmL^{-1}, 25 µgmL^{-1}, 12.5 µgmL^{-1}, 6.25 µgmL^{-1}, 3.125 µgmL^{-1} and1.5625 µgmL^{-1} concentrations.

A primary screen was conducted at 6.25 µg/ml against *M. tuberculosis* H37Rv by Lowenstein-Jensen (LJ) MIC method[147]where primary 6.25 µg/ml dilution of each test compound were added to liquid Lowenstein-Jensen Medium and then media weresterilized by inspissation method. A culture of *M. tuberculosis* H37Rv growing on Lowenstein-Jensen Medium was harvested in 0.85% saline in bijou bottles. DMSOwas used as vehicle to get desired concentration. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5 × 104 bacilli per tube). These tubes were then incubated at 37°C. Growth of bacilli was seen after 12, 22, and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv.The concentration at which complete inhibition of colonies occurred was taken asactive concentration of test compound. The standard strain *M. tuberculosis* H37Rvwas tested with known drug Isoniazide and Rifampicin.

The screening results are summarized as % inhibition relative to standard drug Isoniazide and Rifampicin. Compounds effecting < 90% inhibition in the primary screen were not evaluatedfurther. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentration (MIC) in a Lowenstein-Jensen medium.

Table 5. Antimycobacterial activity of the compounds

Compound	**% Inhibition (at 250 µg/mL)**	**MIC (µg/mL)**
9a	99	12.5
9b	98	50
10a	54	-----
Rifampicin	98	40

III. RESULTS AND DISCUSSION

The synthetic approach adopted r target compounds is depicted in **Scheme 3.2**. Required *N'*-(3-oxocyclohex-1-enyl)isonicotinohydrazides **6 a,b** were synthesized by nucleophilic additionreaction of 1,3-cyclohexanedione/dimedone **4a,b** and isoniazid **11** at 120 oC for 30minute under solvent free condition. Thebiquinoline-isoniazid hybrids **9a-b**and **10a**were prepared *via* one-pot threecomponent cyclocondensation reaction between 1-allyl-2-oxo-1,2-dihydroquinoline-3-carbaldehydes **3a-d**, β-enaminones **6a, b** and methyl cyanoacetate**8a**/ ethyl cyanoacetate**8b**/isopropyl cyanoacetate **7b/**malononitrile **7a** in ethanolcontaining a catalytic amount of piperidine in good to excellent yields **Table 2**.

The structural elucidation of the synthesized compounds **9a-b**and **10a**was carried out by FT-IR, 1H NMR, 13C NMR (APT), elemental analysis and massspectrometry. In FT-IR spectra, an absorption bands around 1628-1652 and 1695-1705 cm-1 exhibited for –C=O stretching. The absorption bands for compounds **10a**were observed in the range of 2160-2183 cm-1 corresponding to C≡N. The characteristic absorption bands for all the compounds were observed in the range of 3340-3396 and 3209-3286 cm-1 corresponding to asymmetrical and symmetrical stretching of NH2 group. In 1H NMR spectra of compounds **9a-b**and **10a**, a sharp singlet peak of methine proton (H4) was appeared around δ 4.81-5.13 ppm. A most characteristic positive signal observed around δ 29.18-35.85 ppm was indicated methine carbon (C4) in 13C NMR (APT) spectra of compounds **9a-b**and **10a**which confirm the formation of another quinolone ring. A peak around δ 11.40-11.85 was attributed to –NH proton of amide linkage. In 13C NMR (APT) spectra, the negative signals around δ 159.84-163.69, 165.61-165.99, 168.91-169.77 and 195.00- 195.26 were arise for the carbonyl carbon (–C=O) of *N*-allyl quinolone ring, amide linkage, ester and cyclohexanone ring, respectively.

The obtained elemental analysis values are in consonance with theoretical data. Mass spectra of title compounds showed expected molecular ion peak M+ corresponding with proposed molecular mass. Physicochemical properties of all the derivatives are shown in **Table 3**. Physical, analytical and spectroscopic characterization data of the compounds **9a-b**and **10**are given then after.

Upon exploration of antimicrobial activity data **Table 4**, it has been observed that against *B.subtilis*, compound **9b** (MIC = 50 μg/mL) was found to be more potent to ampicillin (MIC = 250 μg/mL), against *E. coli*compound 9b and 10a (MIC = 100 μg/mL) were found to be equipotent to ampicillin (MIC = 100 μg/mL). Overall compound 9a was found to be more potent against all tested pathogens on compare to standard ampicillin, opens up a new door to optimize for new class of antimicrobial agents.

In vitro antituberculosis activity of all the newly synthesized compounds **9a-b**and **10a** was determined against *M. tuberculosis* H37Rv strain by usingLowenstein-Jensen slope method (conventional method) as describe in chapter 1 andthe observed results are presented in **Table 5** in the form of % inhibition, relative tothat of standard antitubercular drugs isoniazid and rifampicin. Compounds effectingless than 90 % inhibition in the primary screen were not evaluated further. Of the compounds screened for antituberculosis activity, compounds **9a** (MIC = 12.5 μg/mL found to possess highest potency against *M. tuberculosis* with 99% inhibition and found to have fabulous activity as compared to rifampicin (MIC = 40 μg/mL).Compounds **9b** (MIC = 50 μg/mL) exhibited excellent inhibition of 98% and compound 10a shows inhibition of 54% so its MIC value was not determined.

CONCLUSION

The present study opens up a scope for the area which deals with isoniazid incorporating derivatives and diversity on quinoline nucleus, for aiming their potent antimicrobial and antitubercular activities. Some magnificent results have been obtained with the biquinoline-INH hybridized scaffold. Compound**9a**was found to be most efficient antimicrobials and most efficient antitubercular.

The electron donating and withdrawing groups at R1 position, H and CH_3 at R2 position and diverse lipophilic groups at R3 position plays a pivotal role in the construction of biquinoline-INH derivatives as potent antimicrobial and antitubercular agents. These results verify a proposed hypothesis of the present work. Furthermore, this work contributes to validate the choice of the biquinoline-INH scaffold, as a template, useful to design new antimicrobial and antitubercular compounds.

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