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Original article

Synthesis, antibacterial and antimycobacterial activities of some new 4-aryl/heteroaryl-2,6-dimethyl-3,5-bis-N-(aryl)-carbamoyl-1,4-dihydropyridines

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ABSTRACT

A novel class of 4-aryl/heteroaryl-2,6-dimethyl-3,5-bis-N-(phenyl/substituted phenyl)-carbamoyl-1,4-dihydropyridines has been synthesized by simple, economical and eco-friendly, modified Hantzsch condensation reaction making use of N-arylacetoacetamides, aryl or heteroaryl aldehydes and ammonium acetate. The newly synthesized compounds were characterized by their spectral (IR, ^{1}H NMR, Mass), elemental analyses data and evaluated for in vitro antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ ATCC 27294 and antibacterial activity against different Gram +ve and Gram −ve bacteria. The preliminary screening results revealed that some of the compounds possess promising antimicrobial activity. Amongst the new series of compounds, **6m** containing pyrrolyl and 4-methylphenyl groups and **6r** possessing 2-pyridyl and 2-methylphenyl groups were found to exhibit a significant antitubercular activity (MIC = 12.5–25 μg/mL) in comparison with the first line drug pyrazinamide.

1. Introduction

Tuberculosis (TB), the 'King of Diseases' is one of the most important global infectious diseases known to the mankind. TB is an ancient enemy and present threat that ranks amongst the foremost killers of the 21st century. *Mycobacterium tuberculosis*, a human pathogen causing TB infects approximately one-third of the world's population, kills more than 2 million a year [1]. The World Health Organization has declared TB to be a 'global emergency' and estimates that about 30 million people will be infected by *M. tuberculosis* within the next 20 years [2]. The incidence of TB infection has steadily risen in the last decade. The reemergence of TB infection has been linked to co-infection with the human immunodeficiency virus (HIV) [3] and to the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *M. tuberculosis* strains which are resistant to the available therapies [4]. Multidrug-resistant TB and extensively drug-resistant TB are refractory to treatment with any of the commonly available drugs, warranting the need for new classes of drugs to counter effectively the threat posed by these strains [5]. Thus to overcome the menace of MDR-TB and XDR-TB, there is an urgent need for the development of new chemical entities with novel mechanisms of action [6].

In this regard, it could be noticed that 1,4-dihydropyridines (1,4-DHPs) are one of the emerging classes of antitubercular agents [7–9]. Recent studies have shown that the 3,5-dicarbamoyl derivatives of 1,4-DHPs containing lipophilic groups possess considerable antitubercular activity against *M. tuberculosis* $H_{37}Rv$ [10,11]. Such compounds were believed to act as precursors, and after penetration into the cell wall, convert to their 3,5-carboxylate anions by enzymatic hydrolysis. It has been reported that the substitution of ester with N-aryl carbamoyl group at 3- and 5-positions of 1,4-DHPs known for cardiovascular property, results in a dramatic reduction in the calcium channel antagonistic activity with an increase in antitubercular activity [12]. Quite recently, Manvar et al. reported that the presence of a phenyl or substituted phenyl group at 4-position and as N-substituents at 3- and 5-positions of the 3,5-dicarbamoyl1,4-DHPs resulted in moderate to good antitubercular activity, in comparison to rifampicin [13]. It has been also reported that DHPs with an electron-donating $-OCH_3$ group and that too being at ortho- or 2-position of the carbamoyl phenyl substituent exhibited highest antitubercular activity (93%). Recently, some new 3,5-bis-N-aryl carbamoyl1,4-DHPs containing 4,5-dichloroimidazole-2-yl moiety at 4-position were reported as weak to moderate inhibitors of *M. tuberculosis* ($H_{37}Rv$) as compared to rifampicin [14]. Similarly, some new 3,5-dicarbamoyl1,4-DHPs containing substituted imidazolyl moieties at 4-position and

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various substituted phenyl or pyridyl carboxamide groups at 3- and 5-positions of the 1,4-DHP ring were also reported to exhibit significant antitubercular activity [15]. It was shown that a substituted imidazole group is a suitable equivalent for nitrophenyl group which was previously reported to be responsible for the significant antitubercular activity. Literature reveals that some 3,5-dicarbamoyl1,4-DHPs to also possess weak to moderate antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* [16]. Gunics et al. [17] have reported that some 4-aryl-3,5-diacetyl-2,6-dimethyl-1,4-dihydropyridines with phenyl group having 4-chloro or 4-thiomethyl substituents at their 4-position showed synergistic antibacterial effect with Erythrocin while four more DHPs exhibited additive effect on MDR clinical isolates of *Escherichia coli* Recently, the synthesis, antitubercular and antimicrobial (antibacterial and antifungal) activity of some N-aryl-1,4-dihydropyridine derivatives has been reported by Rokad et al. [18]. Furthermore, it has been reported that the compounds possessing heterocyclic systems such as pyridine [19], imidazole [20], thiophene [21], furan [22], pyrrole [23] or substituted phenyls [24] were associated with potent bioactivities including antimicrobial [25,26] and antitubercular activities [27–29]. Thus, in continuation of our studies on novel 1,4-DHPs [30–32], it was felt worthwhile to synthesize some new 1,4-DHPs containing the above specified putative pharmacophoric heterocyclic moieties at 4-position of 1,4-DHP ring, and evaluate them for their possible antitubercular and antibacterial activities. In order to derive at a meaningful structure activity relationship, different substituted phenyl moieties are chosen on the carbamoyl nitrogens at 3- and 5-positions. Herein, we present rapid, facile and high yielding syntheses of 24 new 4-aryl/heteroaryl-2,6-dimethyl-3,5-bis-N-(phenyl/substituted phenyl) carbamoyl-1,4-dihydropyridines (**6a**–**x**), along with the data on their antitubercular and antibacterial evaluation.

2. Results and discussion

2.1. Chemistry

The key intermediates, N-arylacetoacetamides (**3a**–**c**) were synthesized by the condensation of ethyl acetoacetate (**1**) with appropriate aryl amines (**2**) in the presence of a trace of catalyst, potassium *tert*-butoxide in ethanol, adopting the reported procedure [31]. This reaction was also carried out by the microwave irradiation method to obtain the respective N-arylacetoacetamides, within 3–5 min. The results are presented in Table 1. Cyclocondensation of the N-arylacetoacetamides (**3a**–**c**) with eight different aldehydes (**4a**–**h**) and ammonium acetate (**5**) under different experimental conditions, viz.; conventional heating, barium nitrate-catalyzed one-pot synthesis [33], microwave irradiation in solvent and solvent-free conditions, afforded a single product in each case, which have been characterized as 4-substituted-2,6-dimethyl-3,5-bis-N-(phenyl/substituted phenyl)-carbamoyl-1,4-dihydropyridines (**6a**–**x**) (Scheme 1), based on their spectral (IR, ^{1}H NMR, Mass) and elemental analyses data as presented in Table 2.

The percentage yields of N-arylacetoacetamides (**3a**–**c**) have been improved considerably with the presence of potassium *tert*-butoxide. Among the four sets of reaction conditions employed for the synthesis of DHPs, the microwave irradiation solvent-free method on a solid support of silica gel or acidic alumina and the microwave heating in a solvent have been found to be almost identical and offered the advantage of being eco-friendly, simple in operation with increased reaction rates over the conventional method. The second in the order has been the barium nitrate-catalyzed one-pot synthesis. Barium nitrate is safe, commercially inexpensive reagent and the reaction in absence of organic solvent makes the process eco-friendly.

