Full Paper

Synthesis and Antimycobacterial Activity of a Novel Series of Isonicotinylhydrazide Derivatives

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A novel series of 14 new isonicotinyl hydrazide derivatives **2a – g**, **3a – g** containing a 4-thiazolidinone / 2-azetidinone nucleus were synthesized by reacting *N′*-substituted arylidene / heteroarylidene isonicotinyl hydrazide **1a – g** with thioglycollic acid in the presence of dry benzene and with chloroacetyl chloride in the presence of triethylamine, respectively. Structures of all newly synthesized compounds were characterized on the basis of elemental analyses and spectral data (IR and ^{1}H-NMR). All the title compounds were tested for their *in-vitro* antimycobacterial activity against *Mycobacterium tuberculosis* H_{37}Rv using Alamar-Blue susceptibility test, and the activity is expressed as the minimum inhibitory concentration (MIC) in μg/mL. Among the series, compounds **2b**, **2g**, **3b**, and **3g** displayed an encouraging antimycobacterial activity profile as compared to that of the reference drugs isoniazid / rifampicin.

Keywords: Antimycobacterial activity / Azetidinones / Isonicotinyl hydrazide / *Mycobacterium tuberculosis* / Thiazolidinones

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Introduction

Tuberculosis (TB) is a chronic necrotizing bacterial infection with a wide variety of manifestations caused by *Mycobacterium tuberculosis*, which has been a scourge of humanity for thousands of years and remains to be one of the prevalent health tribulations in the world [1]. TB is an ancient enemy and present threat that ranks among the foremost killers of the 21st century. About one third of the world's population is infected with *M. tuberculosis* resulting in eight million new cases of tuberculosis and around 2.9 million deaths annually [2]. In developing countries, where rates of both infection and active disease have always been high, the number of cases skyrocketed; the increase was so dramatic that the World Health Organization (WHO) declared TB a global health emergency in 1993, the first time, an infectious disease achieved that dubious distinction [3 – 5] The risk becomes even greater if the person is co-infected with the human immunodeficiency virus (HIV). In addition, life threatening strains of Multidrug-resistant Tuberculosis (MDR-TB) are appearing, some of which can lead to high mortality rates with death occurring in a short period.

The introduction of the first-line drugs like streptomycin, *para*-aminosalicylic acid, isoniazid, etc., for treatment some 50 years ago led to optimism that the disease could be controlled if not eradicated [6]. These drugs, coupled with generally increasing standards of health care, caused a rapid decrease of tuberculosis in many industrialized countries, which produced a climate of indifference to the need for fresh drugs. As a result of this apathy and the perception by the pharmaceutical industry that such agents would be unlikely to generate a suitable return on investment, few new drugs have been

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introduced in the last 30 years [7]. However, since the 1980's, the disease has been undergoing a resurgence driven by a variety of changes in social, medical, and economic factors. Thus, a dramatic increase in the immunosuppressed individuals mainly due to AIDS, coupled increasing urbanization and poverty in developing countries, has compromised primary health care structures and led to large increases in TB incidence [8]. Concomitant with the resurgence of TB has been the occurrence of the multidrug-resistant disease that has exposed the frailties of the current drug armamentarium [9].

There is now recognition that innovative drugs to combat TB are urgently required. With the completion of the genome of *M. tuberculosis* comes the promise of a new generation of potent drugs to combat the emerging epidemic of TB. The emphasis of mycobacterial research now has shifted from gene hunting to interpretation of the biology of the whole organism in an effort to better define which activities are likely to be critical for survival and, thus, amenable to the development of new drugs [10]. Therefore, there is an urgent need for anti-TB drugs with improved properties such as enhanced activity against MDR strains, reduced toxicity, shortened duration of therapy, rapid mycobactericidal mechanism of action, and the ability to penetrate host cells and exert antimycobacterial effects in the intracellular environment.

