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Synthesis and biological evaluation of some new 1,4-dihydropyridines containing different ester substitute and diethyl carbamoyl group as anti-tubercular agents

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ABSTRACT

Tuberculosis is a leading infectious cause of death worldwide. Because of the concern of the resistance to most of the commonly used drugs displayed by the considered mycobacteria, most efforts have been done to introduce new anti-tubercular agents. Recent studies showed that 1,4-dihydropyridine-3,5-dicarbamoyl derivatives with lipophilic groups have significant anti-tubercular activity. In this study, we synthesized new derivatives of 1,4-dihydropyridines in which different alkyl and aryl esters and diethyl carbamoyl are substituted in C-3 and C-5 of the DHP ring. In addition nitroimidazole ring is substitutes at C-4 position. These asymmetric analogues were synthesized by a modified Hantzsh reaction using procedure reported by Meyer. The in vitro anti-tubercular activity of compounds against *Mycobacterium tuberculosis* was evaluated. The results indicate that the compounds containing aromatic esters are more potent than alkyl ones. The most potent aromatic compound (R = 3-phenylpropyl) exhibits comparable anti-tubercular activity (MIC = 1 μmol/ml) with reference compound isoniazide (INH) (MIC = 1 μmol/ml). Conformational analysis, SAR studies of these compounds showed that increasing in lipophilicity and rotable bonds of these compounds resulted in increasing anti-tubercular activity.

1. Introduction

Tuberculosis is today amongst the worldwide health threats and continues to be one of the most important global infectious causes of morbidity and mortality.[1,2] *Mycobacterium tuberculosis* (MTb) infects approximately one-third of the world's population, kills more than 2 million people a year and is comparable only to human immunodeficiency virus (HIV) as an infectious cause of death.[1,3] Despite the development of effective drug therapy for MTb over 50 years ago, there remain several formidable challenges for controlling MTb infection. These obstacles include lengthy treatment regimens of 6–9 months duration, drug resistance, the lack of a highly efficacious vaccine, and an incomplete understanding of what controls infectivity and progression of disease.[1] The problem is being aggravated by increasing resistant against frontline drugs and synergy of this disease with HIV and mycotic infections in Immunocompromised patients.[4]

No new drug against tuberculosis has been developed in the last 30 years. Hence, there is urgent need for new chemotherapeutic agents, preferably having a different mode of action than presently in use to combat the emergence of the resistance. Therefore, the search for new antimycobacterials is the subject of numerous recent studies.[5–10] Recently, studies showed that 3,5-dicarbamoyl derivatives of 1,4-dihydropyridine (DHP) with lipophilic groups have considerable anti-tubercular activity against *M. tuberculosis* H37Rv.[11] It was also observed that esters or substituted isosters of pyridine and pyrazinecarboxylic acids (such as tetrazoles) have been more active than the parent acids specially against resistant strains. These esters are presumably activated by an esterase to parent acid.[12–15] Indeed, esters of pyrazinoic acids have been shown to posses activity against pyrazinamide-resistant isolates which has been attributed to a deficiency of nicotinamidase.[12–15] In addition, nitroimidazolyl moiety (e.g., Metronidazole) and nitroimidazopyran exhibited significant anti-tubercular activity.[16] In view of this, it appeared of interest to design and synthesize new derivatives of 1,4-dihydropyridines in which different alkyl and aryl esters and diethyl carbamoyl are substituted in C-3 and C-5 of the DHP ring, respectively. It seems that such replacements could effectively overcome the resistant isolates which have been attributed to a deficiency of amidase or esterase. In addition, nitroimidazolyl moiety is substituted at C-4 position of dihydropyridine ring. The anti-mycobacterial activity of synthesized compounds was evaluated against *M. tuberculosis* H37Rv strain.

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As the different studies have indicated, 4-aryl-1,4-dihydropyridines (DHPs) are the most studied class of organic calcium channel antagonist. Therefore; as the synthesized compounds have 1,4-DHP structure, they could potentially exhibit calcium channel blocking (CCB) activity. As far as these compounds have been designed as anti-tubercular agents, the CCB activity could be one of the most important side effects of these compounds that cause hypotension, bradycardia and other associated side effects. For this reason; the in vitro CCB activity of synthesized compounds was evaluated to know the structural requirement to decrease the side effect and increase anti-tubercular activity.

2. Chemistry

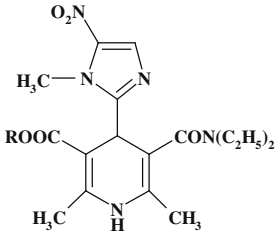
The synthesis of the 1,4-dihydropyridine derivatives **7a–j** was achieved following the steps outlined in Figure 1. Reaction of alcohol **1d–e and 1g,i–j** with 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one **2** afforded the corresponding acetoacetic esters **3d–e** and **3g,i–j** (70–85% yield).[17] Reaction of acetoacetic esters **3** with ammonium acetate afforded the corresponding alkyl 3-amminocrotonate **4**. In addition, reaction of 1-methyl-5-nitroimidazol-2-carboxaldehyde **5** with *N*,*N*-diethyl acetoacetamide afforded the corresponding intermediate, *N*,*N*-diethyl-2-(1-methyl-5-nitro-1*H*-imidazole-2-yl(methylen)3-oxobutan amide **6** (25% yield).[18–20] The asymmetrical analogues **7a–j** were synthesized by a modified Hanstzch reaction, using a procedure reported by Meyer et al. (10–17% yield).[21–23] These compounds were purified by preparative thin-layer chromatography and recrystalization, then characterized by mass spectroscopy, IR and ^{1}H NMR. The yields and melting points of final compounds are summarized in Table 1.