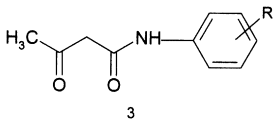
2.2. Antibacterial activity in normal and resistant bacteria

The in vitro antibacterial assay of the compounds (**6a**–**x**) was performed by the disc-diffusion method [34] in nutrient agar medium, at three different concentrations, viz., 12.5, 25 and 50 μg mL^{-1} using two Gram-positive strains: *B. subtilis* (NCIM-2063,ATCC No.6633), *S. aureus* (NCIM-2079,ATCC No.6538P) and two Gram-negative strains: *E. coli* (NCIM-2068,ATCC No.11105), *Proteus vulgaris* (NCIM-2027,ATCC No.13315). The compounds with significant antibacterial activity (**6a**, **6h**, **6j**, **6m** and **6r**) were then tested against *methicillin-resistant S. aureus* (MRSA) at a concentration of 1 mg mL^{-1} (1000 μg mL^{-1}). Tetracyclin and streptomycin were used as reference drugs for comparison and the solvent DMSO as the control. Each compound was assessed in triplicate and an average of the results for each of the active compounds is presented in Table 3. The results obtained indicate that most of these compounds were relatively more active against Gram-negative bacteria. The compound **6m** with 2-pyrrolyl group at 4-position and 4-methylphenyl group as the N-substituent at 3- and 5-positions was almost equipotent to streptomycin/tetracyclin against *E. coli*. However, the compound **6h** with 4-ethylphenyl group at 4-position and phenyl group as N-substituent at 3- and 5- positions was relatively more potent against *B. subtilis*. Compounds **6a**, **6m** and **6r** were also found to show significant inhibition against MRSA (8–10 mm), at 1 mg/mL concentration.

2.3. Antitubercular activity in sensitive and resistant *Mycobacterium tuberculosis*

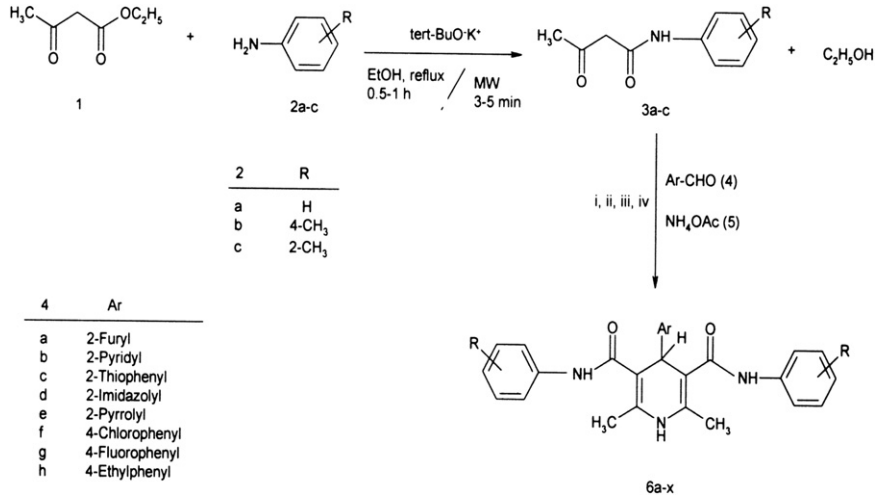
Five compounds (**6a**, **6h**, **6j**, **6m** and **6r**) which exhibited significant antibacterial activity (Table 3) and which showed good

Table 1
Physical and analytical data of Intermediates **3a**–**c**.



3

Compound	Substituent R	Mol. formula (Mol. Wt)	m.p (°C)	Conventional method Yield (%) (Period/h)	Microwave method Yield (%) (Period/min)
3a	H	$C_{10}H_{11}NO_2$ (177.20)	84–86	75 (0.5)	82 (3.0)
3b	4- CH_3	$C_{11}H_{13}NO_2$ (191.23)	88–90	80 (1.0)	85 (5.0)
3c	2- CH_3	$C_{11}H_{13}NO_2$ (191.23)	108–110	72 (0.5)	78 (4.0)



Scheme 1. Synthesis of compounds **6a**–**x**. Reagents and conditions : (i) EtOH, reflux, 20–30 h (ii) 5 mol% $Ba(NO_3)_2$, △, 30–45 min (iii) MWI, EtOH, 4–6 min (iv) Silica gel or acidic alumina, MWI, 4–6min.

clog *P* values amongst the series were tested in vitro against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294, susceptible to rifampicin, pyrazinamide and isoniazid), at graded concentrations: 6.25, 12.5, 25, 50 and 100 μg mL^{-1} and also evaluated against a clinical isolate of multidrug-resistant *M. tuberculosis* (MDR-T.B, C110/09, resistant to rifampicin, pyrazinamide and isoniazid) at concentrations: 64, 128 and 256 μg mL^{-1} using the LJ proportion method [35]. The activity is expressed as minimum inhibitory concentration (MIC), i.e., the lowest concentration of compound that completely inhibited the growth on the culture. The results indicated that some of the tested compounds were promising candidates with uniquely good activity against both sensitive and resistant strains of *M. tuberculosis* ($H_{37}Rv$ and C110/09). The results of three independent determinations are given in Table 4. The data revealed compound **6m** with 2-pyrrolyl group at 4-position and 4-methylphenyl group as the N-substituent at 3- and 5- positions as the most potent one amongst the tested compounds. It was as potent as pyrazinamide against *M. tuberculosis* C110/09 and more potent than pyrazinamide against *M. tuberculosis* $H_{37}Rv$. However, similar compounds with 4-hydroxyphenyl or2-chlorophenyl substitution at 4-position of DHP have earlier been reported to exhibit weak antitubercular activity [13]. Compound **6r** with 2-pyridyl group at 4-position and 2-methylphenyl group as the N-substituent at 3- and 5-positions was also found to exhibit higher antitubercular activity (MIC = 25 μg mL^{-1}) in comparison to pyrazinamide (MIC = 32 μg mL^{-1}) against *M. tuberculosis* $H_{37}Rv$.

3. Conclusions

The novel synthetic methodologies adopted resulted in new 1,4-dihydropyridine-3,5-dicarbamoyl derivatives, in high yields. These new DHPs, based on previous reports were evaluated for their antibacterial and antitubercular activities. The study indicated that the replacement of ester groups at 3- and 5- positions with N-aryl carbamoyl groups in DHPs results in a significant increase in their antimycobacterial activity. Comparison of the antibacterial and antitubercular activities of the compounds **6a**–**x** showed that two compounds: **6m** and **6r** possess potent antibacterial and antitubercular activities. The new DHP **6m** having a 2-pyrrolyl substituent at 4-position and 4-methylphenyl substituent at 3,5-dicarbamoyl nitrogen has been found to be superior in antitubercular activity in comparison to others of the series. This particular substitution pattern, particularly at 3,5-N-phenyl groups, partly is in agreement with the reports of Desai et al. [11] to the extent that para-substitution in phenyl group is favorable for better antitubercular potency since it may influence the enzymatic hydrolysis of carbamoyl groups to carboxylate ions on their penetration into the mycobacterial cell.

