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Synthesis and antitubercular activity of novel 4-substituted imidazolyl-2,6-dimethyl-N^3,N^5-bisaryl-1,4-dihydropyridine-3,5-dicarboxamides

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ABSTRACT

A series of 4-substituted imidazolyl-2,6-dimethyl-N^3,N^5-bisaryl-1,4-dihydropyridine-3,5-dicarboxamides were prepared. They were screened as antitubercular agents against *Mycobacterium tuberculosis* H_{37}Rv. Minimum inhibitory concentrations (MICs) were determined using agar proportion method. Compound **3i** with 1-benzyl-2-methylthio-1*H*-imidazole-5-yl substituent at C-4 position and 4′-chloromophenyl group at C-3 and C-5 positions of the 1,4-dihydropyridine ring was the most potent one among the tested compounds. It was as potent as rifampicin against *M. tuberculosis* H_{37}RV. Compound **3l** also was an active antitubercular agent with the same substituent as compound **3i** at the C-4 position and 3′-pyridyl group at C-3 and C-5 positions of the 1,4-dihydropyridine ring.

1. Introduction

The unique structure of *Mycobacterium tuberculosis* cell wall is responsible for the resistance of mycobacters. The acid-fast bacillus *M. tuberculosis*, the causative agent of tuberculosis (TB), possesses a cell wall that differs significantly in structure from both Gram-negative and Gram-positive bacteria [1]. This cell wall consists of mycolyl arabinogalactan units which are bonded to peptidoglycan nucleus through covalent bonds. Mycolated 1,3-branched arabinofuranoside-based hexasaccharide motif forms the mycolyl–arabinan domain in this structure. Mycolation occurs via ester linkages at each of the four of the primary arabinose hydroxyls [2,3]. The mycolyl moieties are high molecular weight α-alkyl, β-hydroxy fatty acids that are responsible for producing the hydrophobic character of the cell envelope. The unique hydrophobic properties of mycobacterium envelope protect the bacterium from its environment and provide a barrier to the diffusion of commonly used hydrophilic antimicrobial agents [4,5]. According to Global Health Initiative one-third of the world's population has latent tuberculosis, an inactive form of the disease that develops to active form in one-tenth people [6]. Other statistics tell us that every year, approximately 8 million of these people develop active tuberculosis (TB), and almost 2 million of them will die from the disease [7]. Antitubercular drugs available for the treatment were discovered in the period of 1945–1965. No new drugs were synthesized during the last few decades [8]. Moreover the recent emergence of outbreaks of multidrug resistant tuberculosis, MDR-TB, to the first-line drugs: isoniazid (INH), rifampicin (RIF), ethambutol (ETH), streptomycin (STR), and pyrazinamide (PYR) have made the disease hard to be cured [9]. This obstacle in the treatment of tuberculosis and the statistical facts about its prevalence tell us about the necessity of searching and synthesizing new more potent and less prone to resistance compounds with less side-effects. The number of publications providing a better insight about *M. tuberculosis*, the process of the disease and numerous novel molecules as potential leads for TB

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drug discovery have increased since the mid-1990s [10–18]. Among them are reviews on drugs currently used in antituberculosis treatment, compounds undergoing clinical trials and new synthetic molecules with antimycobacterial activity [10,18]. The search for new anti tubercular drugs may be done using the biochemical and chemical methods. Anti tubercular agents or antimycobacterials fall into at least eleven categories according to their mechanisms of action. These categories and prominent examples are: fatty acid biosynthesis inhibitors such as isoniazid, arabinogalactan and peptidoglycan biosynthesis inhibitors with ethambutol as an example, inhibitors of protein synthesis like streptomycin, inhibitors of DNA-based processes such as rifampicin, inhibitors of dihydrofolate reductase or siderophore biosynthesis with *p*-aminosalicylic acid as an example, inhibitors of the proton pump F_0F_1 H^+ ATPase, for example. TMC 207, inhibitors of mycobacterial cytochrome P450 mono-oxygenase, FtsZ protein targeting compounds like carbamoyl-bearing pteridines and related pyridines, inhibitors of branched-chain amino acid biosynthesis, nucleoside monophosphate kinase inhibitors with pyrimidine or purine structure, and signaling kinase inhibitors [18]. It has been shown that substitution of carboxylate ester with aryl carboxamide group in the usual cardiovascular 1,4-dihydropyridines dramatically reduces calcium channel blocking activity but provides them significant antitubercular properties [19]. Preparation and antimycobacterial evaluation of some new 1,4-dihydropyridine-3,5-dicarboxamide derivatives with lipophilic groups were reported by Desai et al. [8] in 2001. They believed that these compounds may act as precursors, and after penetration into the cell wall may lead to the 3,5-carboxylate anions by enzymatic hydrolysis. Previous studies have shown moderate to good activity for phenyl or substituted phenyl at the C-4 position of 1,4-dihydropyridine-3,5-dicarboxamide compounds comparable to rifampicin [8,19–21]. There are a few reports about evaluation of 1,4-dihydropyridine-3,5-dicarboxamide derivatives containing heteroaromatic rings at the C-4 position. Recently Amini et al. reported the synthesis of new 1,4-dihydropyridine-3,5-dicarboxamide derivatives with a 4-(4,5-dichloroimidazole-2-yl) moiety [22]. The reported compounds were weak to moderate inhibitors of *M. tuberculosis* ($H_{37}Rv$) compared with rifampicin.