3. Biology

3.1. Anti-mycobacterial activity

Minimum inhibitory concentration (MIC) of the compounds is found against *M. tuberculosis* H37Rv strain ATCC 27294 by agar proportion method in Middlebrook 7H10 medium. The results of the anti-mycocabacterial activity are listed in Table 1.

Table 1
Physical, and antimycobacterial data for compounds **7a–j**



Compound	R	Mp (°C)	Yield (%)	MIC (μmol/lit)	Log*P*[a]
7a	CH_3	180–184	10.45	>500	−0.98
7b	CH_2CH_3	193–196	12.2	250	−0.64
7c	$(CH_3)_2CH_2$	185–190	15.8	>500	−0.23
7d	$(CH_2)_2CH_3$	205–209	13.52	2	−0.17
7e	$(CH_2)_3CH_3$	207–212	16.2	1	0.22
7f	CH_2–C_6H_5	185–189	11.43	1	0.79
7g	$CH_2)_2C_6H_5)$	166–169	11.8	1	1.04
7h	$CH_2)_3C_6H_5)$	139–143	13.72	1	1.44
7i	$CH_2)_4C_6H_5)$	124–129	12.32	2	1.84
7j	$CH_2)_5C_6H_5)$	113–118	16.7	2	2.23
Isoniazide	—			1	—
Rifampine	—			1	—

[a] Calculated by Hyperchem software.

3.2. Pharmacology

As the earlier discuss, 4-aryl-1,4-dihydropyridines (DHPs) are the most studied class of organic calcium channel modulators, and since their introduction into clinical medicine in 1975, this class has found widespread uses for the treatment of cardiovascular disease.[24–26] Therefore; the calcium channel blocking (CCB) activity could have been the potential side effect of the designed

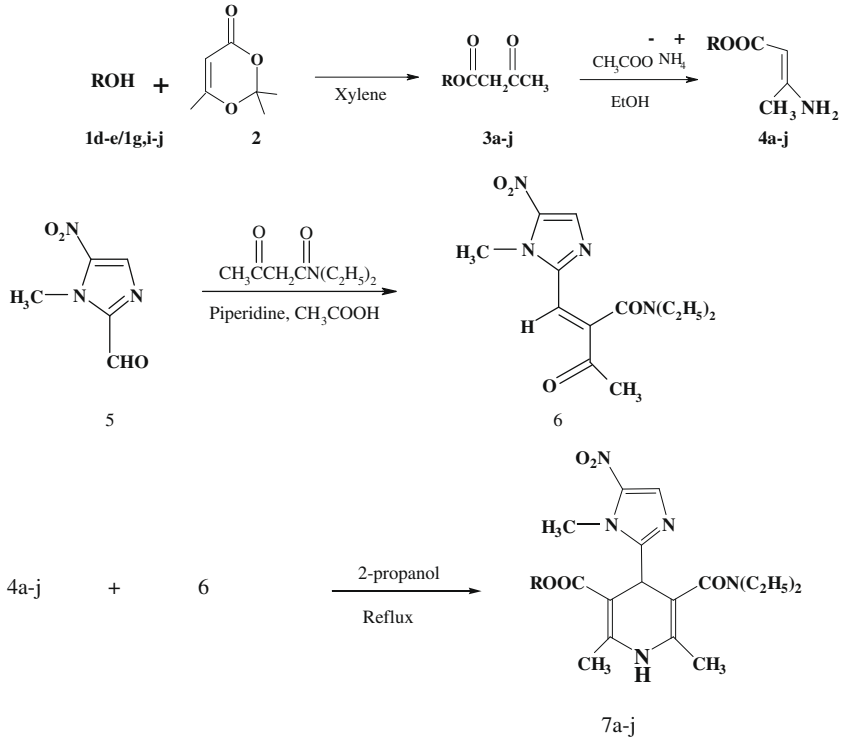


Figure 1. Synthetic pathway for dihydropyridine derivatives used in this study.

compounds. Therefore, it is necessary to evaluate in vitro calcium channel antagonist activity of the compounds in order to understand the minimal structural requirements to decrease CCB activity. The calcium channel antagonist activity of compounds was determined as the concentration needed to produce 50% inhibition of the guinea-pig ileal longitudinal smooth muscle (GPILSM) contractility is summarized in Table 2.

4. Conformational analysis

The optimal 3D structure of the molecules obtained by semi-emprical/AM1 calculations is represented in Figure 2 and some important dihedral angles are listed in Table 3. The most important conformational features considered in this study are:

- *cis* or *trans* orientation of carbonyl groups bonded at C_3 and C_5; C_2–C_3–C_9–O_{10} and C_5–C_6–C_{11}–O_{12} dihedral angles reflect it. If these angles were between −90° and +90°, the orientation was considered *cis*, and the *trans* orientation was assumed when the dihedral angels were between (+90°)–(+180°) or (−90°)–(−180°). The molecules were assigned as *cis–cis*, *cis–trans* and *trans–trans* based on orientation of the individual carbonyl groups.
- The relative position of nitroimidazolyl ring with respect to DHP ring as measured by C'$_2$–C_4–C_3–C_2 dihedral angle.
- The deviation from planarity of DHP ring as measured by C_2–C_3–C_5–C_6 dihedral angle.

In addition numerical calculation of lipophilicity operated with Hyperchem software, is listed in Table 1.

5. Results and discussion

Ten different alkyl and aryl ester derivatives of 5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4-dihydropyridine-3-carboxylate were synthesized and tested for their inhibition activities against *M. tuberculosis*. The results indicate that the overall anti-tubercular activity of aryl ester substituents is more than alkyl ones and could be comparable with INH and rifampine. In aryl series, the compounds **7f**–**h** (R = benzyl, phenethyl, 3-phenyl propyl, respectively) show the most anti-mycobacterial activity (MIC = 1 μM). Moreover, increasing the aliphatic chain of aryl ester substituents (compounds **7i**–**j**; R = 4-phenyl butyl and 5-phenyl pentyl) resulted in decreased activity. In alkyl series; increasing the aliphatic chain from methyl to *n*-butyl, resulted in enhanced activity. But branching the aliphatic chain, significantly abolish anti-mycobacterial activity (compound **7d**; R = *n*-propyl vs **7c**; R = isopropyl). Therefore, 1,4-dihydropyridines containing diethyl carbamoyl and aryl esters with optimal aliphatic chain could posses the suitable anti-mycobacterial activity.