4. Experimental

4.1. Chemistry

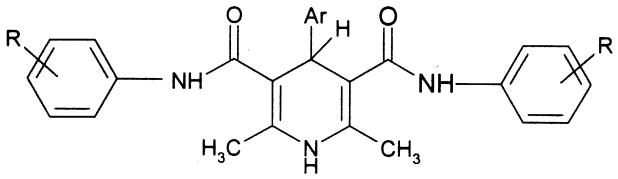
Melting points were determined in open capillaries using Toshniwal electrical melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FTIR-8700 spectrometer in KBr discs. ^1H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 with tetramethylsilane (TMS) as an internal standard on a Bruker 80 MHz FT-NMR (300 MHz) spectrometer and the chemical shifts are reported as δ (ppm). The ^{13}C NMR spectra of some representative compounds were recorded in $CDCl_3$ or DMSO on AVANCE 300 MHz NMR spectrometer. Mass spectra were recorded on a GC-MS QP-1100 Shimadzu instrument (70 eV). Elemental analyses (C, H, N) of the compounds were obtained from Perkin Elmer 240B analyzer and were within ±0.4% of theoretical values. The "Little Chef" domestic microwave oven (LG) was used for microwave irradiation. The progress of the reactions and purity of the products were checked by thin-layer chromatography (TLC) on precoated silica gel 60 F_{254} aluminum plates (Merck, Germany). Column chromatography was performed on neutral aluminum oxide 90 (activity I-II, 70–230 mesh. ASTM, Merck).

4.1.1. General procedure for the synthesis of N-arylacetoacetamides (*3a*–*c*)

Conventional method: A mixture of ethyl acetoacetate (1, 1.3 g, 10 mmol), an appropriate amine (**2a**–**c**, 10 mmol) and a catalytic amount of potassium tert-butoxide was taken into a 250 mL RB flask and dissolved in 25 mL of ethanol. The reaction mixture was heated under reflux for 0.5–1 h. The solvent was removed from the reaction mixture to a possible extent, under reduced pressure and the residue was cooled and triturated with dry ether. The product was filtered and washed with small portions of dry ether. Purification was effected by recrystallization from ethanol to obtain colorless crystalline solid.

Microwave method: A mixture of ethyl acetoacetate (1, 1.3 g, 10 mmol), an appropriate amine (**2a**–**c**, 10 mmol) and a catalytic

Table 2
Physical and analytical data of 1,4-DHPs **6a**–**x**.



6a-x

Compd	R	Ar	Mol. formula (Mol. wt)	m.p (°C)	Conventional method[a]	Barium nitrate catalyzed method[b]	Microwave method[c]		
					Yield (%) (Period/h)	Yield (%) (Period/min)	Method I Yield (%)	Method II Yield (%)	Method I and II (Period/min)
6a	H	2-Furyl	$C_{25}H_{23}N_3O_3$ (413.464)	260 (dec)	70 (20.0)	78 (35.0)	80	84	4.0
6b	H	2-Pyridyl	$C_{26}H_{24}N_4O_2$ (424.472)	242–244	60 (25.0)	65 (30.0)	75	80	4.0
6c	H	2-Thienyl	$C_{25}H_{23}N_3O_2S$ (429.454)	246 (dec)	68 (25.0)	75 (40.0)	80	82	4.0
6d	H	2-Imidazolyl	$C_{24}H_{23}N_5O_2$ (413.444)	252 (dec)	65 (30.0)	68 (40.0)	70	72	4.0
6e	H	2-Pyrrolyl	$C_{25}H_{24}N_4O_2$ (412.462)	208–210	72(30.0)	75(35.0)	80	78	4.0
6f	H	4-ClC_6H_4	$C_{27}H_{24}ClN_3O_2$ (457.982)	292 (dec)	82 (25.0)	85 (40.0)	85	85	4.5
6g	H	4-FC_6H_4	$C_{27}H_{24}FN_3O_2$ (441.482)	282–284	74 (30.0)	79 (45.0)	84	82	4.5
6h	H	4-$C_2H_5C_6H_4$	$C_{29}H_{29}N_3O_2$ (451.532)	266 (dec)	78 (30.0)	82 (40.0)	80	78	4.0
6i	4-CH_3	2-Furyl	$C_{27}H_{27}N_3O_3$ (441.516)	176–180	68 (30.0)	72 (40.0)	70	72	4.5
6j	4-CH_3	2-Pyridyl	$C_{28}H_{28}N_3O_2$ (438.524)	238–240	75 (30.0)	78 (45.0)	80	80	5.0
6k	4-CH_3	2-Thienyl	$C_{27}H_{27}N_3O_2S$ (457.506)	220 (dec)	76 (25.0)	78 (40.0)	80	78	4.5
6l	4-CH_3	2-Imidazolyl	$C_{26}H_{27}N_5O_2$ (440.488)	262–264	84 (30.0)	86 (40.0)	88	86	4.5
6m	4-CH_3	2-Pyrrolyl	$C_{27}H_{28}N_4O_2$ (440.514)	234 (dec)	76 (30.0)	78 (45.0)	78	80	5.0
6n	4-CH_3	4-ClC_6H_4	$C_{29}H_{28}ClN_3O_2$ (486.034)	238 (dec)	78 (30.0)	82 (40.0)	85	85	4.5
6o	4-CH_3	4-FC_6H_4	$C_{29}H_{28}FN_3O_2$ (469.534)	240 (dec)	82 (25.0)	85 (40.0)	84	80	4.5
6p	4-CH_3	4-$C_2H_5C_6H_4$	$C_{31}H_{33}N_3O_2$ (479.594)	272–274	73 (30.0)	75 (40.0)	78	78	5.5
6q	2-CH_3	2-Furyl	$C_{27}H_{27}N_3O_3$ (441.516)	210–212	68 (25.0)	73 (40.0)	80	82	5.0
6r	2-CH_3	2-Pyridyl	$C_{28}H_{28}N_3O_2$ (438.524)	258–260	70 (28.0)	72 (40.0)	75	72	4.5
6s	2-CH_3	2-Thienyl	$C_{27}H_{27}N_3O_2S$ (457.506)	224–226	62 (29.0)	70 (40.0)	70	75	4.5
6t	2-CH_3	2-Imidazolyl	$C_{26}H_{27}N_5O_2$ (440.488)	268–270	82 (30.0)	84 (40.0)	85	88	5.0
6u	2-CH_3	2-Pyrrolyl	$C_{27}H_{28}N_4O_2$ (440.514)	218–220	55 (30.0)	58 (45.0)	68	65	6.0
6v	2-CH_3	4-ClC_6H_4	$C_{29}H_{28}ClN_3O_2$ (486.034)	256 (dec)	72 (25.0)	75 (35.0)	80	82	5.0
6w	2-CH_3	4-FC_6H_4	$C_{29}H_{28}FN_3O_2$ (469.534)	278–280	76 (30.0)	74 (38.0)	65	60	4.5
6x	2-CH_3	4-$C_2H_5C_6H_4$	$C_{31}H_{33}N_3O_2$ (479.594)	238 (dec)	75 (30.0)	78 (30.0)	82	82	4.0

[a] Reflux in ethanol for 40–45 h.
[b] Heating in presence of $Ba(NO_3)_2$ for 30–45 min.
[c] Microwave irradiation in solvent (method I) and solvent-free (method II).

amount of potassium tert-butoxide was taken into a 250 mL Pyrex beaker with an inverted glass funnel and irradiated in a domestic microwave oven for 3–5 min with 30 s pulses at 480W while monitoring the progress of the reaction by TLC. On completion of the reaction, the reaction mixture was cooled and triturated with ice-cold ether. The product separated was filtered, washed with small portions of ice-cold ether and dried. Purification by recrystallization from ethanol afforded a colorless crystalline solid.

Table 3
Antibacterial activity of some 4-aryl/hetero aryl-3,5-bis-N-(phenyl/substituted phenyl)-carbamoyl-1,4-diydropyridines (**6a**–**x**)*.