In order to understand the structure–antitubercular activity relationship, we decided to prepare some novel 1,4-dihydropyridine-3,5-dicarboxamide derivatives and determined their inhibitory activity against *M. tuberculosis* $H_{37}Rv$. The prepared compounds had 1-benzyl-2-methylthio-1*H*-imidazole-5-yl or 1-phenylamino-2-methylthio-1*H*-imidazole-5-yl at the C-4 position and various aryl carboxamide derivatives at C-3 and C-5 positions of the 1,4-dihydropyridine ring.

Here, we report the synthesis of 12 novel 4-substituted imidazolyl-2,6-dimethyl-N^3,N^5-bisaryl-1,4-dihydropyridine-3,5-dicarboxamides. Ten of these derivatives were screened for their antitubercular activity against *M. tuberculosis* ($H_{37}Rv$) (ATCC 27294; American type culture collection, Rockville, MD).

2. Results and discussion

2.1. Chemistry

1,4-Dihydropyridine-3,5-dicarboxamide derivatives **3a**–**l** were prepared in 55–86% from condensation of substituted imidazole-5-carboxaldehydes 1, appropriate *N*-aryl-acetoacetamide derivatives **2a**–**f** and ammonium acetate in absolute ethanol (Scheme 1).

Preparation of substituted imidazole-5-carbaldehydes **1** (X = NH or CH_2) is described [23]. *N*-Arylacetoacetamide derivatives **2a**–**f** were prepared according to modified method of Clemens by simple condensation of 2,2,6-trimethyl-1,3-dioxine-4-one with the appropriate arylamine [24]. Structural properties of the final compounds are summarized in Table 1.

The structures of the title compounds were confirmed by IR, ^1H NMR, ESI-MS mass spectrometry, and elemental analysis. Some of the characterization data of the prepared compounds are summarized in Table 2.

All compounds showed in the IR spectra a shouldered absorption band at 1645–1680 cm^{-1}, typical of the stretch vibrations of the two carboxamide C═O groups. The reason of shouldered absorption bond is that the two carboxamide groups are not the same due to different geometrical isomerism of the two carbonyl groups at C-3 and C-5 positions of the 1,4-dihydropyridine ring (Fig. 1).

The ^1H NMR spectra of the final compounds contained a singlet in the δ 1.95–2.22 ppm region due to CH_3 protons at C-2 and C-6 positions and another singlet at 5.05–5.20 ppm, due to the C-4 proton of the 1,4-dihydropyridine ring. These two singlets are indicatives for the presence of the 1,4-dihydropyridine ring. Other ^1H NMR spectral signals were in accordance with the proposed structures.

Two different modes, ESI+ and ESI−, were used in obtaining mass spectra, so molecular ions are M + H or M − H. In the case of compound **3l** there is a peak for M − H: 550.15 and a base peak at 586.15 which is an adduct ion ($M + Cl^-$) that was presumably an impurity of the solvent.