Table 2
Calcium channel blocking data for compounds **7a**–**j**

Compound	R	Calcium channel antagonist activity IC_{50} ± SEM (n = 3)
7a	CH_3	$3.22 \pm 0.011 \times 10^{-5}$
7b	CH_2CH_3	$2.97 \pm 0.008 \times 10^{-5}$
7c	$(CH_3)_2CH_2$	$1.88 \pm 0.008 \times 10^{-5}$
7d	$(CH_2)_2CH_3$	$1.20 \pm 0.007 \times 10^{-5}$
7e	$(CH_2)_3CH_3$	$8.57 \pm 0.015 \times 10^{-6}$
7f	CH_2–C_6H_5	$4.2 \pm 0.118 \times 10^{-6}$
7g	$CH_2)_2C_6H_5)$	$2.4 \pm 0.209 \times 10^{-6}$
7h	$CH_2)_3C_6H_5)$	$2.7 \pm 0.064 \times 10^{-6}$
7i	$CH_2)_4C_6H_5)$	$3.5 \pm 0.246 \times 10^{-6}$
7j	$CH_2)_5C_6H_5)$	$2.4 \pm 0.110 \times 10^{-5}$
Nifedipine	—	$1.07 \pm 0.170 \times 10^{-8}$

The results of CCB activity (Table 2) indicate that all compounds **7a**–**j** are weak CCB agents in compared with reference drug nifedipine ($P < 0.05$) (100–1000 times weaker than nifedipine). Structure–activity relationship study of 1,4-dihydropyridines as CCB agents also indicate that the ester groups are important to the pharmacological effect. Not only does variation of the ester groups lead to quantitative changes in the activity, but replacement of an ester groups by other groups can also produce dramatic qualitative changes.[25] Therefore, we can deduced that substitution of ester group with amide type could significantly decrease CCB activity and this result is favorable for design of DHPs as anti-tubercular agents. Comparison of the activities of aryl ester compounds **7f–i** and alkyl ester series **7a–e** indicates that aryl esters are more active (in CCB effect) than alkyl compounds. As far as, aryl ester groups exhibit much higher calcium channel blocking and anti-mycobacterial activity in compared to alkyl ester group, it could be deduced that lipophilicity and/or aromaticity of ester group have an increasing effect on both biological activities. However; the lipophilicity should be optimal. As we can see in Tables 1 and 2; increasing in aliphatic chain of aryl esters and hence increasing the lipophilicity (Log*P*) of compound (compound **7j**); resulted in decreased biological activities.

Comparison of conformational parameters of the compounds (Table 3) with nifedipine indicate**s** that the obvious conformational feature of the designed compounds is significant deviation of DHP ring from planarity (C_2–C_3–C_5–C_6 angle is deviated from nifedipine angle); while, different X-ray structural investigation, computational and statistical calculations confirm a preference for a flattened boat conformation of the DHP ring in nifedipine and its analogues as CCB agents.[25–28] In addition, different studies of 1,4-DHPs indicates that *cis/trans* and *cis/cis* arrangements are preferred conformation of ester groups for CCB activity.[25–29] Conformational analysis of compound **7a–j** indicates that the ester and amide groups positioned at C_3 and C_5 of DHP ring of designed compounds, specially in the most potent anti-tubercular agents (**7e–i)** prefer the *trans/trans* orientation (C_2–C_3–C_9–O_{10} and C_5–C_6–C_{11}–O_{12} dihedral angles are between +90 and +180); Figure 2. While *cis/cis* orientation is preferred in nifedipine ester groups. Furthermore, the conformation of nitroimidazolyl group is also deviated slightly from pseudoaxial form which is predominant in nifedipine analogues.

The most important conformational parameters for different compounds specially the *trans/trans* conformation of carbamoyl and ester substitutes, pseudoaxail orientation of nitroimidazolyl group are represented in Figure 2.

It could be deduced that the conformational structure of designed compounds deviated from CCB activity, makes them suitable anti-mycobacterial candidate.

6. Conclusion

The results indicate that replacement of ester substitute with amide type in Nifedipine analogues significantly reduces muscle relaxing activity and increase anti-mycobacterial effect. Therefore; 1,4-dihydropiridines with *N,N*-diethyl carbamoyl and aryl esters with optimal lipophilicity could be a suitable candidates against *M. tuberculosis* in vivo anti-mycobacterial evaluation is needed for further optimization. In addition, in vitro evaluation of designed compounds against resistant strain of *M. tuberculosis* could be valuable.