Compounds	clog *P*[a]	Concentration (μg mL^{-1})	Diameter of zone of inhibition (mm)				
			Gram positive			Gram negative	
			B. subtilis	*S. aureus*	*MRSA*	*E. coli*	*P. vulgaris*
6a	4.399	12.5	10	8	–	12	8
		25	14	12	–	15	13
		50	16	14	–	18	17
		1000	–	–	10	–	–
6h	5.366	12.5	11	8	–	10	9
		25	13	11	–	13	14
		50	21	16	–	15	16
		1000	–	–	–	–	–
6j	4.724	12.5	9	8	–	8	9
		25	10	10	–	10	12
		50	12	12	–	14	16
		1000	–	–	–	–	–
6m	4.827	12.5	7	9	–	14	10
		25	11	11	–	19	14
		50	13	12	–	22	18
		1000	–	–	10	–	–
6r	3.424	12.5	–	–	–	14	10
		25	8	12	–	17	12
		50	10	15	–	19	16
		1000	–	–	8	–	–
Streptomycin	–	50	22	21	–	23	26
		1000	–	–	18	–	–
Tetracyclin	–	50	20	22	–	25	21
		1000	–	–	15	–	–

MRSA-Methicillin-resistant Staphylococcus aureus.
* The data of the compounds showing significant zones of inhibition at the concentration tested is shown. The rest of molecules are not active at the concentration tested.
[a] Calculated by ChemDraw software (6.0 version).

*4.1.1.1. N-Phenyl acetoacetamide (**3a**).* IR (KBr) ν (cm^{-1}): 3267 (N–H), 2921 (C–H, aromatic), 1705 (C=O, amide), 1675 (C=O), 1585 (C=C), 1232; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.30 (s, 3H, $-CH_3$), 3.61 (s, 2H, $-CH_2$), 7.1–7.29 (m, 5H, Ar-H), 9.11 (s, 1H, CO–NH); MS *m*/*z* (%): 177 (37) [M$^+$], 162(19), 134(25), 120(42), 92(53). Anal. Calcd. (%) for $C_{10}H_{11}NO_2$: C, 67.79; H, 6.21; N, 7.9. Found: C, 67.81; H, 6.19; N, 7.88.

*4.1.1.2. N-(4-Methylphenyl)acetoacetamide (**3b**).* IR (KBr) ν (cm^{-1}): 3293 (N–H), 1657 (C=O), 1162, 1001, 818, 786; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.29 (s, 6H, 2x-CH_3), 3.54 (s, 2H, $-CH_2$), 7.09–7.40 (m, 4H, Ar-H), 8.98 (s, 1H, CO–NH); MS *m*/*z* (%): 191(26) [M$^+$], 148(3), 133 (73), 120(10), 107(76), 91(9), 77(53), 65(17). Anal. Calcd. (%) for $C_{11}H_{13}NO_2$: C, 69.10; H, 6.80; N, 7.32. Found: C, 69.12; H, 6.83; N, 7.35.

*4.1.1.3. N-(2-Methylphenyl)acetoacetamide (**3c**).* IR (KBr) ν (cm^{-1}): 3286 (N–H str), 3056 (C–H, aromatic), 1762 (C=O, amide), 1600 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.32 (s, 3H, Ar-CH_3), 3.64 (s, 3H, CO–CH_3), 3.98 (s, 2H, $-CH_2$), 6.78–7.12 (m, 4H, Ar-H), 9.25 (s, 1H, CO–NH); MS *m*/*z* (%): 191(30) [M$^+$], 133(20), 106(42), 77(10), 65(5), 43(40). Anal. Calcd. (%) for $C_{11}H_{13}NO_2$: C, 69.10; H, 6.80; N, 7.32. Found: C, 69.08; H, 6.79; N, 7.32.

Table 4
Antibacterial activity of compounds **6a**, **6h**, **6j**, **6m** and **6r** against sensitive and resistant strains of *Mycobacterium tuberculosis*.

Compounds	*M. tuberculosis* ($H_{37}Rv$) MIC (μg mL^{-1})	*M. tuberculosis* (C110/09) MIC (μg mL^{-1})
6a	>25	–
6h	>25	>256
6j	>25	>256
6m	12.5	128
6r	25	256
Pyrazinamide (clog *P* = 0.676)	32	128

*4.1.2. General procedure for the synthesis of 4-aryl/heteroaryl-2,6-dimethyl-3,5-bis-N-(aryl)-carbamoyl-1,4-dihydropyridines **6a**–**x***

Conventional method: A mixture of N-arylacetoacetamide (**3a**–**c**, 20 mmol), an appropriate aldehyde (**4a**–**h**, 10 mmol) and ammonium acetate (5, 20 mmol) in ethanol (25 mL) was heated under reflux, on a water-bath for 20–30 h while monitoring the reaction by TLC. On completion of the reaction, the solvent was removed to the possible extent by distillation under reduced pressure and the residue was cooled. The product was filtered, washed with small portions of ice-cold ethanol and dried. Further purification was effected by column chromatography using pet.ether-chloroform (3:1) as eluent.

Barium nitrate-catalyzed one-pot synthesis: A mixture of N-arylacetoacetamide (**3a**–**c**, 20 mmol), an appropriate aldehyde (**4a**–**h**, 1 mmol) and ammonium acetate (5, 20 mmol) in presence of 5 mol % barium nitrate was heated on a hot water-bath for 30–45 min while monitoring the reaction by TLC. On completion, the reaction mixture was poured into ice-cold water (25 mL), while stirring. The product was filtered, washed with small portions of cold water and dried. It was purified by column chromatography using pet.ether-chloroform (3:1) as eluent.

In Solvent Microwave irradiation (Method I): To a mixture of an N-arylacetoacetamide (**3a**–**c**, 20 mmol) and an appropriate aldehyde (**4a**–**h**, 10 mmol) in ethanol (10 mL) taken in a 250 mL Pyrex beaker, ammonium acetate (5, 20 mmol) was added and irradiated in the microwave oven at 480W, for 4–6 min with 30 s to 1 min pulses. After completion of the reaction (TLC), the reaction mixture was cooled in a refrigerator, and the product obtained was filtered, washed with ice-cold water and dried. Purification of the product was effected by recrystallization from ethanol.

Solvent-free microwave irradiation method (Method II): N-arylacetoacetamide (**3a**–**c**, 20 mmol), an appropriate aldehyde (**4a**–**h**, 10 mmol) and ammonium acetate (5, 20 mmol) were mixed thoroughly in a mortar with silica gel ($\sim$10g). The reaction mixture was

transferred to a 250 mL Pyrex beaker and irradiated in a microwave oven at 480W for 3–6 min. The progress of the reaction was monitored by TLC, the reaction mixture was cooled, on completion and triturated with dichloromethane (3 × 50 mL). Silica gel was removed by filtration, washed with small portions of dichloromethane and the washings were added to the filtrate. The dichloromethane extracts were dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure using a rotary vacuum evaporator to obtain the product **6a**–**x**. Further purification was effected by recrystallization from ethanol.

*4.1.2.1. 4-(2-Furyl)-2,6-dimethyl-3,5-bis-N-(phenyl)-carbamoyl-1,4-dihydropyridine (**6a**).* IR (KBr) ν (cm^{-1}): 3248 (N–H), 1652 (C=O), 1539 (N–H bend, amide), 1443, 1316 (C–N), 753, 692; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.45 (s, 6H, 2x-CH_3), 5.15 (s, 1H, H4-DHP), 6.34 (d, J = 3.5 Hz, 1H, H4-furyl), 6.87 (d, J = 3.5 Hz, 1H, H3-furyl), 7.13–7.63 (m, 12H, Ar-H + H5-furyl + NH-DHP), 8.81 (s, 2H, 2x CO–NH); MS m/z (%): 412(100) $[M-H]^+$, 320(4), 226(10), 186(2). Anal. Calcd. (%) for $C_{25}H_{23}N_3O_3$: C, 72.63; H, 5.56; N, 10.16. Found: C, 72.66; H, 5.54; N, 10.18.

