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Synthesis and antimycobacterial activity of 5-aryl-1-isonicotinoyl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives☆

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Abstract

5-Aryl-1-isonicotinoyl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives were synthesized and tested for their in vitro antimycobacterial activity. The compounds showed an interesting activity against a strain of *Mycobacterium tuberculosis* and a human strain of *M. tuberculosis* H4. © 2001 Elsevier Science S.A. All rights reserved.

Keywords: 5-Aryl-1-isonicotinoyl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives; Antimycobacterial activity

1. Introduction

4,5-Dihydro-1*H*-pyrazole derivatives were described for their antibacterial [1–5] and antifungal [1,4,6] activities. In our search for new antimycobacterial agents we synthesized a series of 1,3,5-trisubstituted 4,5-dihydro-1*H*-pyrazoles (**3a**–**3m**) (Table 3), in which the nitrogen at position 1 of the pyrazoline cycle was linked to the isonicotinoyl residue in order to verify whether that substitution might confer antimycobacterial properties to the compounds. Moreover, with position 3 was connected the 2-pyridinyl substituent which, together with the nitrogen atoms on the cycle, partially resembled the 2-pyridinecarboxamidrazone moiety present in compounds characterized by antimycobacterial properties [7–12]. All the synthesized compounds were tested for their antimycobacterial activity toward a strain of *Mycobacterium tuberculosis* $H_{37}Rv$ and toward a strain of *M. tuberculosis* H4, isolated from human bronchial aspirates. The activity of these compounds toward strains of *M. gordonae*, *M. bovis*, *Candida albicans*, *Escherichia coli* and *Staphylococcus epidermidis* was also determined.

2. Chemistry

The synthesis of 5-aryl-1-isonicotinoyl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazoles (**3a**–**3m**) (Table 3) was carried out (Scheme 1) by reacting isonicotinoyl chloride with the corresponding 5-aryl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazoles (**2a**–**2m**) (Table 2) which in turn were prepared from the corresponding 3-aryl-1-(pyridin-2-yl)-propenones (**1a**–**1m**) (Table 1) by treatment with hydrazine hydrate. However, when α,β-unsaturated ketones **1c**, **1f**, **1h**, **1i** and **1k**–**1m** were allowed to react with hydrazine hydrate, very unstable products were obtained from which the corresponding 4,5-dihydro-1*H*-pyrazoles (**2**) could not be isolated, according to the literature findings for similar compounds [13]. The crude products were directly used for the following reaction (see Section 3). Any attempt to obtain compounds **3a**–**3m** by reacting propenone derivatives **1a**–**1m** with isoniazid was unsuccessful.

☆ A preliminary account of this work was presented at the Italian–Hungarian–Polish Joint Meeting on Medicinal Chemistry (Divisione di Chimica Farmaceutica), Giardini Naxos-Taormina, 28 September–1 October 1999.

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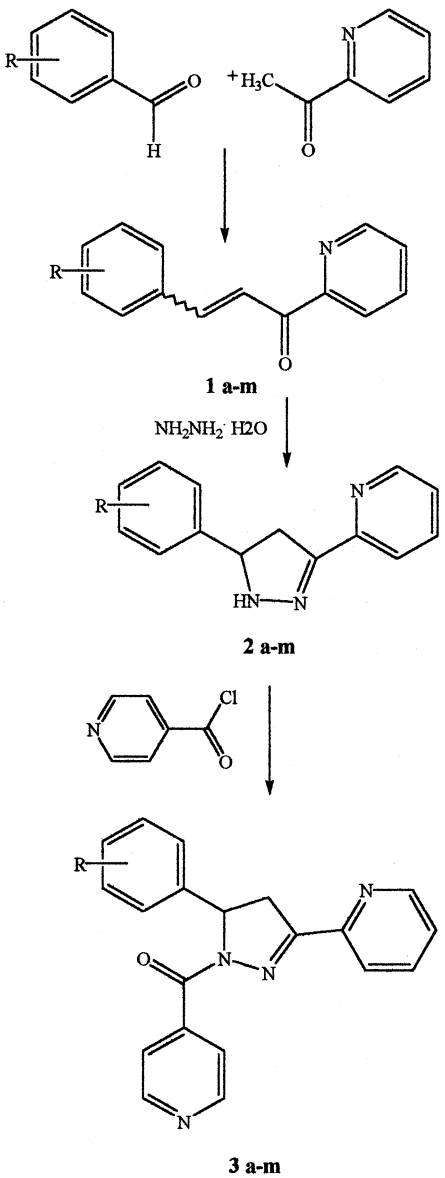
The ^{1}H NMR spectra of compounds **2a**, **2b**, **2d**, **2e**, **2g**, **2j** and **3a**–**3m** reveal the presence of three doublets of doublet signals due to the magnetically nonequivalent protons H_A (upfield H of CH_2) and H_B (downfield H of CH_2), and to the vicinal methine proton H_X. The values of the coupling constants between the protons are consistent with the expected structures. However, the pyrazoline derivatives **2a**–**2m** may react with isonicotinoyl chloride in two of the possible tautomeric forms, viz. 5-aryl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazoles (**A**) or 3-aryl-5-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazoles (**B**), to give the corresponding 1-isonicotinoyl derivatives whose ^{1}H NMR spectra would be very similar. From the ^{1}H NMR spectra of the obtained compounds it appears that there exists a difference in the chemical shifts between the methine protons of the pyrazolines with *ortho*-substituted phenyl residues, whose δ values are in the range 5.98–6.10 ppm, and the corresponding methine protons of the pyrazolines bearing *meta*- and *para*-substituted phenyl rings, whose δ values are in the range 5.72–5.77 ppm. This spectral behavior is not in agreement with compounds derived from the tautomeric form **B**, in which the methine protons have a more homogeneous magnetic environment. Moreover, the *ortho*-substituted phenyl groups present in the pyrazoline derivatives **2b**, **2e** produce a similar downfield shift of the methine protons with respect to the resonance of the corresponding protons of the *para*-substituted derivatives **2d**, **2g**. On the basis of these findings, we assigned to the obtained compounds **3a**–**3m** the structure corresponding to the 5-aryl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives **2a**–**2m**. The known α,β-unsaturated ketones **1a**–**1d**, **1g**, **1k**, **1m** were prepared according to the literature. The general synthetic procedure is described in Section 3 for the preparation of the new compounds **1e**, **1f**, **1h**, **1i**, **1j**, **1l** (Table 1).