7. Experimental

Melting points were determined on an Electrothermal 9200 apparatus (England) and are uncorrected. ^{1}H NMR spectra were obtained with a Bruker-Avance DPX-250 MHz spectrometer (Germany) in chloroform-d_1 and tetramethylsilane (TMS) was used as an internal standard. The mass spectra were measured with a

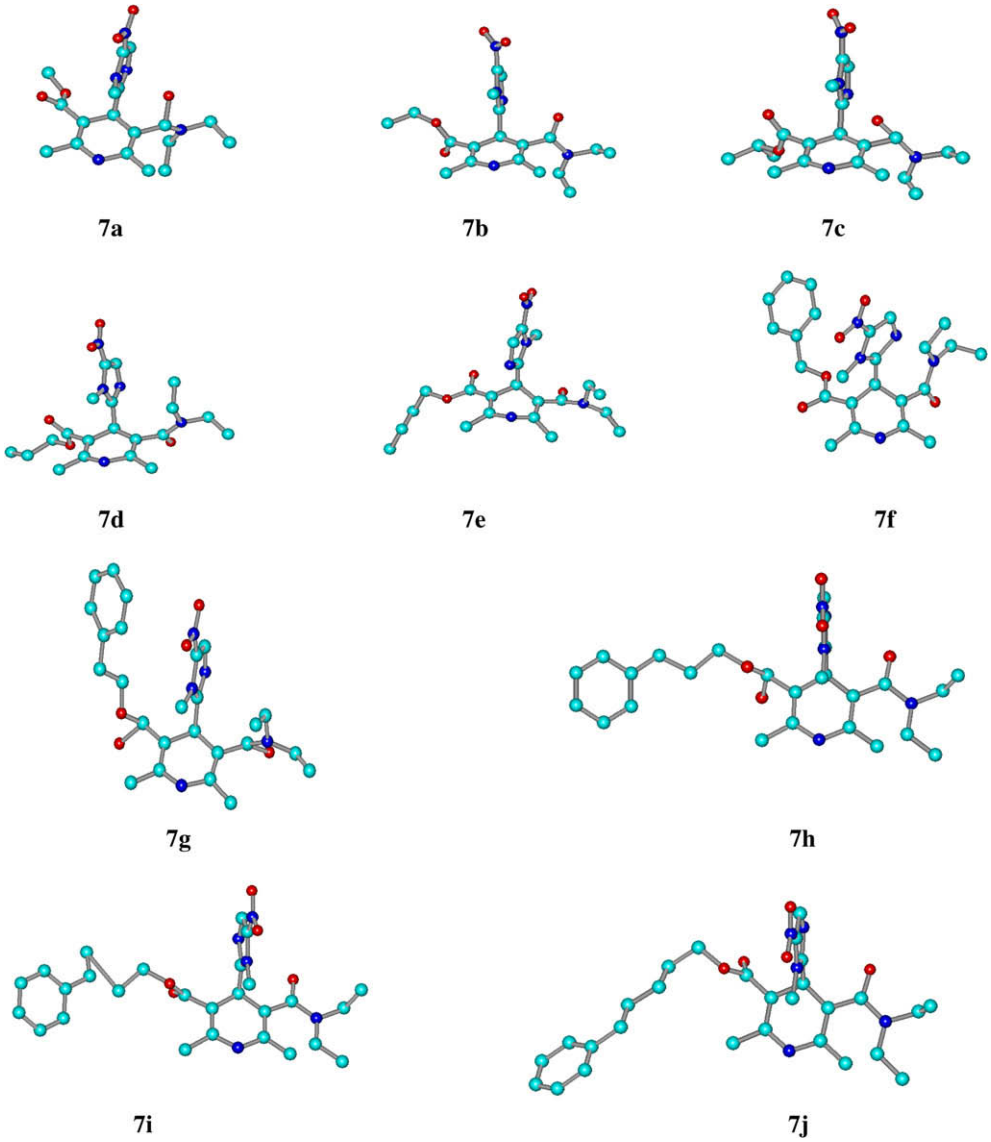


Figure 2. The optimized 3D structures of the compounds obtained by semiemprical/AM$_1$ calculation.

Hewlett Packard (HP) 6890 (Germany) at 70 eV. The IR spectra were obtained by using a Perkin Elmer (KBr disks) (England). The results of elemental analyses (C, H and N) were within ±0.4% of the calculated values. Silica gel HT-254 (E. Merck, Darmstadt, Germany) was used for thin-layer chromatography. All spectra were consistent with the assigned structures. Methyl, ethyl, isopropyl, benzyl and 3-phenyl propyl acetoacetates **1a–c**, **1f** and **1h**, 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one **2** and *N*,*N*-diethyl acetoecetamide purchased from the Aldrich Chemical Co. (Sigma–Aldrich Chemie GmbH, Deisenhofon, Germany).

7.1. General procedure for the synthesis of acetoacetic esters (3d–e and 3g,i–j)

A solution of alcohol **1d–e and 1g,i–j** (50 mmol) and 2,2,6-trimethyl-4*H*-1,3 dioxine-4-one 2 (7.1 g, 50 mmol) in 10 ml xylene was placed in a 50 ml Erlenmeyer flask. The flask was immersed in an oil bath that had been preheated to 150 °C, and the solution was vigorously stirred. Heating was continued for a total 30 min. The reaction mixture was cooled. The xylene was removed. Distillation of the mixture afforded **3d–e** and **3g**,**i–j** which were used immediately in subsequent reactions**.**

7.1.1. *n*-Propyl acetoacetate (3d)

^{1}H NMR ($CDCl_3$): δ 4.10 (t, J = 6.5 Hz, 2H, CO_2CH_2), 3.45 (s, 2H, $COCH_2CO_2$), 2.27 (s, 3H, CH_3CO), 1.63 (m, 2H, CH_2), 0.94 (t, J = 6.5 Hz, 3H, CH_3).

IR (KBr): ν 1743 (C=O, ester), 1714 cm^{-1} (C=O, ketone).

7.1.2. *n*-Butyl acetoacetate (3e)

^{1}H NMR ($CDCl_3$): δ 4.15 (t, J = 6.4 Hz, 2H, CO_2CH_2), 3.44 (s, 2H, $COCH_2CO_2$), 2.16 (s, 3H, CH_3CO), 1.41 (m, 4H, CH_2CH_2), 1.01 (t, J = 6.4 Hz, 3H, CH_3).

IR (KBr): ν 1740 (C=O, ester), 1717 cm^{-1} (C=O, ketone).