*4.1.2.2. 4-(2-Pyridyl)-2,6-dimethyl-3,5-bis-N-(phenyl)-carbamoyl-1,4-dihydropyridine (**6b**).* IR (KBr) ν (cm^{-1}): 3332 (N–H), 1675 (C=O), 1534 (N–H bend, amide), 1356, 1325 (C–N), 789; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.55 (s, 6H, 2x-CH_3), 5.02 (s, 1H, H4-DHP), 6.54–6.67 (m, 3H, H3, H4 and H5-pyridyl), 7.21–7.35 (m, 11H, Ar-H + H6-pyridyl), 7.88 (s, 1H, NH-DHP), 8.93 (s, 2H, 2x CO–NH); MS m/z (%): 424(15) $[M^+]$, 332(23), 304(48), 240(45), 212(12), 120(100), 93(33). Anal. Calcd. (%) for $C_{26}H_{24}N_4O_2$: C, 73.58; H, 5.66; N, 13.20. Found: C, 73.55; H, 5.63; N, 13.17.

*4.1.2.3. 4-(2-Thienyl)-2,6-dimethyl-3,5-bis-N-(phenyl)-carbamoyl-1,4-dihydropyridine (**6c**).* IR (KBr) ν (cm^{-1}): 3276 (N–H), 1654 (C=O str), 1550 (N–H bend, amide), 1444, 1318 (C–N), 756; ^{1}H NMR ($CDCl_3$ + DMSO) δ (ppm): 2.42 (s, 6H, 2x-CH_3), 5.11 (s, 1H, H4-DHP), 6.33–6.45 (m, 2H, H3, H4-thienyl), 6.98 (d, J = 5.5 Hz, 1H, H5-thienyl), 7.13–7.32 (m, 10H, Ar-H), 7.64 (s, 1H, NH-DHP), 8.66 (s, 2H, 2x CO–NH); ^{13}C NMR (75 MHz, DMSO) δ (ppm): 17.43, 104.46, 114.81, 122.43, 124.47, 126.86, 128.4, 129.2, 134.15, 137.74, 141.88, 146.11, 151.08, 151.58, 167.65; MS m/z (%): 429(13) $[M^+]$, 346(4), 337 (25), 309(12), 244(45), 216(100), 202(31), 133(16), 93(5), 77(56), 65 (8). Anal. Calcd. (%) for $C_{25}H_{23}N_3O_2S$: C, 69.93; H, 5.36; N, 9.79. Found: C, 69.91; H, 5.33; N, 9.76.

*4.1.2.4. 4-(2-Imidazolyl)-2,6-dimethyl-3,5-bis-N-(phenyl)-carbamoyl-1,4-dihydropyridine (**6d**).* IR (KBr) ν (cm^{-1}): 3275 (N–H), 1653 (C=O), 1542 (N–H bend, amide), 1320 (C–N), 754, 691; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.39 (s, 6H, 2x-CH_3), 5.12 (s, 1H, H4-DHP), 6.98 (d, J = 2.6 Hz, 2H, H4 and H5-imidazolyl), 7.35–7.47 (m, 10H, Ar-H), 7.53 (s, 1H, NH-DHP, D_2O exchangeable), 7.66–7.71 (d, J = 2.3 Hz, 1H, NH-imidazolyl), 8.89 (s, 2H, 2x CO–NH, D_2O exchangeable); MS m/z (%): 413(2) $[M^+]$, 345(61), 320(27), 305(11), 253(100), 226(15), 212(8), 199(36), 186(11), 160(33), 120(21), 106(47), 93(72), 77(63), 65(57). Anal. Calcd. (%) for $C_{24}H_{23}N_5O_2$: C, 69.73; H, 5.56; N, 16.94. Found: C, 69.75; H, 5.53; N, 16.92.

*4.1.2.5. 4-(2-Pyrrolyl)-2,6-dimethyl-3,5-bis-N-(phenyl)-carbamoyl-1,4-dihydropyridine (**6e**).* IR (KBr) ν (cm^{-1}): 3245 (N–H), 1642 (C=O), 1511 (N–H bend, amide), 1320 (C–N), 812; ^{1}H NMR (DMSO) δ (ppm): 2.34 (s, 6H, 2x-CH_3), 5.23 (s, 1H, H4-DHP), 6.24–6.39 (m, 2H, H3 and H4-pyrrolyl), 6.85 (s, 1H, NH-DHP), 7.12–7.59 (m, 11H, Ar-H + H5-pyrrolyl), 7.85 (d, J = 2.2 Hz, 1H, NH-pyrrolyl), 10.12 (s, 2H, 2x CO–NH); MS m/z (%): 412(8) $[M^+]$, 346(21), 320(12), 292 (40), 227(15), 199(51), 185(33), 120(17), 92(24), 77(100), 51(16). Anal. Calcd. (%) for $C_{25}H_{24}N_4O_2$: C, 72.81; H, 5.82; N, 13.59. Found: C, 72.79; H, 5.80; N, 13.56.

*4.1.2.6. 4-(4-Chlorophenyl)-2,6-dimethyl-3,5-bis-N-(phenyl)-carbamoyl-1,4-dihydropyridine (**6f**).* IR (KBr) ν (cm^{-1}): 3239 (N–H), 1654 (C=O), 1554 (N–H bend, amide), 1316 (C–N str), 841, 749; ^{1}H NMR ($CDCl_3$ + DMSO) δ (ppm): 2.64 (s, 6H, 2x-CH_3), 5.45 (s, 1H, H4-DHP), 7.11–7.24 (m, 12H, Ar-H), 7.51–7.63 (m, 2H, Ar-H), 7.87 (s, 1H, NH-DHP), 10.11 (s, 2H, 2x CO–NH); MS m/z (%): 459(8) $[M^+]$, 366(27), 338(40), 217(12), 116(7), 121(100), 91(15). Anal. Calcd. (%) for $C_{27}H_{24}ClN_3O_2$: C, 70.89; H, 5.25; N, 9.19. Found: C, 70.85; H, 5.23; N, 9.16.

*4.1.2.7. 4-(4-Fluorophenyl)-2,6-dimethyl-3,5-bis-N-(phenyl)-carbamoyl-1,4-dihydropyridine (**6g**).* IR (KBr) ν (cm^{-1}): 3257 (N–H), 1662 (C=O), 1536 (N–H bend, amide), 1336 (C–N), 1229 (C–F), 722; ^{1}H NMR ($CDCl_3$ + DMSO) δ (ppm): 2.73 (s, 6H, 2x-CH_3), 5.53 (s, 1H, H4-DHP), 7.19–7.28 (m, 12H, Ar-H), 7.62–7.68 (m, 2H, Ar-H), 7.92 (s, 1H, NH, DHP), 10.17 (s, 2H, 2x CO–NH); MS m/z (%): 441(15) $[M^+]$, 349(34), 321(41), 256(52), 239(12), 228(9), 214(66), 209(42), 147(100), 77 (30). Anal. Calcd. (%) for $C_{27}H_{24}FN_3O_2$: C, 73.46; H, 5.44; N, 9.52. Found: C, 73.44; H, 5.41; N, 9.51.