2.2. Antitubercular activity

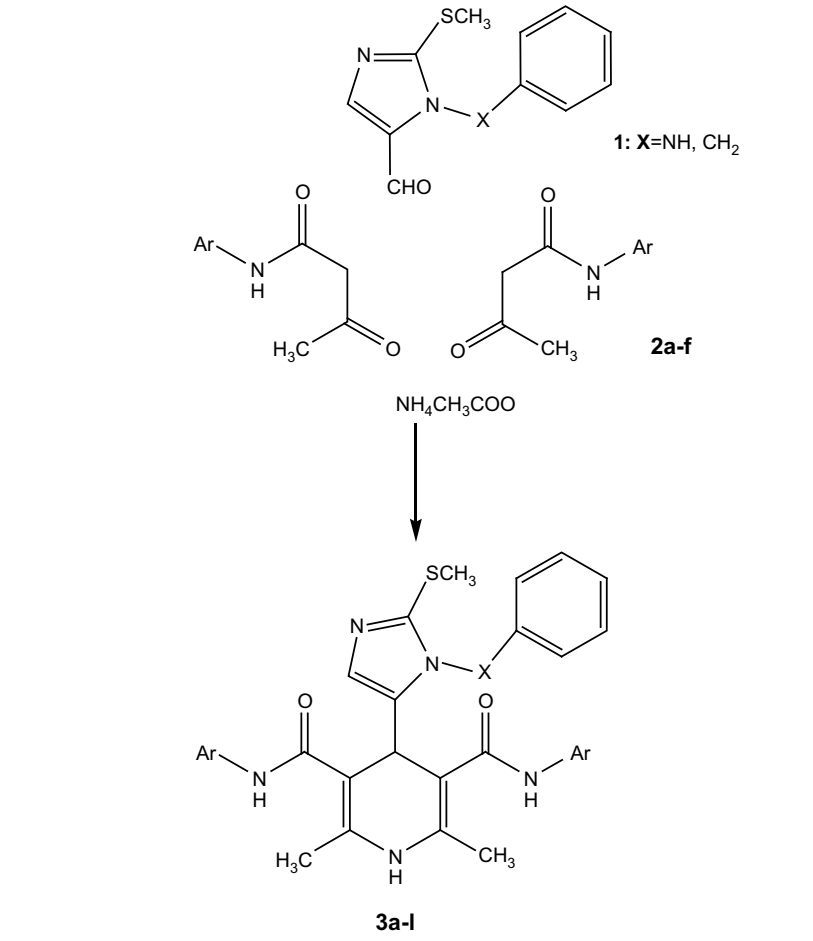
Ten compounds (**3a**–**i**, **3l**) were tested in vitro against *M. tuberculosis* $H_{37}RV$ strain ATCC 27294 which is susceptible to rifampicin and isoniazid. Minimum inhibitory concentration (MIC) was determined using agar proportion method in Middlebrook 7H10 medium. Rifampicin and isoniazid (Sigma Chemical Co.) were used as reference drugs. The antitubercular activity of the tested compounds is provided in Table 2.

2.3. MTT-based cytotoxicity assay

The cytotoxic effect of six compounds (**3a**, **3e**, **3i**, **3h**, **3k**, **3l**) against four different cell lines was determined by MTT assay method. Doxorubicin was used as a reference drug. Assay procedure and cell survival calculations were performed according to previous studies [25]. The cytotoxicity of compounds is listed in Table 3 as IC_{30} (μg/mL).

3. Conclusions

Comparison of the activities of tested compounds (**3a**–**i**, **3l**) indicates that compound **3i** with 1-benzyl-2-methylthio-1*H*-imidazole-5-yl substituent at the C-4 position and 4′-chlorophenyl group at C-3 and C-5 positions of the 1,4-dihydropyridine ring was the most potent one among the tested compounds. It was as potent as rifampicin against *M. tuberculosis* $H_{37}RV$. Compound **3l** also was an active antitubercular agent with MIC = 2 μg/mL with the same substituent as compound **3i** at the C-4 position and 3′-pyridyl group at C-3 and C-5 positions of the 1,4-dihydropyridine ring. The other substituents did not show good antitubercular activity. The results demonstrate that a substituted imidazole group is a suitable equivalent for nitro phenyl group which was previously reported in the structure of anti tubercular 1,4-dihydropyridinedicarboxamides [19].



3a-l

3a: Ar=2-Chlorophenyl, **X=**NH
3b: Ar=3-Chlorophenyl, **X=**NH
3c: Ar=4-Chlorophenyl, **X=**NH
3d: Ar=4-Bromophenyl, **X=**NH
3e: Ar=2-Pyridyl, **X=**NH
3f: Ar=3-Pyridyl, **X=**NH
3g: Ar=2-Chlorophenyl, **X=**CH_2
3h: Ar=3-Chlorophenyl, **X=**CH_2
3i: Ar=4-Chlorophenyl, **X=**CH_2
3j: Ar=4-Bromophenyl, **X=**CH_2
3k: Ar=2-Pyridyl, **X=**CH_2
3l: Ar=3-Pyridyl, **X=**CH_2

Scheme 1. Synthesis of 1,4-dihydropyridine3,5-dicarboxamide derivatives.