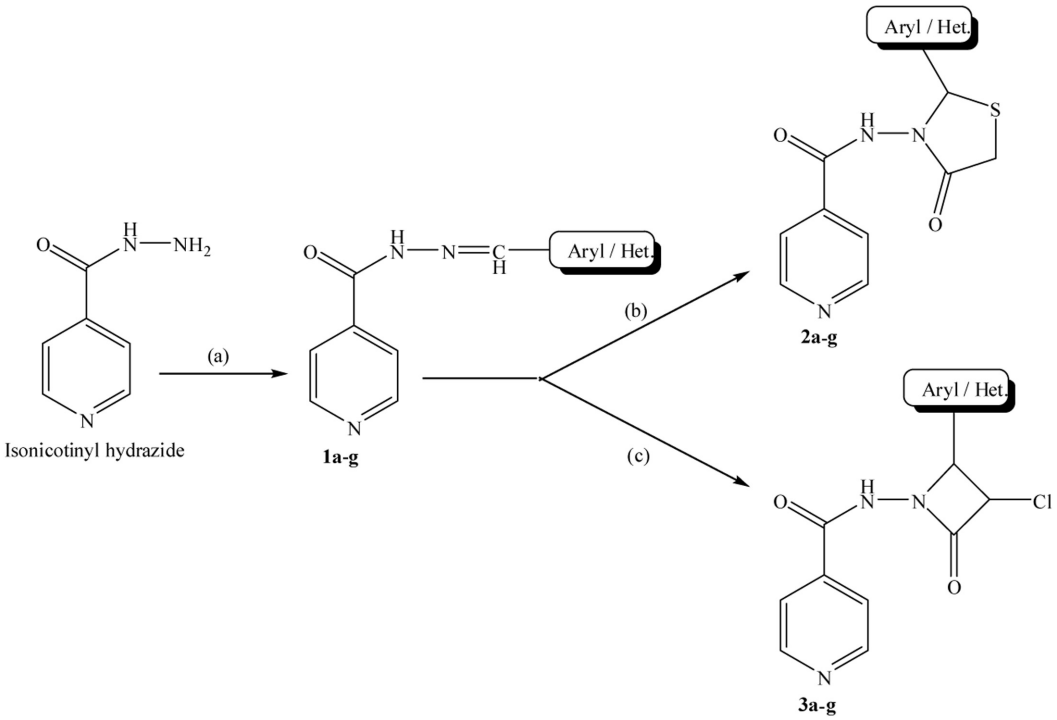
Isonicotinic acid hydrazide (INH) has very high *in-vivo* inhibitory activity towards *M. tuberculosis* H37Rv. Iproniazide, like INH, is used in the treatment of tuberculosis. In the search of new antimycobacterial agents, INH derivatives have been found to possess potential tuberculostatic activities [11–13]. Studies suggest that INH, a prodrug which is converted into its active form by mycobacterial catalase-eperoxidase, acts on the mycobacterial cell wall by preventing the FAS-II (fatty acid synthetase II) system from producing long-chain fatty-acid precursors for mycolic acid synthesis [14, 15].

4-Thiazolidinone is an imperative scaffold that is not only synthetically important but also possesses a wide range of promising biological activities. 4-Thiazolidinone derivatives are known to possess antibacterial [16], antifungal [17], antiviral [18], and antituberculosis [19] properties. 4-Thiazolidinones have been reported as novel inhibitors of the bacterial enzyme Mur B which is a precursor acting during the biosynthesis of peptidoglycan [20]. 2-Azetidinones, commonly known as β-lactams, are well-known heterocyclic compounds among the organic and medicinal chemists [21]. The activity of the famous antibiotics such as penicillins, cephalosporins, and carbapenems are attributed to the presence of 2-azetidinone ring in them. Recently, some other types of biological activities such as antifungal, antitubercular, antitumor, cholesterol absorption inhibition, etc. have been reported in compounds containing an 2-azetidinone ring [22]. The β-lactams also serve as synthons for many biologically important classes of organic compounds [23, 24]. In view of these observations and in continuation of our research program on the synthesis of heterocyclic compounds [25–28], we report herein the synthesis of some new INH derivatives possessing 4-thiazolidinone and 2-azetidinone moieties, which have been synthesized, see Scheme 1, and evaluated for their *in-vitro* antimycobacterial activity against *M. tuberculosis*.