7.1.3. Phenethyl acetoacetate (3g)

^{1}H NMR ($CDCl_3$): δ 7.32 (m, 5H, Phenyl), 4.18 (t, J = 6.5 Hz, 2H, CO_2CH_2), 3.39 (s, 2H, $COCH_2CO_2$), 2.69 (t, J = 6.5, 2H, CH_2Phenyl), 2.46 (s, 3H, CH_3CO).

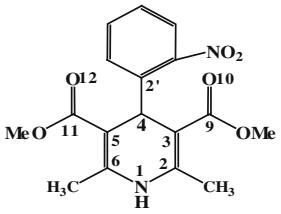
IR (KBr): ν 1747 (C=O, ester), 1715 cm^{-1} (C=O, ketone).

7.1.4. 4-Phenyl butyl acetoacetate (3i)

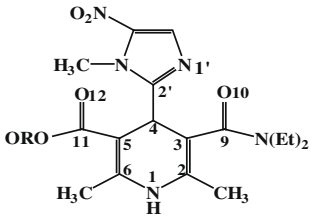
^{1}H NMR ($CDCl_3$): δ 7.23 (m, 5H, Phenyl), 4.19 (t, J = 6.2 Hz, 2H, CO_2CH_2), 3.26 (s, 2H, $COCH_2CO_2$), 2.69 (t, J = 6.7, 2H, CH_2Phenyl), 2.27 (s, 3H, CH_3CO), 1.83 (m, 4H, CH_2–CH_2).

IR (KBr): ν 1736 (C=O, ester), 1716 cm^{-1} (C=O, ketone).

Table 3
Dihedral angles for the most stable conformers of the studied DHP derivatives, obtained by AM1 calculations



Nifedipine



7a-7j

Compound	R	C_2–C_3–C_5–C_6	C_2–C_3–C_9–O_{10}	C_6–C_5–C_{11}–O_{12}	C'_2–C_4–C_3–C_2
7a	CH_3	0.21	96.31	65.09	106.89
7b	CH_2CH_3	0.11	102.53	92.86	102.26
7c	$(CH_3)_2CH_2$	0.82	128.13	87.89	100.32
7d	$(CH_2)_2CH_3$	0.72	103.22	81.10	98.37
7e	$(CH_2)_3CH_3$	0.79	159.09	124.22	94.63
7f	CH_2–C_6H_5	0.27	175.64	105.24	102.08
7g	$CH_2)_2C_6H_5)$	0.90	176.61	107.98	105.99
7h	$CH_2)_3C_6H_5)$	0.31	177.57	102.7	115.95
7i	$CH_2)_4C_6H_5)$	0.37	163.64	110.24	115.09
7j	$CH_2)_5C_6H_5)$	0.80	87.89	128.13	119.77
Nifedipine	—	0.004	52.90	8.76	92.91

7.1.5. 5-Phenyl pentyl acetoacetate (3j)

^{1}H NMR ($CDCl_3$): δ 7.25 (m, 5H, Phenyl), 4.21 (t, J = 6.6 Hz, 2H, CO_2CH_2), 3.53 (s, 2H, $COCH_2CO_2$), 2.71 (t, J = 6.2, 2H, CH_2Phenyl), 2.25 (s, 3H, CH_3CO), 1.91 (m, 6H, CH_2–CH_2–CH_2).

IR (KBr): ν 1737 (C=O, ester), 1713 cm^{-1} (C=O, ketone).

7.2. General procedure for the synthesis of alkyl 3-aminocrotonate (4a–j)

A solution of alkyl acetoacetic esters **3a**–**j** (4 mmol) and ammonium acetate (6 mmol) in 5 ml ethanol was placed in a 10 ml Erlenmeyer flask. The flask was immersed in an oil bath that had been preheated to 90 °C, and the solution was vigorously stirred. Heating was continued for a total 24 h. The reaction mixture was cooled. The ethanol was removed and IR spectra of the compounds were recorded to confirm the structure of **4a**–**j**. Then, the compounds were immediately used in subsequent reactions.

7.2.1. Methyl 3-aminocrotonate (4a)

IR (KBr): ν 3449 and 3370 (NH_2), 1709 cm^{-1} (C=O, ester).

7.2.2. Ethyl 3-aminocrotonate (4b)

IR (KBr): ν 3456and 3400 (NH_2), 1712 cm^{-1} (C=O, ester).

7.2.3. Isopropyl 3-aminocrotonate (4c)

IR (KBr): ν 3454 and 3338 (NH_2), 1712 cm^{-1} (C=O, ester).

7.2.4. *n*-Propyl 3-aminocrotonate (4d)

IR (KBr): ν 3454 and 3330 (NH_2), 1710 cm^{-1} (C=O, ester).

7.2.5. *n*-Butyl 3-aminocrotonate (4e)

IR (KBr): ν 3443 and 3329 (NH_2), 1708 cm^{-1} (C=O, ester).

7.2.6. Benzyl 3-aminocrotonate (4f)

IR (KBr): ν 3412 and 3341 (NH_2), 1714 cm^{-1} (C=O, ester).

7.2.7. Phenethyl 3-aminocrotonate (4g)

IR (KBr): ν 3460 and 3341 (NH_2), 1709 cm^{-1} (C=O, ester).

7.2.8. 3-Phenyl propyl 3-aminocrotonate (4h)

IR (KBr): ν 3461 and 3334 (NH_2), 1717 cm^{-1} (C=O, ester).

7.2.9. 4-Phenyl butyl 3-aminocrotonate (4i)

IR (KBr): ν 3454 and 3329 (NH_2), 1711 cm^{-1} (C=O, ester).

7.2.10. 5-Phenyl pentyl 3-aminocrotonate (4j)

IR (KBr): ν 3446 and 3341 (NH_2), 1709 cm^{-1} (C=O, ester).