4. Experimental protocols

4.1. Chemistry

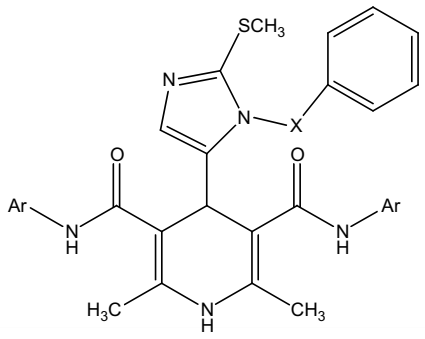
Chemicals from Merck and Sigma–Aldrich Chemical Co. were used without further purification. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel (60F254) plates. Column chromatography was performed on silica gel 60 (Merck, 70–235 mesh). Melting points were determined on a Mettler capillary melting point apparatus and were uncorrected. The IR spectra were recorded with a Perkin Elmer 1420 Ratio Recording IR spectrometer as a KBr disc (γ, cm^{-1}). The ^1H NMR spectra (DMSO-d_6) were recorded on a Bruker 300 MHz spectrometer. Chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane (TMS). Electrospray mass spectra (ESI-MS) were obtained in negative and positive ion mode on a SHIMADZU LCMS-2010 EV spectrometer using methanol and dimethylsulfoxide as solvent, a capillary voltage of 4500 V and a cone voltage of 10 V. Elemental microanalyses were within ±0.4% of the theoretical values for C, H and N. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plate using chloroform and methanol.

4.1.1. General procedure for the preparation of 1,4-dihydropyridine-3,5-dicarbamoyl derivatives (**3a**–**l**)

A mixture of 1-phenylamine (benzyl)-2-methylthio-1*H*-imidazole-5-carbaldehyde **1** (1 mmol), appropriate *N*-arylacetoacetanilide **2a**–**f** (2 mmol) and ammonium acetate (1 mmol) was refluxed in 6 mL of absolute ethanol for 24 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography using chloroform/methanol as eluent. Crystallization from acetone and petroleum ether gave pure compounds **3a**–**l**.

*4.1.1.1. 4-(2-(Methylthio)-1-(phenylamino)-1*H*-imidazol-5-yl)-2,6-dimethyl-*N^3,N^5*-di(2′-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (**3a**).* IR: (KBr) cm^{-1}: 3260 (NH), 1660 (C═O); ^1H NMR (DMSO-d_6): δ 2.22 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.49 (s, 3H, SCH_3), 5.11 [s, 1H, dihydropyridine C_4–H], 7.11 (t, $J = 7.50$ Hz, 2H,

Table 1
Structural properties of compounds **3a–l**.



Compd. no.	Ar	X	Mol. formula	Mol. weight
3a	2-Chlorophenyl	NH	$C_{31}H_{28}Cl_2N_6O_2S$	619.56
3b	3-Chlorophenyl	NH	$C_{31}H_{28}Cl_2N_6O_2S$	619.56
3c	4-Chlorophenyl	NH	$C_{31}H_{28}Cl_2N_6O_2S$	619.56
3d	4-Bromophenyl	NH	$C_{31}H_{28}Br_2N_6O_2S$	708.47
3e	2-Pyridyl	NH	$C_{29}H_{28}N_8O_2S$	552.65
3f	3-Pyridyl	NH	$C_{29}H_{28}N_8O_2S$	552.65
3g	2-Chlorophenyl	CH_2	$C_{32}H_{29}Cl_2N_5O_2S$	617.58
3h	3-Chlorophenyl	CH_2	$C_{32}H_{29}Cl_2N_5O_2S$	617.58
3i	4-Chlorophenyl	CH_2	$C_{32}H_{29}Cl_2N_5O_2S$	617.58
3j	4-Bromophenyl	CH_2	$C_{32}H_{29}Br_2N_5O_2S$	707.48
3k	2-Pyridyl	CH_2	$C_{30}H_{29}N_7O_2S$	551.66
3l	3-Pyridyl	CH_2	$C_{30}H_{29}N_7O_2S$	551.66

2 × $C_{5'}$–H), 7.29 (t, J = 7.50 Hz, 2H, 2 × $C_{4'}$–H), 7.43–7.58 (m, 8H, –NHC$_6$H$_5$, dihydropyridine NH, 2 × $C_{6'}$–H), 7.92 (d, J = 8.10, Hz, 2H, 2 × $C_{3'}$–H), 8.73 (s, 1H, imidazole C_4–H), 9.17 (s, 2H, 2 × amide –NH). ESI-MS (+) m/z (%): 620.2 ($M + H^+$) (5), 478.10 (100).