Scheme 1.

3. Experimental

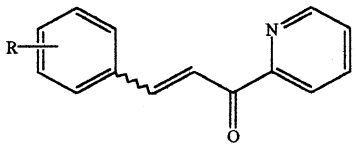
3.1. Chemistry

Melting points were determined with a Büchi 510 capillary apparatus, and are uncorrected. Infrared spectra in nujol mulls were recorded on a Jasco FT 200 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Varian Gemini 200 spectrometer; chemical shifts are reported as δ (ppm) relative to tetramethylsilane as internal standard, deuterochloroform as solvent. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography on silica gel precoated F_{254} Merck plates. EI MS spectra (70 eV) were taken on a VG 7070 spectrometer. Elemental analyses (C, H, N) were performed on a Carlo Erba analyzer and were within ± 0.3 of the theoretical value.

3.1.1. 3-Aryl-1-(pyridin-2-yl)-propenones (*1e*, *1f*, *1h*, *1i*, *1j*, *1l*)

To a mixture of 27 mmol of the appropriate aromatic aldehyde (dissolved in 5 ml of methanol) and 22 ml of 10% sodium hydroxide, 3.27 g (27 mmol) of 2-acetylpyridine was added dropwise under cooling (0–5°C) and stirring. After the addition was complete the reaction mixture was stirred for 2 h keeping the temperature below 10°C. The resulting solid was collected by filtration, washed thoroughly with ice-cold water, dried in a vacuum dessicator and recrystallized from absolute ethanol (Table 1).

Table 1
Compounds **1a**–**1m**



1 a-m

Compound	R	m.p. (°C)	IR (cm^{-1}) (C=O)	Formula (C, H, N)	Ref.
1a	H	70–72	1685	$C_{14}H_{11}NO$	[14]
1b	2-Cl	90–92	1698	$C_{14}H_{10}NOC1$	[14]
1c	3-Cl	94–96	1678	$C_{14}H_{10}NOC1$	[14]
1d	4-Cl	100–102	1674	$C_{14}H_{10}NOC1$	[15]
1e	2-Br	95–97	1698	$C_{14}H_{10}NOBr$	
1f	3-Br	84–86	1697	$C_{14}H_{10}NOBr$	
1g	4-Br	97–99	1699	$C_{14}H_{10}NOBr$	[16]
1h	2-F	72–74	1680	$C_{14}H_{10}NOF$	
1i	3-F	72–74	1670	$C_{14}H_{10}NOF$	
1j	4-F	84–86	1672	$C_{14}H_{10}NOF$	
1k	2-CH_3	74–76	1677	$C_{15}H_{13}NO$	[16]
1l	3-CH_3	73–75	1684	$C_{15}H_{13}NO$	
1m	4-CH_3	83–85	1663	$C_{15}H_{13}NO$	[15]

*3.1.2. 5-Phenyl-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazole (**2a**)*

To a solution of 3 g (14 mmol) of **1a** in 25 ml of ethanol, 1.43 g (28 mmol) of 98% hydrazine monohydrate was added dropwise under stirring. After addition the reaction mixture was further stirred for 1 h and concentrated under reduced pressure. The solid precipitate was collected by filtration and immediately crystallized from absolute ethanol to obtain 1.8 g (85%) of **2a**; m.p. 55°C.