Results and discussion

Chemistry

4-Thiazolidinones are derivatives of thiazolidine with a carbonyl group at the 4-position. Several protocols for the synthesis of 4-thiazolidinones are available in the literature [29–31]. Essentially they are three-component reactions involving an amine, a carbonyl compound, and a mercapto-acid. The process can be either a one-pot three-component condensation or a two-step process [32, 33]. Likewise, the most common method for the synthesis of 2-azetidinones is the Staudinger-keteneimine cyclo-addition, which involves the reaction of imines with acid chloride in the presence of a tertiary base [34]. The synthesis of *N*-(4-oxo-2-(substituted)aryl/heteroarylthiazolidin-3-yl)isonicotinamide **2a–g** and *N*-(3-chloro-2-oxo-4-(substituted)aryl/heteroarylazetidin-1-yl)isonicotinamide **3a–g** was achieved through the versatile and efficient synthetic route outlined in Scheme 1. It is apparent from the scheme that the new heterocyclic compounds contain 2-azetidinone and 4-thiazolidinone moieties. Reaction of *N*′-substituted arylidene / heteroarylidene isonicotinyl hydrazide (Schiff base) with thioglycollic acid and chloroacetyl chloride seemed to be a convenient route to fulfill this aim. Starting materials *N*′-substituted arylidene / heteroarylidene isonicotinyl hydrazide **1a–g** were synthesized by condensation of INH with appropriately substituted aromatic / heteroaromatic aldehydes in the presence of ethanol and glacial acetic acid. The various *N*-(4-oxo-2-(substituted)aryl/heteroarylthiazolidin-3-yl)isonicotinamide derivatives **2a–g** were synthesized by cyclo-condensation of **1a–g** with thioglycollic acid in the presence of dry benzene while the *N*-(3-chloro-2-oxo-4-(substituted)aryl/heteroarylazetidin-1-yl)isonicotinamides **3a–g** were synthesized by cyclo-addition of **1a–g** with chloroacetyl chloride in the presence of triethylamine. The structure of all newly synthesized 4-thiazolidinone and 2-azetidinone deriv-



Reagents and conditions: (a) H–C–Aryl / Het., ethanol, glacial acetic acid, reflux, 3 h; (b) thioglycollic acid, dry benzene, reflux, 15 h; (c) chloroacetyl chloride, triethylamine, dry dioxan, stirring, 20 h.

Scheme 1. Synthesis of a novel series of isonicotinylhydrazide derivatives **2a–g** and **3a–g**.

atives of INH were confirmed on the basis of analytical and spectral data.

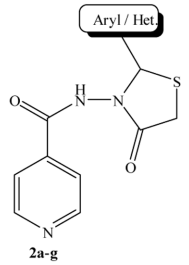
The synthesis of *N′*-(substituted)arylidene/heteroarylidene isonicotinylhydrazide (Schiff base) derivatives **1a–g** involved the reaction between appropriately substituted aromatic / heteroaromatic aldehydes and isoniazid, as described in the general procedure. Further, we have synthesized a novel series of *N*-(4-oxo-2-(substituted)aryl/heteroaryl thiazolidin-3-yl)isonicotinamide **2a–g** and *N*-(3-chloro-2-oxo-4-phenylazetidin-1-yl)isonicotinamide **3a–g** by reacting the appropriately substituted Schiff bases with thioglycollic acid and chloroacetyl chloride, respectively, as illustrated in Scheme 1. Structures of the synthesized compounds were established on the basis of physicochemical, elemental analysis, and spectral data (IR and ^{1}H-NMR), which are presented in Tables 1–4.