*4.1.2.8. 4-(4-Ethylphenyl)-2,6-dimethyl-3,5-bis-N-(phenyl)-carbamoyl-1,4-dihydropyridine (**6h**).* IR (KBr) ν (cm^{-1}): 3240 (N–H), 1647 (C=O), 1557 (N–H bend, amide), 1319 (C–N), 751; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.09 (t, 3H, $-CH_2-\mathbf{CH_3}$), 2.54–2.56 (q, 2H, $-\mathbf{CH_2}-CH_3$), 2.65 (s, 6H, 2x-CH_3), 5.25 (s, 1H, H4-DHP), 7.07–7.30 (m, 15H, Ar-H + NH-DHP), 9.95 (s, 2H, 2x CO–NH); MS m/z (%): 449(26) $[M-2H]^+$, 357(100), 345(2), 264(77), 236(18), 222(11), 208(8), 180(10), 165(6), 139(7), 93(24), 77(19), 65(32). Anal. Calcd. (%) for $C_{29}H_{29}N_3O_2$: C, 77.16; H, 6.43; N, 9.31. Found: C, 77.14; H, 6.41; N, 9.33.

*4.1.2.9. 4-(2-Furyl)-2,6-dimethyl-3,5-bis-N-(4-methylphenyl)-carbamoyl-1,4-dihydropyridine (**6i**).* IR (KBr) ν (cm^{-1}): 3233 (N–H), 1665 (C=O), 1542 (N–H bend, amide), 1435, 1336 (C–N), 721; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.25 (s, 6H, Ar-CH_3), 2.31(s, 6H, 2x-CH_3), 5.03 (s, 1H, H4-DHP), 6.13 (d, J = 3.3 Hz, 1H, H4-furyl), 6.56 (d, J = 3.3 Hz, 1H, H3-furyl), 6.97–7.03 (m, 10H, Ar-H + H5-furyl + NH-DHP), 8.64 (s, 2H, 2x CO–NH); MS m/z (%): 439(43) $[M-2H]^+$, 373(12), 332(26), 267(42), 240(21), 225(32), 133(5), 120(43), 107(100), 91(27). Anal. Calcd. (%) for $C_{27}H_{27}N_3O_3$: C, 73.46; H, 6.12; N, 9.52. Found: C, 73.48; H, 6.14; N, 9.54.

*4.1.2.10. 4-(2-Pyridyl)-2,6-dimethyl-3,5-bis-N-(4-methylphenyl)-carbamoyl-1,4-dihydro-pyridine (**6j**).* IR (KBr) ν (cm^{-1}): 3312 (N–H), 1663 (C=O), 1525 (N–H bend, amide), 1353 (C–N), 701; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.21 (s, 6H, Ar-CH_3), 2.62 (s, 6H, 2x-CH_3), 5.21 (s, 1H, H4-DHP), 6.34–6.47 (m, 3H, H3, H4 and H5-pyridyl), 7.15–7.25 (m, 9H, Ar-H + H6-pyridyl), 7.65 (s, 1H, NH-DHP), 8.64 (s, 2H, 2x CO–NH); MS m/z (%): 438(5) $[M^+]$, 360(15), 332(27), 225(100), 204 (45), 197(32), 107(12), 91(33). Anal. Calcd. (%) for $C_{28}H_{28}N_3O_2$: C, 76.71; H, 6.39; N, 9.58. Found: C, 76.73; H, 6.41; N, 9.56.

*4.1.2.11. 4-(2-Thienyl)-2,6-dimethyl-3,5-bis-N-(4-methylphenyl)-carbamoyl-1,4-dihydro-pyridine (**6k**).* IR (KBr) ν (cm^{-1}): 3345 (N–H), 1653 (C=O), 1520 (N–H bend, amide), 1352 (C–N), 735; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.18 (s, 6H, Ar-CH_3), 2.42 (s, 6H, 2x-CH_3), 5.49 (s, 1H, H4-DHP), 6.25–6.32 (m, 2H, H3, H4-thienyl), 6.82 (d, J = 5.9 Hz, 1H, H5-thienyl), 7.01–7.12 (m, 8H, Ar-H), 7.45 (s, 1H, NH-DHP), 8.57 (s, 2H, 2x CO–NH); MS m/z (%): 457(8) $[M^+]$, 374(12), 323(5), 351(51), 244(100), 216(15), 202(33), 134(42), 107(12). Anal. Calcd. (%) for $C_{27}H_{27}N_3O_2S$: C, 70.89; H, 5.90; N, 9.19. Found: C, 70.85; H, 5.92; N, 9.18.

*4.1.2.12. 4-(2-Imidazolyl)-2,6-dimethyl-3,5-bis-N-(4-methylphenyl)-carbamoyl-1,4-dihydro-pyridine (**6l**).* IR (KBr) ν (cm^{-1}): 3362 (N–H), 1652 (C=O), 1576 (N–H bend, amide), 1342 (C–N), 740; ^1H NMR ($CDCl_3$) δ (ppm): 2.31 (s, 6H, Ar-CH_3), 2.58 (s, 6H, 2x-CH_3), 5.18 (s, 1H, H4-DHP), 6.82 (m, 2H, H4 and H5-imidazolyl), 7.34–7.41 (m, 9H, Ar-H + NH-imidazolyl), 7.52 (s, 1H, NH-DHP, D_2O exchangeable), 8.67 (s, 2H, 2x CO–NH, D_2O exchangeable); MS *m/z* (%): 441 (6) [M^+], 374(12), 335(34), 307(100), 200(7), 186(27), 92(48). Anal. Calcd. (%) for $C_{26}H_{27}N_5O_2$: C, 70.74; H, 6.12; N, 15.87. Found: C, 70.76; H, 6.10; N, 15.88.

*4.1.2.13. 4-(2-Pyrrolyl)-2,6-dimethyl-3,5-bis-N-(4-methylphenyl)-carbamoyl-1,4-dihydro-pyridine (**6m**).* IR (KBr) ν (cm^{-1}): 3356 (N–H), 1645 (C=O), 1547 (N–H bend, amide), 1363 (C–N), 809; ^1H NMR (DMSO) δ (ppm): 2.17 (s, 6H, Ar-CH_3), 2.44 (s, 6H, 2x-CH_3, DHP), 5.26 (s, 1H, H4-DHP), 7.15 (m, 2H, H3 and H4-pyrrolyl), 7.22–7.38 (m, 9H, Ar-H + H5-pyrrolyl), 7.52 (d, J = 2.1 Hz, 1H, NH-pyrrolyl), 7.91 (s, 1H, NH-DHP), 10.14 (s, 2H, 2x CO–NH); MS *m/z* (%): 438(5) [M-2H]$^+$, 373(39), 331(100), 265(41), 237(63), 206(10), 133(15), 91(75). Anal. Calcd. (%) for $C_{27}H_{28}N_4O_2$: C, 73.63; H, 6.36; N, 12.72. Found: C, 73.65; H, 6.34; N, 12.73.

*4.1.2.14. 4.4-(4-Chlorophenyl)-2,6-dimethyl-3,5-bis-N-(4-methylphenyl)-carbamoyl-1,4-dihydropyridine (**6n**).* IR (KBr) ν (cm^{-1}): 3260 (N–H), 1645 (C=O), 1514 (N–H bend, amide), 1317 (C–N), 816; ^1H NMR ($CDCl_3$ + DMSO) δ (ppm): 2.18 (s, 6H, Ar-CH_3), 2.55 (s, 6H, 2x-CH_3, DHP), 5.35 (s, 1H, H4-DHP), 6.90–7.49 (m, 12H, Ar-H), 7.78 (s, 1H, NH-DHP), 9.94 (s, 2H, 2x CO–NH); ^{13}C NMR (75 MHz, DMSO) δ (ppm): 20.43, 22.13, 119.6, 119.78, 127.6, 129.04, 130.32, 130.45, 132.95, 134.45, 135.87, 142.82, 153.45, 165.18; MS *m/z* (%): 485(20) [M^+], 483(50), 379(36), 377(100), 270(59), 244(18), 106(98), 91(25), 77(44), 65(12), 51(13). Anal. Calcd. (%) for $C_{29}H_{28}ClN_3O_2$: C, 71.60; H, 5.76; N, 8.64. Found: C, 71.61; H, 5.74; N, 8.66.