IR (Nujol, cm^{-1}): 3312. ^1H NMR ($CDCl_3$, TMS): δ 3.23 (dd, 1H, H_A, upfield H of CH_2; $J_{AB} = 17.14$ Hz, $J_{AX} = 8.71$ Hz), 3.65 (dd, 1H, H_B, downfield H of CH_2; $J_{BA} = 17.14$ Hz, $J_{BX} = 10.90$ Hz), 4.98 (dd, 1H, H_X, CH; $J_{XA} = 8.71$ Hz, $J_{XB} = 10.90$ Hz), 6.17 (br s, 1H, NH, disappearing on deuteration), 7.06–8.57 (m, 9H, arom. and pyr.). MS; m/z: 223 [M^+]. *Anal.* ($C_{14}H_{13}N_3$): C, H, N.

Analogously, the compounds **2b**, **2d**, **2e**, **2g**, **2j** were prepared. Yields, melting points and spectral data are reported in Table 2.

The very unstable compounds **2c**, **2f**, **2h**, **2i** and **2k**–**2m** were not isolated. At the end of the reaction the solution was evaporated to dryness in vacuo and the oily residue was directly used for the following reaction.

*3.1.3. 1-Isonicotinoyl-5-phenyl)-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazole (**3a**)*

To a solution of 3.74 g (21 mmol) of isonicotinoyl chloride hydrochloride in 15 ml of dichloromethane, 3.1 g (14 mmol) of 5-phenyl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazole **2a** dissolved in 15 ml of absolute ethanol was added at room temperature (r.t.). To the stirred solution, 4.35 g (43 mmol) of triethylamine was added dropwise and the reaction mixture was further stirred for 3 h. The precipitated solid was filtered, washed with water, dried in a vacuum dessicator and recrystallized from absolute ethanol to obtain 2.4 g (52%) of **3a**; m.p. 187–89°C.

IR (Nujol, cm^{-1}): 1647. ^1H NMR ($CDCl_3$, TMS): δ 3.46 (dd, 1H, H_A, upfield H of CH_2; $J_{AB} = 18.68$ Hz, $J_{AX} = 5.13$ Hz), 3.92 (dd, 1H, H_B, downfield H of CH_2; $J_{BA} = 18.68$ Hz, $J_{BX} = 11.72$ Hz), 5.80 (dd, 1H, H_X, CH; $J_{XA} = 5.13$ Hz, $J_{XB} = 11.72$ Hz), 7.25–8.75 (m, 13H, arom. and pyr.). MS; m/z: 328 [M^+]. *Anal.* ($C_{20}H_{16}N_4O$): C, H, N.

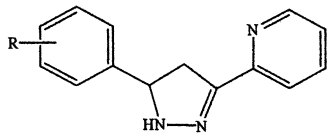
Analogously, the compounds **3b**–**3m** were prepared. Yields and melting points of compounds **3a**–**3m** are reported in Table 3 and their spectral data are recorded in Table 4.

Yields of compounds **3c**, **3f**, **3h**, **3i**, **3k**–**3m**, prepared starting from the crude products of the reaction between the corresponding α,β-unsaturated ketones 1 and hydrazine hydrate, were calculated on the basis of the amount of ketone employed.

3.2. Microbiology

The determination of the antitubercular and antimycobacterial activity was performed by the agar dilution method [17] in quadrant plates containing Middlebrook