7.3. General procedure for the synthesis of *N*,*N*-diethyl-2-(1-methyl-5-nitro-1*H*-imidazole-2-yl (methylen)3-oxobutan amide (6)

A solution of 1-methyl-5-nitroimidazol-2-carboxaldehyde **5** (300 mg, 2 mmol), *N*,*N*-diethyl acetoacetamide (314 mg, 2 mmol), glacial acetic acid (0.5 ml), piperidine (0.75 ml), and dry benzene (5 ml) was refluxed for 7 h, during which the resultant water was removed via a Dean–Stark trap. After cooling, the benzene was removed and the residue was purified by chromatography on silica gel with chloroform–methanol–ethylacetate (92/4/4), to give pure compound **6** (24% yield) as solid (mp 115–118 °C).

MS: *m*/*z*(%) 294(M^+, 54), 222(35), 206(13), 194(100), 180(70), 167(35), 154(17), 134(29), 106(20), 97(19), 83(16), 72(66), 58(51), 43(26).

Elemental Anal. Calcd for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.16; N, 19.04.

7.4. General procedure for the synthesis of alkyl/aryl 5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7a–j)

A solution of compounds **4a–j** (1.2 mmol) and *N*-diethyl-2-(1-methyl-5-nitro-1*H*-imidazole-2-yl (methylen)3-oxobutan amide **6** (353 mg, 1.2 mmol) in 2-propanol (5 ml) was protected from light and refluxed for 24–30 h. After cooling, the solution was

concentrated under reduced pressure and purified by thin-layer chromatography on silica gel with chloroform–methanol (94–6%). The product was recrystallized from methanol to give pure compounds **7a–j**.

7.4.1. Methyl-5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7a)

^{1}H NMR ($CDCl_3$): δ 8.45 (br s, 1H, DHP NH), 8 (s, 1H, Imidazole H-4), 5.2 (s, 1H, C_4–H), 3.9 (s, 3H, N–CH_3), 3.7 (q, J = 7.2 Hz, 4H, ((CH_2CH_3)$_2$), 3.68 (s, 3H, CO_2CH_3), 2.3 (s, 3H, C6–CH_3), 1.79 (s, 3H, C_2–CH_3), 1.2 (t, J = 7.2 Hz, 6H, N(CH_2–CH_3).

IR (KBr): ν 3244 (NH), 1701 (C=O, ester), 1608 (C=O, amide), 1518, 1373 cm^{-1} (NO_2).

MS: *m/z*(%) 391(M$^+$, 26), 374(18), 318(100), 303(32), 291(8), 259(46), 243(12) 213(20), 192(57), 100(20), 72(37), 42(31).

Elemental Anal. Calcd for $C_{18}H_{25}N_5O_5$: C, 55.23; H, 6.44; N, 17.89.

7.4.2. Ethyl- 5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7b)

^{1}H NMR ($CDCl_3$): δ 9.2 (br s, 1H, DHP NH), 7.9 (s, 1H, Imidazole H-4), 4.5 (s, 1H, C_4–H), 3.9 (m, 7H, N–CH_3 and N((CH_2CH_3)$_2$), 3.7 (q, J = 7.2 Hz, 2H, OCH_2–CH_3, 2.4 (s, 3H, C_6–CH_3), 1.9 (s, 3H,C_2–CH_3), 1.2 (t, J = 7.2 Hz, 3H, OCH_2–CH_3), 0.09 (t, J = 7.2, 6H, N((CH_2CH_3)$_2$).

IR (KBr): ν 3243 (NH), 1697 (C=O, ester), 1616 (C=O, amide), 1529, 1373 cm^{-1} (NO_2).

MS: *m/z*(%) 405(M$^+$, 8), 388(4), 332(26), 317(11), 305(57), 279(10), 259(100) 206(16), 178(16), 71(29), 57(38), 43(24).

Elemental Anal. Calcd for $C_{19}H_{27}N_5O_5$: C, 56.28; H, 6.71; N, 17.27.

7.4.3. Isopropyl-5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7c)

^{1}H NMR ($CDCl_3$): δ *9.1 (br s,* 1H, DHP NH), 7.9 (s, 1H, Imidazole C_4–H), 5.1 (s, 1H, C_4–H), 3.9 (m, 7H, N–CH_3 and N(CH_2–CH_3)$_2$), 3.2 (m, 1H, O–CH (CH_3)$_2$), 2.2 (s, 3H, C_6–CH_3), 1.7 (s, 3H, C_2–CH_3), 1.1 (two d, J = 6.2 Hz, 3H each, CH(CH_3)$_2$,), 0.7(t, 6H, N(CH_2–CH_3)$_2$).

IR (KBr): ν 3241 (NH), 1697 (C=O, ester), 1618 (C=O, amide), 1529 and 1372 cm^{-1} (NO_2).

MS: *m/z*(%) 419(M$^+$ 40), 402(11), 360(8), 346(63), 332(16), 319(38), 304(53), 259(100), 213(20), 178(45), 150(18), 100(17), 72(16), 43(16).

Elemental Anal. Calcuild for $C_{20}H_{29}N_5O_5$: C, 57.27; H, 6.97; N, 16.7.

7.4.4. *n*-Propyl-5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7d)

^{1}H NMR ($CDCl_3$): δ 9.4 (br s, 1H, DHP N–H), 7.9 (s, 1H, Imidazole C_4–H), 5.2 (s, 1H, C_4–H), 3.8–3.9 (m, 9H, N–CH_3 and N(CH_2–CH_3)$_2$ and O–CH_2–CH_2–CH_3), 2.2 (s, 3H, C6–CH_3), 1.6 (s, 3H, C2–CH_3), 1.5 (m, 2H, O–CH_2–CH_2–CH_3), 1.1 (t, 3H, O–CH_2–CH_2–CH_3), 0.7 (t, J = 7.2, 6H, N(CH_2–CH_3)$_2$).