*4.1.1.2. 4-(2-(Methylthio)-1-(phenylamino)-1*H*-imidazol-5-yl)-2,6-di methyl-*N^3,N^5*-di(3′-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (**3b**).* IR: (KBr) cm^{-1}: 3260 (NH), 1680 (C═O); ^{1}H NMR (DMSO-d_6): δ 2.12 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.56 (s, 3H, SCH_3), 5.15 [s, 1H, dihydropyridine C_4–H], 7.04 (d, J = 7.80 Hz, 2H, 2 × $C_{6'}$–H), 7.29 (t, J = 8.10 Hz, 2H, 2 × $C_{5'}$–H), 7.44–7.55 (m, 8H, –NHC$_6$H$_5$, dihydropyridine NH, 2 × $C_{4'}$–H), 7.84 (s, 2H, 2 × $C_{2'}$–H), 8.48 (s, 1H, imidazole C_4–H), 9.81 (s, 2H, 2 × amide –NH). ESI-MS (+) m/z (%): 620.2 ($M + H^+$) (7), 393.15 (100).

*4.1.1.3. 4-(2-(Methylthio)-1-(phenylamino)-1*H*-imidazol-5-yl)-2,6-di methyl-*N^3,N^5*-di (4′-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (**3c**).* IR: (KBr) cm^{-1}: 3290 (NH), 1675 (C═O); ^{1}H NMR (DMSO-d_6): δ 2.12 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.55 (s, 3H, SCH_3), 5.14 [s, 1H, dihydropyridine C_4–H], 7.31 (d, J = 9.00 Hz, 4H, 2 × $C_{2'}$–H, $C_{6'}$–H), 7.44–7.55 (m, 5H, –NHC$_6$H$_5$), 7.65 (d, J = 9.00 Hz, 4H, 2 × $C_{3'}$–H, $C_{5'}$–H), 8.45 (s, 1H, imidazole C_4–H), 9.77 (s, 2H, 2 × amide –NH). ESI-MS (+): m/z 620.2 ($M + H^+$) (5), 478.10 (100).

*4.1.1.4. 4-(2-(Methylthio)-1-(phenylamino)-1*H*-imidazol-5-yl)-2,6-di methyl-*N^3,N^5*-di (4′-bromophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (**3d**).* IR: (KBr) cm^{-1}: 3300 (NH), 1660 (C═O); ^{1}H NMR (DMSO-d_6): δ 2.07 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.49 (s, 3H, SCH_3), 5.14 [s, 1H, dihydropyridine C_4–H], 7.43–7.62 (m, 15H, dihydropyridine NH, –NHC$_6$H$_5$, –NHC$_6$H$_5$, 2 × –C$_6$H$_4$Br), 8.45 (s, 1H, imidazole C_4–H), 9.76 (s, 2H, 2 × amide –NH). ESI-MS (+) m/z (%): 709 ($M + H^+$) (5), 393.20 (100).

*4.1.1.5. 4-(2-(Methylthio)-1-(phenylamino)-1*H*-imidazol-5-yl)-2,6-di methyl-*N^3,N^5*-di(pyridin-2-yl)-1,4-dihydropyridine-3,5-dicarboxamide (**3e**).* IR: (KBr) cm^{-1}: 3300 (NH), 1660 (C═O); ^{1}H NMR (DMSO-d_6): δ 2.16 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.71 (s, 3H, SCH_3), 5.20 [s, 1H, dihydropyridine C_4–H], 7.02 (t, J = 6.00 Hz, 2H, 2 × pyridine C_5–H), 7.40–7.56 (m, 6H, –NHC$_6$H$_5$, dihydropyridine NH), 7.72 (t, J = 7.80 Hz, 2H, 2 × pyridine C_4–H), 8.06 (d, J = 8.40 Hz, 2H, 2 × pyridine C_6–H), 8.26–8.30 (m, 2H, 2 × pyridine C_3–H), 8.68 (s, 1H, imidazole C_4–H), 10.40 (s, 2H, 2 × amide –NH). ESI-MS (+) m/z (%): 553.20 ($M + H^+$) (10), 539.20 (100).

