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Synthesis of novel N-(2-substituted-4-oxothiazolidin-3-yl) isonicotinamide derivatives as anti mycobacterial agents

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ABSTRACT

In the present work, the intermediate Schiff's bases (imines) ***1(a-j)*** *were synthesized by refluxing isoniazid with aromatic and heterocyclic aldehydes in ethanol. Cyclocondensation of* ***1(a-j)*** *(imines) with thioglycolic acid in dimethylformamide as solvent, in presence of anhydrous zinc chloride as a catalyst, was carried out under microwave irradiation, to give a series of N-(2-substituted-4-oxothiazolidin-3-yl) isonicotinamide derivatives* ***2(a-j)*** *in good yield. The synthesized compounds were evaluated for their in vitro anti-mycobacterial activity against M. tuberculosis H37Rv using tube dilution method. Some of the compounds exhibited a significant anti mycobacterial activity when compared with first line drugs such as isoniazid (INH) and rifampicin (RIP).*

Keywords: Isoniazid derivatives; Oxothiazolidin-3-yl; Schiff bases; Anti-mycobacterial.

INTRODUCTION

Tuberculosis is caused by *Mycobacterium tuberculosis*, is a deadly contagious disease. WHO has predicted that by year 2020 there will be one billion new active cases if new anti-TB drugs are not developed [1].Treatment of TB infection that has been caused by multidrug resistant (MDR) *M. Tuberculosis* has become a major concern worldwide and its synergy with HIV in immuno compromised patients has worsened the situation . Cost of the drugs Due to the emergence of resistant strains of *M. tuberculosis*, the pathogenic synergy of the tubercular and non tubercular mycobacterial infections with infections [2-3], there is an unmet medical need to discover newer synthetic molecules and drugs for the treatment of tuberculosis and that will shorten the duration of therapy [4-6]. 4-Thiazolidinones have been shown to have various important biological activities [7]. The pharmacological properties of 4-thiazolidinones encouraged our interest in synthesizing several new compounds featuring various heterocyclic rings, attached to 4-thiazolidinone moieties. In continuation with our research work with the aim to obtain new potent anti mycobacterial agents[8], we planned to synthesized a new series of isoniazid derivatives by substitution on $-N^2$ of the pharmacophore $-CON^1HN^2H_2$; isoniazid was coupled via its Schiff's base formation [9] with mercapto acetic acid to get N-(2-substituted-4-oxothiazolidin-3-yl) isonicotinamide derivatives, as synthesis of such isoniazid molecule coupled with important heterocycles has resulted in enhanced antimicrobial activity [10-12]. All the synthesized compounds were characterized and evaluated for their anti mycobacterial activity.

Chemistry

The title compounds N-(2-substituted-4-oxothiazolidin-3-yl) isonicotinamide derivatives **2(a-j)** were synthesized as per the scheme of synthesis, Scheme1.

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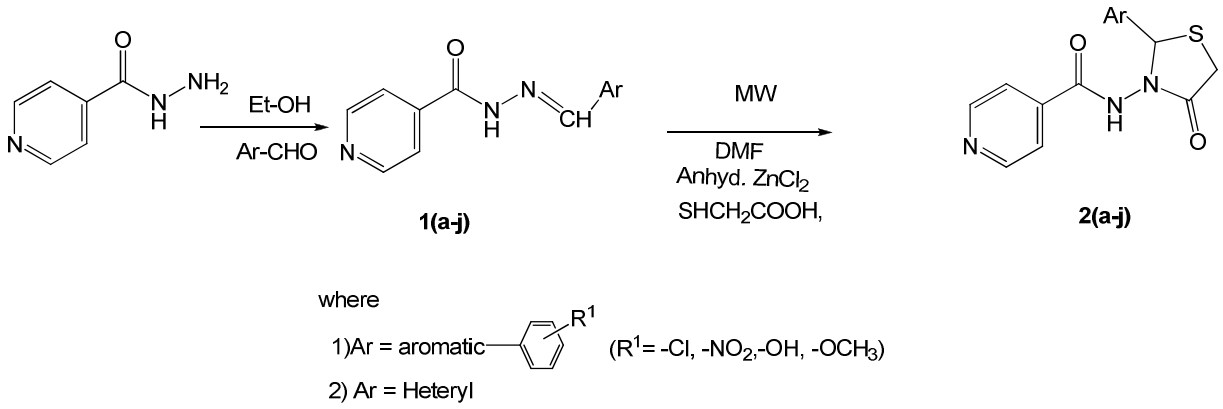