IR (NaCl): ν 3251 (NH), 1699 (C=O, ester), 1609 (C=O, amide), 1518 and 1372cm^{-1} (NO_2).

MS: *m/z*(%) 419(M$^+$ 33), 402(10), 346(100), 331(32), 319(19), 303(27), 293(28), 259(71), 243(20), 213(24), 178(24), 150(17), 100(23), 72(21), 43(12).

Elemental Anal. Calcd for $C_{20}H_{29}N_5O_5$: C, 57.27; H, 6.97; N, 16.7.

7.4.5. *n*-Butyl-5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7e)

^{1}H NMR ($CDCl_3$): δ 8.7(br s, 1H, DHP NH), 7.9 (s, 1H, Imidazole C_4–H), 5 (s, 1H, C_4–H), 3.9 (m, 9H, N–CH_3 and O–CH_2(CH_3)$_3$ and N(CH_2CH_3)$_2$), 2.2 (s, 3H, C_6–CH_3), 1.6 (s, 3H, C_2–CH_3), 1.4 (m, 2H, O–CH_2–CH_2–CH_2–CH_3), 1–1.2 (m, 5H, O–(CH_2)$_2$–CH_2–CH_3), 0.08 (t, J = 7.2, 6H, N(CH_2–CH_3)$_2$).

IR (NaCl): ν 3238 (NH), 1693 (C=O, ester), 1622 (C=O, amide), 1525 and 1373cm^{-1} (NO_2).

MS: *m/z*(%) 433(M$^+$ 53), 416(19), 360(100), 345(43), 333(37), 307(45), 259(100), 234(32), 213(33), 178(49), 150(24), 100(37), 72(39), 42(11).

Elemental Anal. Calcd for $C_{21}H_{31}N_5O_5$: C, 58.18; H, 7.21; N, 16.16.

7.4.6. Benzyl- 5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7e)

^{1}H NMR ($CDCl_3$): δ 9.45 (br s, 1H, NH-DHP), 8.32 (s, 1H, H-Imidazole), 7.15–7.22 (m, 5H-Phenyl), 5.91 (s, 1H, C_4H), 4.93 (3H, N–CH_3), 3.9–3.97 (m, 4H, N(CH_2CH_3)$_2$, 3.16 (s, 2H, CO_2CH_2), 1.70 (s, 6H, C_2–CH3 and C_6–CH3), 0.70–0.80 (m,6H N(CH_2CH_3)$_2$.

IR (KBr): ν 3317 (NH), 3008 (CH, aromatic), 1695 (C=O, ester), 1611 (C=O, amide), 1522 and 1371cm^{-1} (NO_2).

MS: *m/z*(%) 467(M$^+$ 62), 450(16), 394(99), 367(30), 341(48), 259(100), 91(99), 72(28).

Elemental Anal. Calcd for $C_{24}H_{29}N_5O_5$: C, 61.66; H, 6.25; N, 14.98.

7.4.7. Phenethyl-5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7f)

^{1}H NMR ($CDCl_3$): δ 9.14 (br s, 1H, NH-DHP), 7.86 (s, 1H, H-Imidazole), 7.02–7.20 (m, 5H-Phenyl), 5.35 (s, 1H, C_4H), 4.20 (3H, N–CH_3), 3.35–3.55 (m, 4H, N(CH_2CH_3)$_2$, 2.79 (t, 2H, CO_2CH_2), 2.19 (s, 6H, C_2–CH_3 and C_6–CH_3), 1.16 (m, 6H, N(CH_2CH_3)$_2$, 0.75–0.93 (t, $CO_2CH_2CH_2$).

IR (KBr): ν 3252 (NH), 3086 (CH, aromatic), 1696 (C=O, ester), 1654 (C=O, amide), 1550 and 1373cm^{-1} (NO_2).

MS: *m/z*(%) 481(M$^+$ 40), 464(20), 408(100), 393(21), 381(24), 355(32), 259(77), 178(39),105(91),72(37).

Elemental Anal. Calcd for $C_{25}H_{31}N_5O_5$: C, 62.36; H, 6.49; N, 14.54.

7.4.8. 3-Phenyl propyl-5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7g)

^{1}H NMR ($CDCl_3$): δ 8.5 (br s, 1H, NH-DHP), 7.922 (s, 1H, H-Imidazole), 7.07–7.19 (m, 5H-Phenyl), 4.70 (s, 1H, C_4H), 3.9–4.0 (m, 7H, N–CH_3 and N(CH_2CH_3)$_2$), 2.49 (t, CO_2CH_2), 2.28 (s, 6H, C_2CH_3 and C_6CH_3), 1.03 (m, 4H, CO_2–CH_2–(CH_2)$_2$–), 0.80–1.18 (m, 6H, N(CH_2CH_3)$_2$.

IR (KBr): ν 3302 (NH), 3112 (CH, aromatic), 1694 (C=O, ester), 1609 (C=O, amide), 1518 and 1371cm^{-1} (NO_2).

MS: *m/z*(%) 495(M$^+$ 42), 478(18), 422(100), 395(22), 259(59), 213(17), 178(22.95), 91(37.70), 72(16.39).

Elemental Anal. Calcd for $C_{26}H_{33}N_5O_5$: C, 63.01; H, 6.71; N, 14.13.

7.4.9. 4-Phenyl butyl-5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7h)

^{1}H NMR ($CDCl_3$): δ 9.24 (br s, 1H, NH-DHP), 7.87 (s, 1H, H-Imidazole), 7.05–7.21 (m, 5H-Phenyl), 5.03 (s, 1H, C_4H), 4.27 (s. 3H, N–CH_3), 3.97–4.04 (m, 4H, N(CH_2CH_3)$_2$, 2.50 (t, CO_2CH_2), 2.10 (s, 6H, C_2CH_3 and C_6CH_3), 1.49–1.59 (m, 6H, CO_2–CH_2–(CH_2)$_3$–), 1.18–1.27 (m, 6H, N(CH_2CH_3)$_2$.

