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Synthesis and biological evaluation of some novel *N*-aryl-1,4-dihydropyridines as potential antitubercular agents

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ABSTRACT

1,4-Dihydropyridines are the emerging class of antitubercular agent. Recently, studies have revealed that 1,4-dihydropyridine-3,5-dicarbamoyl derivatives with lipophilic groups have demonstrated excellent antitubercular activity. We have synthesized new *N*-aryl-1,4-dihydropyridines bearing carbethoxy and acetyl group at C-3 and C-5 of the DHP ring. In addition, 1*H*-pyrazole ring is substituted at C-4 position. The lowest minimum inhibitory concentration value, 0.02 μg/mL, was found for diethyl 1-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyridine-3,5-dicarboxylate **4e** making it more potent than first line antitubercular drug isoniazid. In addition, this compound exhibited relatively low cytotoxicity.

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Tuberculosis (TB) is a common and often deadly infectious disease caused by various strains of mycobacterium, usually *Mycobacterium tuberculosis*. Tuberculosis has been considered to be a disease of poverty for many years with quite rare occurrence in the developed countries. Unfortunately recently more people in the developed world are contracting tuberculosis because their immune systems are compromised by immunosuppressive drugs, substance abuse or AIDS. Several decades ago effective anti-TB drugs have been launched and one could hardly find a TB case to be demonstrated at the medicinal universities. But TB stroke back![1] The return of tuberculosis was declared by World Health Organization (WHO) as a global emergency compared to a hypothetic third world war with 9 million new TB cases and two million deaths reported each year[2,3]; about one-third of the world's population is already infected with *M. tuberculosis*.[4] Furthermore, in recent times the occurrence of multidrug-resistant TB (MDR-TB), a form of TB that does not respond to the standard treatments, is more common. It is a shocking revelation that MDR-TB is present in almost all countries as per the recent survey, made by the World Health Organization (WHO) and its partners.

Despite the efforts of academic institutions and the pharmaceutical companies engaged in the design, synthesis, and development of new antitubercular regimens, the current TB therapeutic arsenal is poor. Only a few derivatives were found endowed with some antimycobacterial activity, including fluoroquinolones (gatifloxacin and moxifloxacin),[5–8] diarylquinoline (TMC207),[9] nitroimidazoles (OPC67683 and PA824),[10,11] pyrrole (LL3858)[12] and 1,2-diamine (SQ109).[13] All the above facts reveal that there is an urgent need for development of new drugs with unique and divergent structure and with a novel mechanism of action.

Recently, studies showed that 3,5-dicarbamoyl derivatives of 1,4-dihydropyridine (DHP) with lipophilic groups have considerable antitubercular activity against *M. tuberculosis* $H_{37}Rv$.[14–18] It was also observed that esters or substituted isosters of pyridine and pyrazine carboxylic acids (such as tetrazoles) have been more active than the parent acids especially against resistant strains. These esters are presumably activated by an esterase to parent acid.[19–22] Indeed, esters of pyrazinoic acids have been shown to possess activity against pyrazinamide-resistant isolates which has been attributed to a deficiency of nicotinamidase.[19–22] In addition, pyrazoles exhibited significant antitubercular activity.[23] However, to the best of our knowledge, antitubercular activity of 1,4-dihydropyridines bearing N-aryl-substitution or C-3 and C-5 acetyl group is not much explored. Recognizing these facts and in continuation of our work on antitubercular agents,[24–28] it appeared of interest to design and synthesize new derivatives of *N*-substituted 1,4-dihydropyridines bearing carbethoxy and acetyl group at 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, pyrazole moiety is substituted at C-4 position of dihydropyridine ring. The antimycobacterial activity of synthesized compounds was evaluated against *M. tuberculosis* $H_{37}Rv$ (MTB).