Scheme 1: Synthesis of N-(2-substituted-4-oxothiazolidin-3-yl) isonicotinamide

The intermediate Schiff's bases (imines) **1(a-j)** were synthesized by refluxing isoniazid with aromatic and heterocyclic aldehydes in ethanol [13]. The novel N-(2-substituted-4-oxothiazolidin-3-yl) isonicotinamide derivatives **2(a-j)** were synthesized by cyclo condensation of intermediate Schiff's bases (imines) with thioglycolic acid in presence of 50 mg anhydrous zinc chloride as a catalyst as described in general procedure. The reaction was carried out under microwave irradiation, in dimethylformamide as solvent, with 70-85% yields. All the compounds were identified by spectral data. In general, IR spectra of Schiff's bases **1(a-j)** showed bands at 3448cm^{-1} (N-H stretch) and 1633cm^{-1} (C=N stretch). The ^{1}H-NMR showed signals at δ12.10 and 8.47 ppm due to exo cyclic NH and N=CH, respectively. The **2(a-j)** series showed bands at 3100- 3000 cm^{-1} (CH=CH stretch of aromatic), 1760-1700 cm^{-1} (C=O stretch) and 690-590 cm^{-1} (C-S stretch). The ^{1}H-NMR shows signals at δ 3.83, 6.84 and 7.3-8.76 ppm due to -OCH_3, -CH_2 and Ar, respectively. Physical characterization data of the synthesized derivatives **2(a-j)** is given in Table 1.

MATERIALS AND METHODS

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled unless otherwise noted. Melting points were determined by open capillary method and are uncorrected. Infrared spectra were recorded on JASCO FT IR (PS-4000) using KBr powder technique and frequencies are expressed in cm^{-1}. Mass spectra were recorded on Micro mass Q-Tof Micro system mass spectrometer. ^{1}H-NMR spectra were recorded on Varian Mercury YH 300 FT-NMR Spectrometer operating at 300 MHz (^{1}H) and on BRUKER AVANCE II 400 spectrometer operating at 400 MHz (^{1}H) in deuterated dimethyl sulphoxide. Chemical shifts are reported in ppm (d) relative to tetramethylsilane. Proton spectra were typically obtained at room temperature. For TLC, plates coated with silica gel were run in chloroform/methanol mixture and spots were developed in iodine chamber.

3.1.1. General procedures for the synthesis of N'-(substituted- aryl/heterylidene) isonicotinohydrazide **1 (a-j**) Intermediate Schiff's base

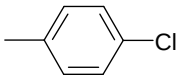
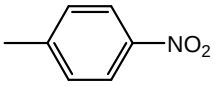
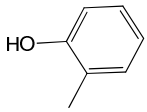
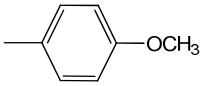
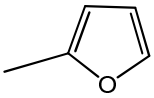
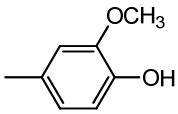
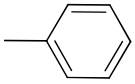
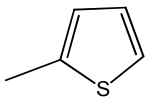
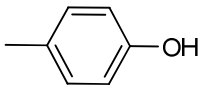
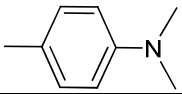
The isonicotinoyl hydrazide derivatives were prepared by reaction between equimolar quantities of isoniazid (1.0 equiv.) and substituted aldehyde (1.0 equiv.) in ethanol. The resulting mixture was refluxed for 2-2.5 h. The reaction mixture was then concentrated and cooled. Thus obtained solid was filtered and dried. The yield and mp of the product was recorded. The crude products were recrystallized from ethanol.

3.1.2. General procedures for the synthesis of N-(2-substituted-4-oxothiazolidin-3-yl) isonicotinamide **2(a-j)**

A mixture of Schiff base **1(a-j)** (0.01mole) and thioglycolic acid (0.7ml, 0.01 mole) in dimethylformamide were taken in Erlenmeyer flask, 50mg anhydrous zinc chloride was added as a catalyst. The mixture was irradiated in Microwave oven at 700W for about 4-6 min. The mixture was diluted with ice cold water and solid product

precipitated. The crude compound was recrystallized from ethanol.Spectral data and elemental analysis data of the synthesized derivatives is given below.