In general, IR spectra of compounds **2a–g** and **3a–g** showed two absorption bands ranging around 1705–1728 and 1672–1698 cm^{-1} indicating the presence of two C=O groups in their structure (one C=O group as -CONH- and the other is in the cyclic ring) and also the NH stretching vibrations appeared between 3226–3273 cm^{-1}. In the nuclear magnetic resonance spectra (^{1}H-NMR), the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, multiplicities, and coupling constants. In particular, it must be pointed out that in compounds **1a–g** the presence of a singlet between δ = 8.85–8.37 ppm indicate the formation of imine / Schiff base (>CH=N-) by a simple condensation process; this singlet was not observed in the title compounds. Further, the appearance of two singlets at around δ = 4.3 and 2.9 ppm in compounds **2a–g** evidently confirms the formation of 4-thiazolidinone. Similarly, compounds **3a–g** showed two doublets around δ = 4.9 and 4.6 ppm demonstrating the formation of 2-azitidinone. The peaks appearing at δ = 2.73–2.82, 3.78–3.88, 5.25–5.42, and 6.83–7.92 ppm confirm the presence of -N(CH$_3$)$_2$, -OCH$_3$, -OH, and aromatic protons, respectively.

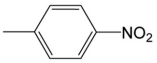
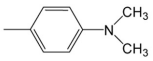
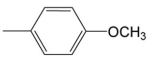
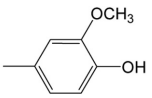
Antimycobacterial activity

The Minimum Inhibitory Concentration (MIC) was determined for compounds **2a–g** and **3a–g** against the *M. tuberculosis* strain H37Rv using the micro plate Alamar Blue assay (MABA) [35] (Table 5). This methodology is nontoxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric methods [36, 37]. The purpose of the screening program

Table 1. Physicochemical data of *N*-(4-oxo-2-(substituted)aryl/heteroarylthiazolidin-3-yl) isonicotinamide **2a–g**.



2a-g

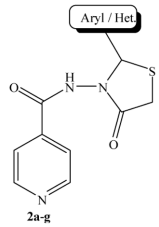
Compound	Aryl / Het.	Yield (%)	Mp (°C)	R_f Value[a)]	Mol. Formula	Mol. Wt.
2a		45	168–170	0.42	$C_{15}H_{13}N_3O_2S$	299.32
2b		59	208–210	0.54	$C_{15}H_{13}N_3O_3S$	315.36
2c		42	272–275	0.5	$C_{13}H_{11}N_3O_3S$	289.29
2d		55	256–260	0.62	$C_{15}H_{12}N_4O_4S$	344.35
2e		39	120–122	0.52	$C_{17}H_{18}N_4O_2S$	342.44
2f		52	130–133	0.49	$C_{16}H_{15}N_3O_3S$	329.37
2g		48	212–214	0.51	$C_{16}H_{15}N_3O_4S$	345.40

[a)] All synthesized compounds were purified by column chromatography using chloroform / methanol (9.3 : 0.7) as mobile phase and iodine vapor as visualizing agent.

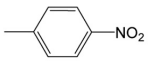
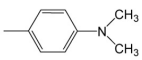
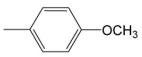
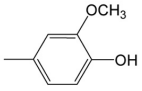
is to provide a resource whereby new experimental compounds can be tested for their capacity to inhibit the growth of virulent *M. tuberculosis*.

The synthesized compounds **2a–g** and **3a–g** were evaluated for their *in-vitro* antimycobacterial activity against *M. tuberculosis* strain H37Rv by using MABA method. The result of antimycobacterial activity is presented in Table 5. All the synthesized compounds exhibited an interesting activity profile against the tested mycobacterial strain. The results reveal that the activity is considerably affected by various substituents on the aromatic ring of either 4-thiazolidinone or 2-azetidinone nucleus. It has been observed that compounds **2a** and **3a** having no substitution on the aromatic ring did not show any considerable activity. It is interesting to note that the introduction of a hydroxyl or methoxyl group on aromatic ring (compounds **2b**, **2f**, **2g**, **3b**, **3f** and **3g**), resulted in compounds with an enhanced antimycobacterial activity (with MIC values ranging from 0.31–5.0 μg/mL). Amongst them, compounds **2g** and **3g** (MIC = 0.31 μg/mL) exhibited a significant activity and also compounds **2b** and **3b** (MIC = 0.62 μg/mL) showed a respectable activity when compared with first-line drugs such as INH (MIC = 0.2 μg/mL) and rifampicin (RIP, MIC = 1.0 μg/mL). However, when a nitro group was introduced on the aromatic ring (compounds **2d** and **3d**) and also when the substituted aromatic ring was replaced by a heterocyclic group (compounds **2c** and **3c**), a moderate antimycobacterial activity was observed. We have also studied the influence of the 4-thiazolidinone nucleus in compounds **2a–g** and the 2-azetidinone nucleus in compounds **3a–g** on the biological activity. We observed that the replacement in