*4.1.1.6. 4-(2-(Methylthio)-1-(phenylamino)-1*H*-imidazol-5-yl)-2,6-di methyl-*N^3,N^5*-di(pyridin-3-yl)-1,4-dihydropyridine-3,5-dicarboxamide (**3f**).* IR: (KBr) cm^{-1}: 3280 (NH), 1650 (C═O); ^{1}H NMR (DMSO-d_6): δ 2.14 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.49 (s, 3H, SCH_3), 5.20 [s, 1H, dihydropyridine C_4–H], 7.30 (dd, J = 8.16 Hz, J = 4.66 Hz, 2H, 2 × pyridine C_5–H), 7.40–7.55 (m, 6H, –NHC$_6$H$_5$, dihydropyridine NH), 8.04 (d, J = 8.40 Hz, 2H, 2 × pyridine C_4–H), 8.20 (d, J = 4.50 Hz, 2H, 2 × pyridine C_6–H), 8.50 (s, 1H, imidazole C_4–H), 8.76 (s, 2H, 2 × pyridine C_2–H), 9.82 (s, 2H, 2 × amide –NH). ESI-MS (+) m/z (%): 553.20 ($M + H^+$) (7), 437.20 (100).

*4.1.1.7. 4-(1-Benzyl-2-(methylthio)-1*H*-imidazol-5-yl)-2,6-dimethyl-N^3,N^5-bis (2′-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (**3g**).* IR: (KBr) cm^{-1}: 3350 (NH), 1660 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.95 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.35 (s, 3H, SCH_3), 5.20 (s,

Table 2
Characterization data and antitubercular screening results of the compounds **3a–l**.

Compd. no.	M.P. (°C)	Yield (%)	Analysis (%)			MIC (μg/mL)
			Found (calculated)			
			C	H	N	
3a	230–231	86	60.22 (60.10)	4.44 (4.56)	13.78 (13.56)	>16
3b	231–232	81	59.95 (60.10)	4.36 (4.56)	13.65 (13.56)	>16
3c	221–223	73	59.89 (60.10)	4.55 (4.56)	13.45 (13.56)	>16
3d	239–240	76	52.21 (52.55)	4.02 (3.98)	12.12 (11.86)	>16
3e	221–223	86	63.37 (63.03)	4.98 (5.11)	20.54 (20.28)	>16
3f	198–199	60	63.26 (63.03)	4.86 (5.11)	20.12 (20.28)	>16
3g	235–236	88	59.95 (62.13)	4.65 (4.73)	11.45 (11.32)	>16
3h	238–239	80	62.45 (62.13)	4.45 (4.73)	11.64 (11.32)	>16
3i	218–220	73	59.03 (62.13)	4.82 (4.73)	11.21 (11.32)	1
3j	240–242	73	54.71 (54.33)	3.96 (4.13)	10.12 (9.90)	ND[a]
3k	211–212	85	64.93 (65.32)	5.58 (5.30)	18.01 (17.77)	ND
3l	200–201	55	65.12 (65.32)	5.06 (5.30)	17.72 (17.77)	2
Rifampicin	–	–	–	–	–	1
Isoniazide	–	–	–	–	–	0.2

[a] ND: Not determined.

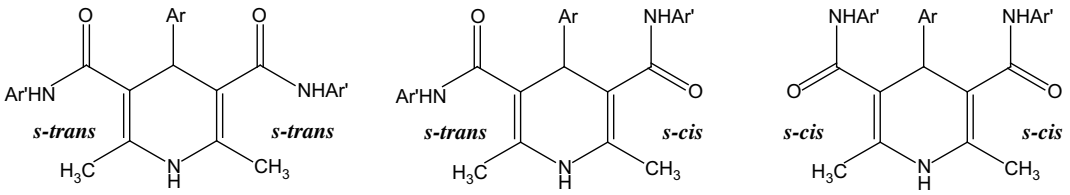


Fig. 1. Different geometrical isomerisms of the two carbonyl groups of 1,4-dihydropyridinedicarboxamides.

2H, $-CH_2-C_6H_5$), 5.23 (s, 1H, dihydropyridine C_4–H), 6.92 (m, 3H, C_2–H, C_4–H, C_6–H of $-CH_2-C_6H_5$), 7.10–7.16 (m, 5H, C_3–H and C_5–H of $-CH_2-C_6H_5$, dihydropyridine NH, 2 × $C_{5'}$–H), 7.29 (t, $J = 8.10$ Hz, 2H, 2 × $C_{4'}$–H), 7.44 (d, $J = 7.80$ Hz, 2H, 2 × $C_{6'}$–H), 7.62 (d, $J = 8.10$, Hz, 2H, 2 × $C_{3'}$–H), 8.44 (s, 1H, imidazole C_4–H), 8.80 (s, 2H, 2 × amide –NH). ESI-MS (+) *m*/*z* (%): 618.15 ($M + H^+$) (100).