Table 1: Characterization data of isonicotinamide derivatives 2(a-j)

Code	Ar	Molecular Weight	% yield	Melting Point (^{0}C)	Rf Value*
2a		335	69	320	0.52
2b		346	76	290-293	0.64
2c		317	78	270	0.58
2d		331	86	225-227	0.61
2e		291	75	245	0.54
2f		347	91	316	0.64
2g		301	82	245	0.58
2h		307	76	330	0.54
2i		317	74	340	0.48
2j		344	82	280-282	0.62

Solvent system for Rf value were Chloroform : methanol(9:1)

3.1.2.1. N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl) isonicotinamide **(2a).**
IR (KBr) in cm^{-1}: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. Calc. For $C_{15}H_{12}ClN_3O_2S$: Calculated (Found):C, 53.97(52.20); H, 3.62(3.49); N, 12.59(11.19).

3.1.2.2. N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl) isonicotinamide **(2b).**
IR (KBr) in cm^{-1}: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H), 3.10 (s, 6H,-CH_3). Anal. Calc. For $C_{15}H_{12}N_4O_4S$: Calculated (Found): C, 52.32(52.12); H, 3.51(3.22); N, 16.27(15.54).

3.1.2.3. N-(2-(2-hydroxyphenyl)-4-oxothiazolidin-3-yl) isonicotinamide **(2c).**
IR (KBr) in cm^{-1}: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. Calc. For $C_{15}H_{13}N_3O_3S$: Calculated (Found): C, 57.13(55.12); H, 4.16(3.89); N, 13.33(12.34).

3.1.2.4. N-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl) isonicotinamide **(2d).**
IR (KBr) in cm^{-1}: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. Calc. For $C_{16}H_{15}N_3O_3S$: Calculated (Found): C, 58.34(58.23); H, 4.59(4.21); N, 12.76(11.23).

3.1.2.5. N-(2-(furan-2-yl)-4-oxothiazolidin-3-yl) isonicotinamide **(2e).**
IR (KBr) in cm^{-1}: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. Calc. For $C_{13}H_{11}N_3O_3S$: Calculated (Found): C, 53.97(53.11); H, 3.83(3.21); N, 14.52(13.12).

3.1.2.6. N-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl) isonicotinamide **(2f).**
IR (KBr) in cm^{-1}: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. Calc. For $C_{16}H_{15}N_3O_4S$: Calculated (Found): C, 55.64(54.24); H, 4.38(4.26); N, 12.17(12.10).

3.1.2.7. N-(4-oxo-2-phenylthiazolidin-3-yl) isonicotinamide **(2g).**
IR (KBr) in cm^{-1}: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. Calc. For $C_{15}H_{13}N_3O_2S$: Calculated (Found): C, 60.18(59.45); H, 4.38(4.12); N, 14.04(13.89).

3.1.2.8. N-(4-oxo-2-(thiophen-2-yl) thiazolidin-3-yl) isonicotinamide **(2h).**
IR (KBr) in cm^{-1}: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. Calc. For $C13H_{11}N_3O2S_2$: Calculated (Found): C, 51.13(50.33); H, 3.63(3.33); N, 13.76(13.55).

3.1.2.9. N-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-yl) isonicotinamide **(2i).**
IR (KBr) in cm^{-1}: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. Calc. For $C_{15}H_{13}N_3O_3S$: Calculated (Found): C, 57.13(5.12); H, 4.16(3.89); N, 13.33(12.23).

3.1.2.10. N-(2-(4-(dimethylamino) phenyl)-4-oxothiazolidin-3-yl) isonicotinamide **(2j).**
IR (KBr) in cm^{-1}: 3328.25 (N-H), 1710.46 (C=O), 1593.78 (–CONH), 1633.0 (C=N); ^{1}H NMR (DMSO, 300 MHz) δ ppm: 11.5(s, 1H, –NH) 8.6-8.9 (m, 4H, CH–Pyridine ring), 8.67(s, 1H,CH=N), 7.1-7.7(m, 4H, Ar–H),3.10 (s, 6H,-CH_3). Anal. Calc. For $C_{17}H_{18}N4O_2S$: Calculated (Found): C, 59.63(58.9); H, 5.30(5.23); N, 16.36(16.21).

4. Pharmacology

4.1 Biological investigation

The anti-mycobacterial activities were assessed against *Mycobacteria Tuberculosis* H37Rv using the tube dilution method. This methodology is nontoxic, uses a thermally- stable reagent. All the synthesized compounds were dissolved, separately, in dimethyl sulphoxide to prepare a stock solution containing 1000 µg/mL. The successive concentrations like 500, 200, 100, and 50 and µg/mL so on were prepared in a similar manner up to 6 dilutions. A sweep of Mycobacterial tuberculosis H37Rv strain culture was discharged with the help of 22 S.G.W. nichrome wire loop with a 3mm external diameter, into a sterile distilled bijou bottle containing six 3mm glass beads and 4 ml distilled water.