Table 2. Analytical data of *N*-(4-oxo-2-(substituted)aryl/heteroaryl thiazolidin-3-yl)isonicotinamide **2a – g**.



2a-g

Compound	Aryl / Het.	IR (KBr, cm^{-1})	^{1}H-NMR (DMSO-d_6, δ, ppm)	Elemental Analysis[a)]
2a		3268.34 (N – H), 1719.84 (C=O of amide), 1685. 49 (C=O of thiazolidinone), 1604.06 (C=N).	10.22 (s, 1H, NH), 7.25–7.92 (m, 9H, Ar-H), 4.45 (s, 1H, CH-N), 2.83 (s, 2H, CH_2).	$C_{15}H_{13}N_3O_2S$
2b		3462.33 (O – H), 3245.82 (N – H), 1728.01 (C=O of amide), 1696.75 (C=O of thiazolidinone), 1599.13 (C=N).	10.41 (s, 1H, NH), 7.18–7.79 (m, 8H, Ar-H), 5.25 (s, 1H, Ar-OH), 4.39 (s, 1H, CH-N), 2.92 (s, 2H, CH_2).	$C_{15}H_{13}N_3O_3S$
2c		3252.88 (N – H), 1721.46 (C=O of amide), 1690.18 (C=O of thiazolidinone), 1602.89 (C=N).	10.05 (s, 1H, NH), 6.91–7.67 (m, 7H, Ar-H), 4.34 (s, 1H, CH-N), 2.87 (s, 2H, CH_2)	$C_{13}H_{11}N_3O_3S$
2d		3258.64 (N – H), 1717.90 (C=O of amide), 1686.33 (C=O of thiazolidinone), 1596.14 (C=N).	10.52 (s, 1H, NH), 7.15–7.84 (m, 8H, Ar-H), 4.32 (s, 1H, CH-N), 2.89 (s, 2H, CH_2)	$C_{15}H_{12}N_4O_4S$
2e		3261.25 (N – H), 1705.13 (C=O of amide), 1679.21 (C=O of thiazolidinone), 1595.47 (C=N).	10.37 (s, 1H, NH), 7.17–7.79 (m, 8H, Ar-H), 4.35 (s, 1H, CH-N), 2.90 (s, 2H, CH_2), 2.73 (s, 6H, Ar-N(CH_3)$_2$).	$C_{17}H_{18}N_4O_2S$:
2f		3273.27 (N – H), 1712.42 (C=O of amide), 1679.21 (C=O of thiazolidinone), 1601.51 (C=N).	10.25 (s, 1H, NH), 7.16–7.79 (m, 8H, Ar-H), 4.36 (s, 1H, CH-N), 3.78 (s, 3H, Ar-OCH_3), 2.85 (s, 2H, CH_2).	$C_{16}H_{15}N_3O_3S$
2g		3477.43 (O – H), 3265.41 (N – H), 1708.10 (C=O of amide), 1675.69 (C=O of thiazolidinone), 1600.04 (C=N).	10.09 (s, 1H, NH), 6.85–7.68 (m, 7H, Ar-H), 5.29 (s, 1H, Ar-OH), 4.27 (s, 1H, CH-N), 3.85 (s, 3H, Ar-OCH_3), 2.87 (s, 2H, CH_2).	$C_{16}H_{15}N_3O_4S$

[a)] All compounds were within acceptable range in the elemental analyses (± 0.4%).

the core nucleus did not alter the antimycobacterial activity to a greater extent.