*4.1.1.8. 4-(1-Benzyl-2-(methylthio)-1*H*-imidazol-5-yl)-2,6-dimethyl-*N^3,N^5*-bis (3′-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (**3h**).* IR: (KBr) cm^{-1}: 3335 (NH), 1645 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.98 (s, 6H, dihydropyridine C_2,C_6, CH_3), 2.27 (s, 3H, SCH_3), 5.04 [s, 1H, dihydropyridine C_4–H], 5.17 (s, 2H, $-CH_2-C_6H_5$), 6.8–6.84 (m, 3H, C_2–H, C_4–H, C_6–H of $-CH_2-C_6H_5$), 6.99–7.06 (m, 5H, dihydropyridine NH, C_3–H, C_5–H of $-CH_2-C_6H_5$, 2 × $C_{6'}$–H), 7.27 (t, $J = 8.10$ Hz, 2H, 2 × $C_{5'}$–H), 7.42 (d, $J = 8.40$ Hz, 2 × $C_{4'}$–H), 7.70 (s, 2H, 2 × $C_{2'}$–H), 8.38 (s, 1H, imidazole C_4–H), 9.57 (s, 2H, 2 × amide –NH). ESI-MS (+) *m*/*z* (%): 617.95 ($M + H^+$) (100).

*4.1.1.9. 4-(1-Benzyl-2-(methylthio)-1*H*-imidazol-5-yl)-2,6-dimethyl-*N^3,N^5*-bis (4′-chlorophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (**3i**).* IR: (KBr) cm^{-1}: 3180 (NH), 1650 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.97 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.27 (s, 3H, SCH_3), 5.04 [s, 1H, dihydropyridine C_4–H], 5.15 (s, 2H, $-CH_2-C_6H_5$), 6.82–6.85 (m, 2H, C_3–H and C_5–H of $-CH_2-C_6H_5$), 7.00–7.02 (m, 3H, C_2–H, C_4–H, C_6–H of $-CH_2-C_6H_5$), 7.30 (d, $J = 8.70$ Hz, 4H, 2 × $C_{2'}$–H,$C_{6'}$–H), 7.55 (d, $J = 8.70$ Hz, 4H, 2 × $C_{3'}$–H,$C_{5'}$–H) 8.32 (s, 1H, imidazole C_4–H), 9.51 (s, 2H, 2 × amide –NH). ESI-MS (+) *m*/*z* (%): 618.05 ($M + H^+$) (100).

*4.1.1.10. 4-(1-Benzyl-2-(methylthio)-1*H*-imidazol-5-yl)-2,6-dimethyl-*N^3,N^5*-bis (4′-bromophenyl)-1,4-dihydropyridine-3,5-dicarboxamide (**3j**).* IR: (KBr) cm^{-1}: 3200 (NH), 1660 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.96 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.26 (s, 3H, SCH_3), 5.03 [s, 1H, dihydropyridine C_4–H], 5.15 (s, 2H, $-CH_2-C_6H_5$), 6.79 (s, 1H, dihydropyridine NH), 6.85 (m, 2H, C_3–H and C_5–H of $-CH_2-C_6H_5$), 7.02 (m, 3H, C_2–H, C_4–H, C_6–H of $-CH_2-C_6H_5$), 7.42 (d, $J = 9.00$ Hz, 4H, 2 × $C_{3'}$–H,$C_{5'}$–H), 7.50 (d, $J = 9.00$ Hz, 4H, 2 × $C_{2'}$–H,$C_{6'}$–H), 8.33 (s, 1H, imidazole C_4–H), 9.51 (s, 2H, 2 × amide –NH). ESI-MS (+) *m*/*z* (%): 707.95 ($M + H^+$) (100).

Table 3
Cytotoxicity assay results of six of the prepared compounds.

Compound	Cell line			
	Jurkat	Raji	Hela	Kga-1
3a	7.76[a]	43.65	>100	>100
3e	13.48	>100	67.6	>100
3i	12.30	85.11	>100	58.88
3h	47.86	69.18	>100	26.3
3k	43.65	60.25	>100	19.051
3l	37.15	75.85	91.2	27.54
Doxorubicin	2.13	1.51	ND[b]	ND

[a] IC_{30} (μg/mL).
[b] Not determined.