The quest for the synthesis of *N*-aryl-1,4-dihydropyridines **4a–j** and **5a–j** was carried out by heating the 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **1** (1 equiv), ethyl acetoacetate/acetyl acetone **2** (2 equiv) and substituted anilines **3** (1 equiv), on a steam bath for 2–3 h. Then after, methanol (25 mL) was added to the reaction mixture; refluxing for an appropriate time culminated in the synthesis

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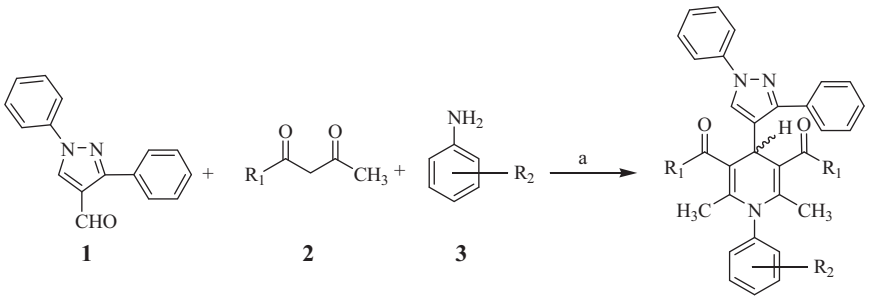

4a-j $R_1 = OC_2H_5$; R_2 = H, 2-CH_3, 3-CH_3, 4-CH_3, 2-Cl, 3-Cl, 4-Cl, 2-OCH_3, 3-OCH_3, 4-OCH_3
5a-j $R_1 = CH_3$; R_2 = H, 2-CH_3, 3-CH_3, 4-CH_3, 2-Cl, 3-Cl, 4-Cl, 2-OCH_3, 3-OCH_3, 4-OCH_3

Scheme 1. Synthesis of *N*-aryl-1,4-dihydropyridines **4a–j** and **5a–j**. Reagents and conditions: (a) MeOH, reflux.

Table 1
Physical constants and In vitro antitubercular screening data of dihydropyridines **4a–j** and **5a–j**

Sr. No.	R_1	R_2	Yield (%)	M.P. (°C)	% Inhibition	MIC[a] (μg/mL)	IC_{50}[b] VERO cells (μg/mL)	SI[c] (SI = IC_{50}/MIC)	*mi* Log *P*[d]
4a	OC_2H_5	H	67	68	96	1.56	>10	>6.4	7.179
4b	OC_2H_5	2-CH_3	71	118	95	3.13	>10	>3.2	7.580
4c	OC_2H_5	3-CH_3	62	157	98	1.56	>10	>6.4	7.604
4d	OC_2H_5	4-CH_3	70	90	48	48	n.d.	n.d.	7.628
4e	OC_2H_5	2-Cl	69	146	100	0.02	>10	>500	7.809
4f	OC_2H_5	3-Cl	75	80	97	1.56	7.08	4.5	7.833
4g	OC_2H_5	4-Cl	64	133	74	n.d	n.d	n.d	7.857
4h	OC_2H_5	2-OCH_3	66	95	39	n.d	n.d	n.d	7.188
4i	OC_2H_5	3-OCH_3	68	99	55	n.d	n.d	n.d	7.212
4j	OC_2H_5	4-OCH_3	74	112	33	n.d	n.d	n.d	7.236
5a	CH_3	H	72	168	48	n.d	n.d	n.d	5.794
5b	CH_3	2-CH_3	69	172	97	3.13	>10	>3.2	6.194
5c	CH_3	3-CH_3	73	188	98	6.25	>10	>1.6	6.218
5d	CH_3	4-CH_3	70	224	34	n.d	n.d	n.d	6.242
5e	CH_3	2-Cl	71	198	59	n.d	n.d	n.d	6.424
5f	CH_3	3-Cl	67	248	76	n.d	n.d	n.d	6.448
5g	CH_3	4-Cl	71	254	29	n.d	n.d	n.d	6.472
5h	CH_3	2-OCH_3	74	178	25	n.d	n.d	n.d	5.803
5i	CH_3	3-OCH_3	75	186	77	n.d	n.d	n.d	5.827
5j	CH_3	4-OCH_3	68	194	69	n.d	n.d	n.d	5.851
INH	—	—	—	—	—	0.03	—	—	—

[a] Minimum inhibitory concentration against $H_{37}Rv$ strain of *M. tuberculosis* (μg/mL).
[b] Measurement of cytotoxicity in VERO cells: 50% inhibitory concentrations (μg/mL).
[c] Selectivity index (in vitro): IC_{50} in VERO cells/MIC against *M. tuberculosis*.
[d] *mi* Log P: Molinspiration Cheminformatics (www.molinspiration.com) calculated log *P* using online Molinspiration Property Engine v2009.01.