*4.1.2.15. 4-(4-Fluorophenyl)-2,6-dimethyl-3,5-bis-N-(4-methylphenyl)-carbamoyl-1,4-dihydropyridine (**6o**).* IR (KBr) ν (cm^{-1}): 3264 (N–H), 1657 (C=O), 1534 (N–H bend, amide), 1356 (C–N), 1227 (C–F), 731; ^1H NMR ($CDCl_3$ + DMSO) δ (ppm): 2.28 (s, 6H, Ar-CH_3), 2.65 (s, 6H, 2x-CH_3), 5.44 (s, 1H, H4-DHP), 7.11–7.23 (m, 10H, Ar-H), 7.45–7.57 (m, 2H, Ar-H), 7.89 (s, 1H, NH, DHP), 10.11 (s, 2H, 2x CO-NH); MS *m/z* (%): 441(15) [M^+], 349(34), 321(41), 256(52), 239(12), 228(9), 214 (66), 209(42), 147(100), 77(30). Anal. Calcd. (%) for $C_{29}H_{28}FN_3O_2$: C, 74.20; H, 5.97; N, 8.95. Found: C, 74.23; H, 5.96; N, 8.93.

*4.1.2.16. 4-(4-Ethylphenyl)-2,6-dimethyl-3,5-bis-N-(4-methylphenyl)-carbamoyl-1,4-dihydro-pyridine (**6p**).* IR (KBr) ν (cm^{-1}): 3237 (N–H), 1639 (C=O), 1560 (N–H bend, amide), 1315 (C–N), 723; ^1H NMR ($CDCl_3$) δ (ppm): 1.05 (t, 3H, $-CH_2-$**CH_3**), 2.45–2.47 (q, 2H, $-$**CH_2**$-CH_3$), 2.26 (s, 6H, Ar-CH_3), 2.56 (s, 6H, 2x-CH_3), 5.16 (s, 1H, H4-DHP), 6.97–7.13 (m, 13H, Ar-H + NH-DHP), 9.77 (s, 2H, 2x CO–NH); MS *m/z* (%): 449(26) [M-2H]$^+$, 357(100), 345(2), 264(77), 236(18), 222(11), 208(8), 180(10), 165(6), 139(7), 93(24), 77(19), 65 (32). Anal. Calcd. (%) for $C_{31}H_{33}N_3O_2$: C, 77.66; H, 6.88; N, 8.76. Found: C, 77.64; H, 6.86; N, 8.74.

*4.1.2.17. 4-(2-Furyl)-2,6-dimethyl-3,5-bis-N-(2-methylphenyl)-carbamoyl-1,4-dihydro-pyridine (**6q**).* IR (KBr) ν (cm^{-1}): 3270 (N–H), 1694 (C=O), 1585 (N–H bend, amide), 1375 (C–N), 713; ^1H NMR ($CDCl_3$) δ (ppm): 1.98 (s, 6H, Ar-CH_3), 2.36 (s, 6H, 2x-CH_3), 5.70 (s, 1H, H4-DHP), 5.90–5.94 (m, 1H, H4-furyl), 6.12–6.24 (m, 1H, H3-furyl), 6.36 (s, 1H, NH-DHP), 7.08–7.15 (m, 1H, H5-furyl), 7.18–7.56 (m, 8H, Ar-H), 8.71 (s, 2H, 2x CO–NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 19.5, 22.69, 104.43, 111.3, 120.9, 127.8, 128.6, 129.4, 130.2, 136.25, 139.6, 141.22, 143.9, 153.5, 158.5, 166.4; MS *m/z* (%): 439(25) [M-2H]$^+$, 333 (60), 266(72), 238(15), 174(100), 91(80). Anal. Calcd. (%) for $C_{27}H_{27}N_3O_3$: C, 73.46; H, 6.12; N, 9.52. Found: C, 73.45; H, 6.11; N, 9.50.

*4.1.2.18. 4-(2-Pyridyl)-2,6-dimethyl-3,5-bis-N-(2-methylphenyl)-carbamoyl-1,4-dihydro-pyridine (**6r**).* IR (KBr) ν (cm^{-1}): 3365 (N–H), 1689 (C=O), 1575 (N–H bend, amide), 1368 (C–N), 716; ^1H NMR ($CDCl_3$) δ (ppm): 2.01 (s, 6H, Ar-CH_3), 2.24 (s, 6H, 2x-CH_3), 5.15 (s, 1H, H4-DHP), 6.23–6.37 (m, 3H, H3, H4 and H5-pyridyl), 7.09 (s, 1H, NH-DHP), 7.26–7.34 (m, 9H, Ar-H + H6-pyridyl), 9.11 (s, 2H, 2x CO–NH); MS *m/z* (%): 438(15) [M^+], 360(5), 332(45), 226(100), 204 (38), 197(62), 91(12). Anal. Calcd. (%) for $C_{28}H_{28}N_3O_2$: C, 76.71; H, 6.39; N, 9.58. Found: C, 76.69; H, 6.36; N, 9.60.

*4.1.2.19. 4-(2-Thienyl)-2,6-dimethyl-3,5-bis-N-(2-methylphenyl)-carbamoyl-1,4-dihydro-pyridine (**6s**).* IR (KBr) ν (cm^{-1}): 3355 (N–H), 1659 (C=O), 1561 (N–H bend, amide), 1325 (C–N), 765; ^1H NMR ($CDCl_3$) δ (ppm): 2.11 (s, 6H, Ar-CH_3), 2.38 (s, 6H, 2x-CH_3), 5.09 (s, 1H, H4-DHP), 6.21–6.29 (m, 2H, H3, H4-thienyl), 7.02 (m, 1H, H5-thienyl), 7.21–7.27 (m, 8H, Ar-H), 7.53 (s, 1H, NH-DHP), 8.89 (s, 2H, 2x CO–NH); MS *m/z* (%): 457(9) [M^+], 374(41), 323(22), 268(11), 240(100), 216(65), 173(31), 107(17). Anal. Calcd. (%) for $C_{27}H_{27}N_3O_2S$: C, 70.89; H, 5.90; N, 9.19. Found: C, 70.91; H, 5.88; N, 9.17.

*4.1.2.20. 4-(2-Imidazolyl)-2,6-dimethyl-3,5-bis-N-(2-methylphenyl)-carbamoyl-1,4-dihydro-pyridine (**6t**).* IR (KBr) ν (cm^{-1}): 3349 (N–H), 1661 (C=O), 1545 (N–H bend, amide), 1362 (C–N), 762; ^1H NMR ($CDCl_3$) δ (ppm): 2.24 (s, 6H, Ar-CH_3), 2.42 (s, 6H, 2x-CH_3), 5.05 (s, 1H, H4-DHP), 6.67 (d, J = 3 Hz, 2H, H4 and H5-imidazolyl), 7.27–7.31 (m, 8H, Ar-H), 7.45 (s, 1H, NH-DHP, D_2O exchangeable), 7.56–7.61 (d, J = 2.1 Hz, 1H, NH-imidazolyl), 8.45 (s, 2H, 2x CO–NH, D_2O exchangeable); MS *m/z* (%): 441(6) [M^+], 374(12), 335(34), 307(100), 228(55), 200(7), 185(27), 92(48). Anal. Calcd. (%) for $C_{26}H_{27}N_5O_2$: C, 70.74; H, 6.12; N, 15.87. Found: C, 70.72; H, 6.15; N, 15.90.