Conclusion

In the present paper, we report the synthesis, spectral studies, and antimycobacterial activity of some new series of 4-thiazolidinone and 2-azitidinone derivatives of INH. The various 4-thiazolidinone derivatives of INH were synthesized by cyclo-condensation of *N′*-substituted arylidene / heteroarylidene isonicotinyl hydrazide with thioglycollic acid in the presence of dry benzene while the 2-azetidinone derivatives of INH were synthesized by cyclo-addition of *N′*-substituted arylidene / heteroarylidene isonicotinyl hydrazide with chloroacetyl chloride in the presence of triethylamine.

The preliminary *in-vitro* antimycobacterial screening results of the title compounds, reported here, evidenced that some of the compounds from both new series have emerged as potential antimycobacterial compounds endowed with moderate to good activity. Further improvements in the antitubercular activity can possibly be achieved by slight modifications in the ring substituents. Yet, extensive additional functioning warrants further investigations. Our findings will have impact on chemists and pharmacists for further investigations in this field in search of potent antimycobacterial agents.

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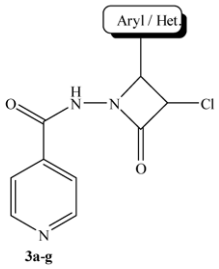
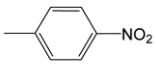
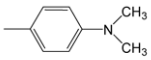
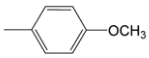
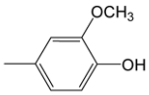
Table 3. Physicochemical data of *N*-(3-chloro-2-oxo-4-(substituted)aryl/heteroarylazetidin-1-yl) isonicotinamide **3a–g**.



3a-g

Compound	Aryl / Het.	Yield (%)	Mp (°C)	R_f Value[a)]	Mol. Formula	Mol. Wt.
3a		52	140–142	0.51	$C_{15}H_{12}ClN_3O_2$	301.69
3b		45	210–212	0.58	$C_{15}H_{12}ClN_3O_3$	317.72
3c		39	198–200	0.61	$C_{13}H_{10}ClN_3O_3$	291.65
3d		48	312–316	0.52	$C_{15}H_{11}ClN_4O_4$	346.74
3e		37	180–182	0.47	$C_{17}H_{17}ClN_4O_2$	344.83
3f		57	152–154	0.63	$C_{16}H_{14}ClN_3O_3$	331.70
3g		44	246–248	0.43	$C_{16}H_{14}ClN_3O_4$	347.78

[a)] All synthesized compounds were purified by column chromatography using chloroform / methanol (8.2 : 1.8) as mobile phase and iodine vapor as visualizing agent.

Professor, K.L.E.S' College of Pharmacy Hubli, for his encouragement. We are also thankful to Lupin Pharmaceutical Industry, Aurangabad, for providing the gift sample of INH and Rifampicin. We are grateful to the Director, SAIF, Punjab University and The Chairman, USIC, Karnataka University, for providing elemental and spectral analysis.

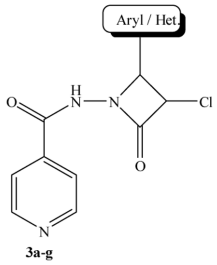
The authors have declared no conflict of interest.

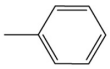
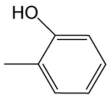
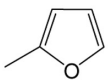
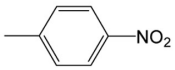
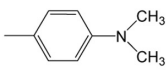
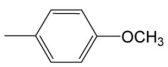
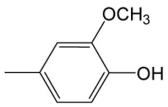
Experimental