of the molecules in good yields (62–75%, Scheme 1). Preparation of 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **1** is described.[29] Designed series of molecules **4a–j** and **5a–j** (Table 1) were characterized by ^{1}H NMR, ^{13}C NMR, and Mass spectrometry techniques, and their purity by elemental analysis. The ^{1}H NMR spectra of DHPs **4a–j** and **5a–j** have the typical singlet of methine group lying in the region 5.42–5.47 ppm and multiplet of aromatic part of molecules occurring in region between 6.21 and 7.93 ppm. The ^{13}C NMR signal of methine group can be observed at 32.0–36.2 ppm. IR spectra of DHP derivatives were also in agreement with the structures.

All compounds were initially screened for their in vitro antimycobacterial activity at 6.25 μg/mL against MTB $H_{37}Rv$ strain by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) in BACTEC 12B medium using the Microplate Alamar Blue Assay.[30] Compounds exhibiting $\geqslant$90% inhibition in the initial screen were retested at and below 6.25 μg/mL using 2-fold dilution to determine the actual MIC.

In the preliminary screening, seven compounds **4a–c, 4e**, **4f**, **5b**, and **5c** inhibited MTB with 90–100%. In the secondary level, one compound **4e** inhibited MTB with MIC of <1 μg/mL and three compounds **4a**, **4c**, and **4f** with MIC of <2 μg/mL. When compared to isoniazid (MIC: 0.03 μg/mL), diethyl 1-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyridine-3,5-dicarboxylate **4e** was found to be the most active compound in vitro with MIC of 0.02 μg/mL against MTB and was more potent than isoniazid. Studies showed that pharmacokinetic properties and cellular permeability of a drug can be modulated by its derivatization to more lipophilic forms and lipophilicity can be considered an important factor for antimycobacterial activity in penetrating the lipid-rich mycobacterial cell-walls.[31] The preliminary antimycobacterial evaluation results showed that compounds with carbethoxy substituents at C-3 and C-5 position of DHP ring exhibited higher antimycobacterial activity than the compounds with acetyl substituents at C-3 and C-5 position, probably due to their comparatively higher lipophilicity (log *P* values) which can assist in penetrating lipid-rich mycobacterial cell-wall. However, lipophilicity cannot be considered as a sole parameter contributing in the higher activity for elaborating the structure–activity relationship. The extensive structure–activity relation could be derived in future with various possible modifications at the present active 1,4-dihydropyridine

skeleton by various advanced methods such as quantitative structure–activity relationship methods.

Having identified good number of active antimycobacterial dihydropyridines, the next step was to examine the toxicity of the drug candidates. Compounds exhibiting reasonably low MICs (from 0.02 to 6.25 μg/mL) were tested for cytotoxicity (IC_{50}) in VERO cells, and a selectivity index (SI), defined as IC_{50}: MIC, was calculated. The IC_{50} and SI values are shown in Table 1. The compound **4f** was somewhat more toxic than the **4a–c, 4e**, **5b**, and **5c**. Generally, compounds with an MIC $\leqslant$6.25 μg/mL and an SI $\geqslant$10 are interesting compounds, and an MIC $\leqslant$1 μg/mL in a novel compound class is considered an excellent lead,[32] which makes the diethyl 1-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyridine-3,5 dicarboxylate **4e** very promising antimycobacterial compound. Further in vitro studies of compound **4e** as well as synthesis of analogues of this lead compounds are currently in progress.

Comparison of the activities of tested compounds **4a–j** and **5a–j** indicated that compound **4e** with 2-chloro substituent at the N-aryl ring and carbethoxy group at C-3 and C-5 positions of the 1,4-dihydropyridine ring was the most potent one among the tested compounds. The results indicate that alkyl ester with optimal lipophilicity could be a suitable candidate against *M. tuberculosis*. In vivo antimycobacterial evaluation is needed for further optimization. In addition, in vitro evaluation of designed compounds against resistant strain of *M. tuberculosis* could be valuable.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.068.

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