*4.1.2.21. 4-(2-Pyrrolyl)-2,6-dimethyl-3,5-bis-N-(2-methylphenyl)-carbamoyl-1,4-dihydro-pyridine (**6u**).* IR (KBr) ν (cm^{-1}): 3266 (N–H), 1654 (C=O), 1514 (N–H bend, amide), 1318 (C–N), 816; ^1H NMR (DMSO) δ (ppm): 2.25 (s, 6H, Ar-CH_3), 2.57 (s, 6H, 2x-CH_3, DHP), 5.15 (s, 1H, H4-DHP), 7.07 (d, 2H, H3 and H4-pyrrolyl), 7.12–7.14 (d, 5H, Ar-H + NH-pyrrolyl), 7.39–7.45 (t, 1H, H5-pyrrolyl), 7.57–7.59 (d, 4H, Ar-H), 7.96 (s, 1H, NH-DHP), 10.29 (s, 2H, 2x CO–NH); MS *m/z* (%): 438(4) [M-2H]$^+$, 373(24), 331(17), 312(58), 267(50), 225(68), 206(100), 133(30), 107(85), 91(21), 77(65). Anal. Calcd. (%) for $C_{27}H_{28}N_4O_2$: C, 73.63; H, 6.36; N, 12.72. Found: C, 73.66; H, 6.34; N, 12.74.

*4.1.2.22. 4-(4-Chlorophenyl)-2,6-dimethyl-3,5-bis-N-(2-methylphenyl)-carbamoyl-1,4-dihydropyridine (**6v**).* IR (KBr) ν (cm^{-1}): 3243 (N–H), 1672 (C=O), 1551 (N–H bend, amide), 1335 (C–N), 756; ^1H NMR ($CDCl_3$ + DMSO) δ (ppm): 2.11 (s, 6H, Ar-CH_3), 2.21 (s, 6H, 2x-CH_3, DHP), 5.56 (s, 1H, H4-DHP), 7.12–7.37 (m, 12H, Ar-H), 7.64 (s, 1H, NH-DHP), 9.14 (s, 2H, 2x CO–NH); MS *m/z* (%): 485(9) [M^+], 483 (15), 379(28), 377(68), 244(100), 106(65), 91(42), 65(72). Anal. Calcd. (%) for $C_{29}H_{28}ClN_3O_2$: C, 71.60; H, 5.76; N, 8.64. Found: C, 71.58; H, 5.77; N, 8.63.

*4.1.2.23. 4-(4-Fluorophenyl)-2,6-dimethyl-3,5-bis-N-(2-methylphenyl)-carbamoyl-1,4-dihydropyridine (**6w**).* IR (KBr) ν (cm^{-1}): 3255 (N–H), 1661 (C=O), 1562 (N–H bend, amide), 1356 (C–N), 1231 (C–F), 714; ^1H NMR ($CDCl_3$ + DMSO) δ (ppm): 2.27 (s, 6H, Ar-CH_3), 2.55 (s, 6H, 2x-CH_3), 5.28 (s, 1H, H4-DHP), 7.21–7.35 (m, 10H, Ar-H), 7.42–7.61 (m, 2H, Ar-H), 7.79 (s, 1H, NH, DHP), 10.05 (s, 2H, 2x CO–NH); MS *m/z* (%): 441(5) [M^+], 349(36), 321(100), 239(82), 228 (18), 209(55), 147(12), 77(67). Anal. Calcd. (%) for $C_{29}H_{28}FN_3O_2$: C, 74.20; H, 5.97; N, 8.95. Found: C, 74.18; H, 5.95; N, 8.97.

*4.1.2.24. 4-(4-Ethylphenyl)-2,6-dimethyl-3,5-bis-N-(2-methylphenyl)-carbamoyl-1,4-dihydropyridine (**6x**).* IR (KBr) ν (cm^{-1}): 3225 (N–H), 1638 (C=O), 1560 (N–H bend, amide), 1320 (C–N), 725; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.14 (t, 3H, $-CH_2-\mathbf{CH_3}$), 2.39–2.47 (q, 2H, $-\mathbf{CH_2}-CH_3$), 2.52 (s, 6H, Ar-CH_3), 2.76 (s, 6H, 2x-CH_3), 5.21 (s, 1H, H4-DHP), 7.01–7.13 (m, 13H, Ar-H + NH-DHP), 9.53 (s, 2H, 2x CO–NH); MS *m/z* (%): 449(7) $[M-2H]^+$, 357(48), 345(23), 264(17), 222(75), 208(51), 165 (6), 93(39), 77(25). Anal. Calcd. (%) for $C_{31}H_{33}N_3O_2$: C, 77.66; H, 6.88; N, 8.76. Found: C, 77.67; H, 6.89; N, 8.77.

5. Biological evaluation

5.1. Antibacterial assay in vitro

The cultures were obtained from NCIM (National Collection of Industrial Microorganisms, National Chemical Laboratories, Pune - 411 003, India). Stock solutions of compounds (**6a**–**x**) (1 mg mL^{-1}) were prepared in DMSO and various dilutions were made from them with DMSO to get the final concentrations: 12.5, 25 and 50 μg mL^{-1}. These were then loaded on a sterile Whatman No.1 filter paper disc with 5 mm diameter and dried, aseptically. Sterile antibiotic discs of tetracyclin and streptomycin (50 μg mL^{-1}) were prepared as above and used as standards. Sterile nutrient agar was poured in sterile petri dishes at 40–45 °C and allowed to solidify. 100 μL of each of 24 h old bacterial suspension (10^8 subcells mL^{-1}) was then spread onto the surface of the agar plates using a sterile glass spreader and allowed to dry for 10 min. Then the compounds-impregnated discs along with the control disc (DMSO) and standard discs were placed on the surface of inoculated agar media and incubated at 37 ± 1 °C for 24 h. After incubation, the plates were observed and the diameters of the zones of inhibition were read and recorded.

5.2. Antitubercular activity in vitro

The antitubercular activity was assayed using the conventional Lowenstein–Jensen (LJ) medium against *M. tuberculosis* $H_{37}Rv$ strain ATCC 27294 (susceptible to rifampicin and isoniazid) and against a clinical isolate of multidrug-resistant strain of *M. tuberculosis* (MDR-TB, C110/09, resistant to rifampicin, pyrazinamide and isoniazid). The stock solutions of the compounds **6a**, **6h**, **6j**, **6m** and **6r** were prepared in dimethylsulfoxide (DMSO). Various dilutions were made from the stock with DMSO, to get the final drug concentrations: 6.25, 12.5, 25, 50 and 100 μg mL^{-1} for $H_{37}Rv$. Likewise, another set of dilutions were made to get 64, 128 and 256 μg mL^{-1} for C110/09. Sterile LJ medium (4 mL) was poured into sterile Mc Cartney bottles containing different concentrations of drug solutions, tightly capped and kept aside in slanting position, for overnight. A standard bacterial suspension of either $H_{37}Rv$ or C110/09 (4 mg mL^{-1}) was inoculated onto LJ medium slants with a loop of 3 mm internal diameter. All the culture bottles were incubated at 37 °C. Readings were taken at the end of 4 weeks of incubation and MIC was determined by counting the colony forming units and comparing with the culture controls. A negative control (LJ media alone), a positive control (inoculated LJ medium) and a solvent control (inoculated LJ medium with DMSO) were included in the assay to make necessary corrections. Pyrazinamide was used as reference drug, for comparison.

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