Chemistry

All research chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) or Lancaster Co. (Ward Hill, MA, USA) and were used as such for the reactions. Solvents, except laboratory reagent grade, were dried and purified according to the literature when necessary. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates from E. Merck and Co. (Darmstadt, Germany). Melting points of the synthesized compounds were determined in a Thermonik melting point apparatus (Mumbai, India) and are uncorrected. IR spectra were recorded on a Thermo Nicolet IR200 FT-IR Spectrometer (Nicolet, Madison, WI, USA) by using KBr pellets. ^{1}H-NMR spectra were recorded on Bruker AVANCE 300 (Bruker, Rheinstetten/Karlsruhe, Germany) using $CDCl_3$ / DMSO-d_6 as solvent. Chemical shifts are reported in δ ppm units with respect to TMS as internal standard. The elemental analysis (C, H, N) of the compounds were performed on Heraus CHN rapid analyzer. Results of elemental analysis were within ± 0.4% of the theoretical values. Purity of the compounds was checked on TLC plates using silica gel G as stationary phase and iodine vapors as visualizing agent.

Table 4. Analytical data of *N*-(3-chloro-2-oxo-4-(substituted)aryl/heteroarylazetidin-1-yl)isonicotinamide **3a – g**.



Compound	Aryl / Het.	IR (KBr, cm^{-1})	^{1}H-NMR (DMSO-d_6, δ, ppm)	Elemental Analysis[a)]
3a		3243.86 (N – H), 1714.89 (C=O of amide), 1689.54 (C=O of azetidinone), 1606.40 (C=N).	10.49 (s, 1H, NH), 7.18–7.86 (m, 9H, Ar-H), 4.93 (d, 1H, CH-Cl), 4.53 (d, 1H, -N-CH).	$C_{15}H_{12}ClN_3O_2$
3b		3433.26 (O – H), 3242.58 (N – H), 1720.18 (C=O of amide), 1687.56 (C=O of azetidinone), 1604.37 (C=N).	10.02 (s, 1H, NH), 7.13–7.77 (m, 8H, Ar-H), 5.31 (s, 1H, Ar-OH), 4.89 (d, 1H, CH-Cl), 4.58 (d, 1H, -N-CH).	$C_{15}H_{12}ClN_3O_3$
3c		3232.23 (N – H), 1716.24 (C=O of amide), 1698.10 (C=O of azetidinone), 1598.02 (C=N).	10.30 (s, 1H, NH), 6.95–7.71 (m, 7H, Ar-H), 4.95 (d, 1H, CH-Cl), 4.62 (d, 1H, -N-CH).	$C_{13}H_{10}ClN_3O_3$
3d		3245.86 (N – H), 1709.71 (C=O of amide), 1681.97 (C=O of azetidinone), 1597.68 (C=N).	10.22 (s, 1H, NH), 7.17–7.88 (m, 8H, Ar-H), 4.88 (d, 1H, CH-Cl), 4.55 (d, 1H, -N-CH).	$C_{15}H_{11}ClN_4O_4$
3e		3251.62 (N – H), 1713.50 (C=O of amide), 1692.17 (C=O of azetidinone), 1595.47 (C=N).	10.10 (s, 1H, NH), 6.83–7.62 (m, 8H, Ar-H), 4.84 (d, 1H, CH-Cl), 4.39 (d, 1H, -N-CH), 2.82 (s, 6H, Ar-N(CH_3)$_2$).	$C_{17}H_{17}ClN_4O_2$
3f		3226.34 (N – H), 1706.27 (C=O of amide), 1672.19 (C=O of azetidinone), 1599.93 (C=N).	10.36 (s, 1H, NH), 6.94–7.66 (m, 8H, Ar-H), 4.89 (d, 1H, CH-Cl), 4.63 (d, 1H, -N-CH), 3.88 (s, 3H, Ar-OCH_3).	$C_{16}H_{14}ClN_3O_3$
3g		3463.54 (O – H), 3274.71 (N – H), 1710.38 (C=O of amide), 1685.26 (C=O of azetidinone), 1604.76 (C=N).	10.20 (s, 1H, NH), 7.19–7.74 (m, 7H, Ar-H), 5.44 (s, 1H, Ar-OH), 4.91 (d, 1H, CH-Cl), 4.60 (d, 1H, -N-CH), 3.82 (s, 3H, Ar-OCH_3).	$C_{16}H_{14}ClN_3O_4$

[a)] All compounds were within acceptable range in the elemental analyses (± 0.4%).