*4.1.1.11. 4-(1-Benzyl-2-(methylthio)-1*H*-imidazol-5-yl)-2,6-dimethyl-*N^3,N^5*-bis(pyridin-2-yl)-1,4-dihydropyridine-3,5-dicarboxamide (**3k**).* IR: (KBr) cm^{-1}: 3420 (NH), 1675 (C═O); ^{1}H NMR (DMSO-d_6): δ 1.94 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.30 (s, 3H, SCH_3), 5.05 (s, 2H, $-CH_2-C_6H_5$), 5.29 (s, 1H, dihydropyridine C_4–H), 6.68 (m, 3H, C_2–H, C_4–H, C_6–H of $-CH_2-C_6H_5$), 6.91–6.97 (m, 3H, C_3–H and C_5–H of $-CH_2-C_6H_5$, dihydropyridine NH), 7.06 (t, $J = 6.00$ Hz, 2H, 2 × pyridine C_5–H), 7.73 (t, $J = 7.50$ Hz, 2H, 2 × pyridine C_4–H), 8.00 (d, $J = 8.40$ Hz, 2H, 2 × pyridine C_6–H), 8.27–8.30 (m, 2H, 2 × pyridine C_3–H), 8.43 (s, 1H, imidazole C_4–H), 10.17 (s, 2H, 2 × amide –NH). ESI-MS (−) *m*/*z* (%): 550.00 ($M - H^+$) (100).

*4.1.1.12. 4-(1-Benzyl-2-(methylthio)-1*H*-imidazol-5-yl)-2,6-dimethyl-*N^3,N^5*-bis(pyridin-3-yl)-1,4-dihydropyridine-3,5-dicarboxamide (**3l**).* IR: (KBr) cm^{-1}: 3200 (NH), 1660 (C═O); ^{1}H NMR (DMSO-d_6): δ 2.00 (s, 6H, dihydropyridine C_2, C_6, CH_3), 2.27 (s, 3H, SCH_3), 5.11 (s, 1H, dihydropyridine C_4–H), 5.18 (s, 2H, $-CH_2-C_6H_5$), 6.81–6.84 (m, 3H, C_2–H, C_4–H, C_6–H of $-CH_2-C_6H_5$), 6.97–6.98 (m, 3H, C_3–H and C_5–H of $-CH_2-C_6H_5$, dihydropyridine NH), 7.28 (dd, $J = 8.16$ Hz, $J = 4.66$ Hz, 2H, 2 × pyridine C_5–H), 7.95 (d, $J = 8.40$ Hz, 2H, 2 × pyridine C_4–H), 8.20 (d, $J = 4.00$ Hz, 2H, 2 × pyridine C_6–H), 8.40 (s, 1H, imidazole C_4–H), 8.67 (s, 1H, 2 × pyridine C_2–H), 9.63 (s, 2H, 2 × amide –NH). ESI-MS (−) *m*/*z* (%): 550.15 ($M - H^+$) (22), 586.15 (100).

4.2. Antitubercular activity

Ten compounds (**3a**–**i**, **3l**) were tested in vitro against *M. tuberculosis* H_{37}RV strain ATCC 27294 which is susceptible to rifampicin and isoniazid. Minimum inhibitory concentration (MIC) was determined using agar proportion method in Middlebrook 7H10 medium. Rifampicin and isoniazid (Sigma Chemical Co.) were used as a reference drugs at 1 μg/mL and 0.2 μg/mL, respectively. Solutions of compounds in dimethylsulfoxide were added to the Middlebrook 7H10 Agar (Quelab Co., UK) medium with glycerol and enriched with OADC (Oleic acid, Albumin, Dextrose Catalase). The following concentrations were used: 0.05, 0.1, 0.2, 0.5, 1, 2, 4, 8 and 16 μg/mL. A culture of *M. tuberculosis* H37Rv cultivated in 7H9 broth (Quelab Co., UK) at 37 °C for a period of 4–7 days, was adjusted, using the same medium, to the optical density of McFarland standard no. 1. Two dilutions of this suspension, 10^{-2} and 10^{-4}, were used as an inoculum, 0.1 mL per each tubes. MIC values were determined after incubation at 37 °C in the presence of 5–7% CO_2 for a period of 21 days. The colonies on each tube were counted, and the numbers of colonies on drug-containing tubes were compared with that on the drug-free control [26,27]. The present results were obtained from two independent measurements.

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