4.2 Anti mycobacterial activity

All the newly synthesized of **2(a-j)**, were assayed in vitro for anti tubercular activity against *Mycobacteria Tuberculosis* H37Rv using the tube dilution method, using isoniazid and rifampicin as a reference standard. The screening was done by the method described by Watt *et.al* [14, 15]. This methodology is nontoxic, uses a thermally-stable reagent. All the synthesized compounds were dissolved, 10 mg of each, separately in dimethyl sulphoxide to prepare a stock solution containing 1000 µg/mL. The successive concentrations like 500, 250, 125, and 62.5 and so on were prepared in a similar manner up to 6 dilutions. A sweep of Mycobacterial tuberculosis H37Rv strain culture was discharged with the help of 22 S.G.W. nichrome wire loop with a 3mm external diameter, into a sterile distilled bijou bottle containing six 3mm glass beads and 4 ml distilled water. Each loopful was supposed to deliver 4 mg moist weight of bacilli. The bottle was shaken with the help of a mechanical shaker for 2 min. Using S.W.G. nichrome wire loop, 3 mm diameter, a loopful of the suspension was inoculated on the surface of each of Lowenstein-Jensen medium containing test compounds. Lowenstein-Jensen medium containing standard drug as well as control was also inoculated with Mycobacterial tuberculosis H37Rv strain. The medium inoculated were incubated at 37°C for six weeks. The presence or absence of growth of organism was observed after six weeks. The MIC (minimal inhibitory concentration) was defined as the lowest drug concentration, which prevented the microbial growth. Result of anti mycobacterial activities with standard drugs isoniazid and rifampicin are discussed in Table 2.

Table 2: Anti mycobacterial activity of isonicotinamide derivatives 2(a-j)

Compound No.	MIC µg/ml
2a	100
2b	100
2c	>500
2d	>500
2e	12.5
2f	50
2g	50
2h	>500
2i	200
2j	200
Isoniazid	0.2
Rifampicin	40

Study revealed that **2d, 2e, 2f** and **2h** exhibited stronger anti-mycobacterial activity. From the present investigation, five compounds have emerged as lead moieties.

RESULTS AND DISCUSSION

In the present investigation, a series of novel isoniazid derivatives, **2(a-j)** have been synthesized under microwave irradiation. Use of synthetic microwave has enhanced the yield and reaction rate and reduced the duration of reaction to 4-6 minutes, as conventional synthesis requires 12-16 hrs refluxing in solvent benzene, which is harmful to health. Moreover, the conventional synthesis requires Dean –Stark apparatus, which results in benzene –water azotrope and needs to be removed continuously from the reaction mixture. All the synthesized derivatives were evaluated for anti mycobacterial activity against *M. Tuberculosis* H_{37}Rv. The compounds have shown moderate anti mycobacterial activity and two of the synthesized compounds namely, **2j** and **2e** have shown good potency and significant in vitro anti mycobacterial activity against *M. Tuberculosis.* Comparative study of the substitution pattern of the aryl and heteryl group towards antimicrobial activity has shown that electron donating groups gives the better activity while electron withdrawing groups causes less activity. The details are given as follows: When substituent on phenyl ring is $–OCH_3$, the compound **2d**, has shown comparable activity with standard drug (rifampicin). The compound containing $(–N\ (CH_3)_2$ group on phenyl ring, the compound **2j** has shown excellent activity when compared with standard drug (rifampicin). When the substituent were electron donating groups such as –OH, $-OCH_3$ i.e., compounds **2c, 2d, 2f**, and **2j** exhibited increased anti mycobacterial activity and with electron withdrawing groups such as –Cl, $-NO_2$, compounds **2a** and **2b** gave decreased anti mycobacterial activity. The compound **2e** containing heterocyclic furan ring as substituents has shown excellent activity as compared with standard drug (rifampicin). However, when compared with the isoniazid as standard drug, only compound no. **2j** has shown moderate activity.

CONCLUSION

The presence of two important moieties i.e. isoniazid- thiazolidinone in the final derivatives coupled via important pharmacophore CONH- has contributed towards better anti mycobacterial activity. However, the synthesized N-(2-substituted-4-oxothiazolidin-3-yl) isonicotinamide derivatives, which were modified isoniazid derivatives obtained by substitution on N^2 of the pharmacophore $–CON^1HN^2H_2$ of the standard anti mycobacterial drug, isoniazid, resulted in decrease in anti mycobacterial activity.

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