*General procedure for N′-(substituted)arylidene/ heteroarylidene isonicotinylhydrazide **1a – g***

To a constantly stirred solution of INH (2.74 g, 0.02 mol) in 30 mL of ethanol containing few drops of glacial acetic acid (2 mL) was added an appropriate aromatic / heteroaromatic aldehyde (0.02 mol). The reaction mixture was refluxed for 3 h, cooled to room temperature, and poured into crushed ice. The resulting mixture was filtered and the solid obtained was washed with cold water and dried. By this procedure, compounds **1a – g** were obtained starting from benzaldehyde, 2-hydroxybenzaldehyde, furan-2-carboxaldehyde, 4-nitrobenzaldehyde, 4-*N*,*N*′-dimethylbenzaldehyde, 4-methoxybenzaldehyde, 3-methoxy-4-hydroxybenzaldehyde, respectively. The solid obtained was recrystallized from appropriate solvent to yield the title compounds.

*General procedure for N-(4-oxo-2-(substituted)aryl/ heteroarylthiazolidin-3-yl) isonicotinamide **2a – g***

To a solution of compounds **1a – g** in dry benzene (50 mL), thioglycolic acid (0.02 mol) was added dropwise and the reaction mixture was refluxed on a water bath for 12 – 15 h. The excess of solvent was distilled off under reduced pressure. The cooled residual mass was filtered, washed with dilute sodium bicarbonate solution, and dried. Recrystallization from suitable solvents afforded the title compounds **2a – g**. The physicochemical, spec-

Table 5. The *in-vitro* antimycobacterial activity of compounds **2a–g** and **3a–g** against *M. tuberculosis* $H_{37}Rv$ strain.

Compound	MIC[a)]
2a	resistant
2b	0.62
2c	1.25
2d	3.12
2e	5.0
2f	1.25
2g	0.31
3a	resistant
3b	0.62
3c	1.25
3d	5.0
3e	5.0
3f	3.12
3g	0.31
Isoniazid	0.2
Rifampicin	1.0

[a)] Minimal inhibitory concentration (MIC) is expressed in μg/mL.

tral, and elemental analysis data of the synthesized compounds are depicted in Tables 1 and 2, respectively.

General procedure for N-(3-chloro-2-oxo-4-(substituted)aryl/heteroarylazetidin-1-yl) isonicotinamide ***3a–g***

To a constantly stirred solution of the particular *N′*-(substituted) arylidene/heteroarylidene isonicotinylhydrazide (**1a–g**, 0.01 mol) and triethylamine (0.01 mol) in dry dioxan (40 mL), chloroacetyl chloride (0.015 mol) was added dropwise at 0-5°C. The reaction mixture was stirred for 15–20 h and the excess of solvent was distilled off under reduced pressure. The resulting residual mass was cooled, poured into ice water, filtered, washed with water, dried. Recrystallization from the proper solvents yielded the title compounds **3a–g**. The physicochemical, spectral, and elemental analysis data of the synthesized compounds are depicted in Tables 3 and 4, respectively.

Antimycobacterial activity

The antimycobacterial activity of the newly synthesized compounds was assessed against *M. tuberculosis* ATTC 27294 using the micro-plate Alamar Blue assay (MABA) [35]. Succinctly, 200 mL of sterile de-ionized water was added to all outer-perimeter wells of sterile 96-well plates (falcon 3072: Becton Dickinson, Lincoln Park, NJ, USA) to minimize evaporation of the medium in the test wells during incubation. The 96-well plates received 100 mL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) and a serial dilution of the compounds **2a–g** and **3a–g** was made directly on the plate. The final drug concentrations tested were 0.01–20.0 μg/mL. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 μL of a freshly prepared 1 : 1 mixture of Alamar Blue reagent (Accumed International, Westlake, OH, USA) and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The MIC was defined as the lowest drug concentration, which prevented a color change from blue to pink.

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