IR (KBr): ν 3337 (NH), 3015 (CH, aromatic), 1711 (C=O, ester), 1656 (C=O, amide), 1585 and 1359cm^{-1} (NO_2).

MS: *m/z*(%) 509(M$^+$, 8), 492(46), 382(37), 367(22), 259(12), 104(27), 91(100), 72(24), 42(22).

Elemental Anal. Calcd for $C_{27}H_{35}N_5O_5$: C, 63.64; H, 6.92; N, 13.74.

7.4.10. 5-Phenyl pentyl-5-(diethyl carbamoyl)2,6-dimethyl-4-(1-methyl-5-nitro-1*H* imidazole-2-yl)1,4 dihydropyridine-3-carboxylate (7i)

^{1}H NMR ($CDCl_3$): δ 9.43 (br s, 1H, NH-DHP), 7.86 (s, 1H, H-Imidazole), 7.04–7.17 (m, 5H-Phenyl), 5.07 (s, 1H, C_4H), 4.15 (s. 3H, N–CH_3), 3.96–4.03 (m, 4H, N(CH_2CH_3)$_2$, 2.46 (t, CO_2CH_2), 2.08 (s, 6H, C_2CH_3 and C_6CH_3), 1.50–1.59 (m, 6H, CO_2–CH_2–(CH_2)$_4$–), 1.20–1.29 (m, 6H, N(CH_2CH_3)$_2$.

IR (KBr): ν 3277(NH), 3021 (CH, aromatic), 1693 (C=O, ester), 1663 (C=O, amide), 1531 and 1369cm^{-1} (NO_2).

MS: m/z(%) 523(M^+ 4), 506(12), 423(28), 324(16), 259(53), 196(16), 146(36), 91(100), 43(18).

Elemental Anal. Calcd for $C_{28}H_{37}N_5O_5$: C, 64.23; H, 7.12; N, 13.37.

7.5. Determination of anti-mycobacterial activity

In vitro antimicrobial activity of the compounds was elevated against *M. tuberculosis* H37Rv strain ATCC 27294 that susceptible to Rifampin and Isoniazid. Minimum inhibitory concentration (MIC) was determined by using agar proportion method. The compounds in dimethylsulfoxide (DMSO) solutions were adding to the Middlebrook 7H10 (Quelab Co. UK) medium with glycerol and enriched with OADC. The following concentrations were used: 1, 2, 4, 8, 16, 32, 62, 125, 250 and 500 μmol/l. 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 to 7% CO_2 for a period of 21 days. MIC shows the lowest substance concentration indicating the inhibitory effect on the *Mycobacterium* growth. The colonies on each tube were counted, and the numbers of colonies on drug-containing tubes were compared with negative control. The present results were obtained from two independent measurements. Isoniazid and rifampicin (Sigma Chemical Co.) were used as a positive control.

7.6. Pharmacology

7.6.1. Determination of calcium channel antagonist activity

Male guinea-pigs, weighing 300–400 g, were killed by a blow on the head. The animals were deprived from food 18 h before killing but had free access to water. The non-terminal part of the ileum was removed and cut into segments of 10–15 mm length. Each ileal segment was suspended in organ bath and connected to an isometric transducer (K30, Hugo Sachs Electronic). The organ bath contained 20 ml physiological solution of the following composition (in mM): NaCl 119, KCl 2.7, $CaCl_2$ 2, $MgCl_2$ 0.88, NaH_2PO_4 0.36, $NaHCO_3$ 12, glucose 5.5. The physiological salt solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2 and its temperature was held at 37 °C. The fluid of the organ bath was changed every 15 min. A resting tension of 0.5 g was applied to the ileal segments and they were allowed to equilibrate for 1 h. Contractions of the ileal segments were recorded, using an amplifier (Plugsys, Hugo Sachs Electronic) and a recorder (Graphtec, model WR3320). To study the effects of synthesized dihydropyridines on KCl-induced contraction of ileum, at the first step, several contractions with KCl (40 mM) were made. No significant differences between KCl induced contractions were considered as stability of tissue and thereafter the main experiments were started. At this step, KCl (40 mM)-induced contraction was recorded again and the peak of the first phase (phasic contraction) was considered as control. Then, tissues were preincubated with one certain concentration of each compound (for 15 min) and then the effect of KCl (40 mM) was assessed once again. Each segment was treated with only one compound. From concentration–response curves, the pIC_{50} ($-\log IC_{50}$) value of each compound was calculated. nifedipine was used as reference compound.[30–32]

7.6.2. Statistical methods

Results are expressed as mean ± SEM The one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons was used to analyze the data. A value of $P < 0.05$ was considered as the significance level between the groups.

7.7. Computational details

All the computations were run on a Pentium IV personal computer (CPU at 2.6 GH) with the windows XP operating system. Chemical structure of each molecule was built by Hyperchem software (Version 7, Hypercube Inc.) for the structural chemistry. The Z-matrices of the structures were constructed by the software and transferred to the GAUSSIAN 98 program.[33] Complete geometry optimization was performed taking the most extended conformations as starting geometries. Semiempirical molecular orbital calculations (AM1) of the structures were performed using the GAUSSIAN 98 program. No molecular symmetry constraint was applied; rather full optimization of all bond lengths and angles was carried out. The root mean square of 0.1 kcal mol^{-1} was used as ending criteria in geometry optimization. Then, the molecules were reloaded to Hyperchem, some dihedrals angles and Log*P* were calculated in this software.

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