Table 2
Spectral data of compounds **2a**–**2m**



2 a-m

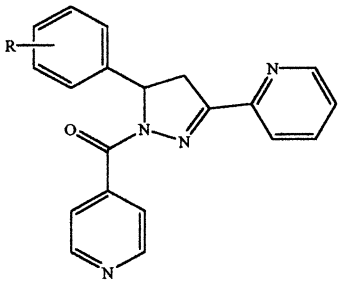
Comp.	R	Yield (%)	m.p. (°C)	Formula (C, H, N)	IR (nujol, cm^{-1})	^1H NMR (DMSO, TMS) (δ)	Mass m/z [M^+]
2a	H	63	55	$C_{14}H_{13}N_3$	3312	3.23 (dd, 1H, HA, upfield H of CH_2; $J_{AB} = 17.14$ Hz, $J_{AX} = 8.71$ Hz), 3.65 (dd, 1H, H_B, downfield H of CH_2; $J_{BA} = 17.14$ Hz, $J_{BX} = 10.90$ Hz), 4.98 (dd, 1H, H_X, CH; $J_{XA} = 8.71$ Hz, $J_{XB} = 10.90$ Hz), 6.17 (br s, 1H, NH, disappearing on deuteration), 7.06–8.57 (m, 9H, arom. and pyr.)	223
2b	2-Cl	61	96–9	$C_{14}H_{12}N_3Cl$	3320	3.09 (dd, 1H, HA; $J_{AB} = 17.21$ Hz, $J_{AX} = 9.52$ Hz), 3.80 (dd, 1H, H_B; $J_{BA} = 17.21$ Hz, $J_{BX} = 10.62$ Hz), 5.37 (overlapped dd, 1H, H_X), 6.20 (br s, 1H, NH, disappearing on deuteration), 7.10–8.40 (m, 8H, arom. and pyr.)	257, 259
2c	3-Cl	[a]	[a]	[a]	[a]	[a]	[a]
2d	4-Cl	58	92–4	$C_{14}H_{12}N_3Cl$	3340	3.10 (dd, 1H, HA; $J_{AB} = 17.21$ Hz, $J_{AX} = 9.52$ Hz), 3.60 (dd, 1H, H_B; $J_{BA} = 17.21$ Hz, $J_{BX} = 11.35$ Hz), 4.90 (overlapped dd, 1H, H_X), 6.17 (br s, 1H, NH, disappearing on deuteration), 7.07–8.60 (m, 8H, arom. and pyr.)	257, 259
2e	2-Br	61	95–7	$C_{14}H_{12}N_3Br$	3343	3.01 (dd, 1H, HA; $J_{AB} = 16.85$ Hz, $J_{AX} = 9.16$ Hz), 3.76 (dd, 1H, H_B; $J_{BA} = 16.85$ Hz, $J_{BX} = 11.35$ Hz), 5.27 (overlapped dd, 1H, H_X), 6.27 (br s, 1H, NH, disappearing on deuteration), 7.02–8.50 (m, 8H, arom. and pyr.)	301, 303
2f	3-Br	[a]	[a]	[a]	[a]	[a]	[a]
2g	4-Br	63	93–5	$C_{14}H_{12}N_3Br$	3300	2.95 (dd, 1H, HA; $J_{AB} = 17.30$ Hz, $J_{AX} = 9.65$ Hz), 3.48 (dd, 1H, H_B; $J_{BA} = 17.30$ Hz, $J_{BX} = 11.20$ Hz), 4.95 (overlapped dd, 1H, H_X), 6.20 (br s, 1H, NH, disappearing on deuteration), 7.10–8.85 (m, 8H, arom. and pyr.)	301, 303
2h	2-F	[a]	[a]	[a]	[a]	[a]	[a]
2i	3-F	[a]	[a]	[a]	[a]	[a]	[a]
2j	4-F	56	96–8	$C_{14}H_{12}N_3F$	3240	3.16 (dd, 1H, HA; $J_{AB} = 17.21$ Hz, $J_{AX} = 9.16$ Hz), 3.63 (dd, 1H, H_B; $J_{BA} = 17.21$ Hz, $J_{BX} = 10.62$ Hz), 4.95 (overlapped dd, 1H, H_X), 6.21(br s, 1H, NH, disappearing on deuteration), 6.96–8.56 (m, 8H, arom. and pyr.)	241
2k	2-CH_3	[a]	[a]	[a]	[a]	[a]	[a]
2l	3-CH_3	[a]	[a]	[a]	[a]	[a]	[a]
2m	4-CH_3	[a]	[a]	[a]	[a]	[a]	[a]

[a] The compounds were not isolated (see Section 3).

and Cohn 7H11 agar, supplemented with Middlebrook ADC or OADC enrichments. Serial dimethylsulfoxide twofold dilutions of the different chemicals tested were included in the agar layer and suspensions of different *Mycobacterium* spp. strains, prepared in sterile saline containing 0.2% fatty acid-free albumin and 0.02% polysorbate 80, were plated on to each quadrant. Control plates with known antitubercular drugs were included and all the plates were incubated at 37°C in 5% CO_2 for 4 weeks, after which viable counting was performed. We employed four different strains of *Mycobacterium* spp.: *M. tuberculosis* H37Rv reference strain, *M. tuberculosis* H4 clinical isolate, *M. gordonae* and *M. bovis* from our bacterial collection. The minimal inhibitory concentration (MIC) was defined for each chemical as the lowest dilution associated with at least a 99% reduction in the number of viable colonies. Results are shown in Table 5.

The other microbial strains tested were *E. coli*, *S. epidermidis* and *C. albicans*. The bacterial strains were grown overnight in Mueller–Hinton broth and the fungal strain was grown overnight in Sabouraud dextrose broth; the test inocula were prepared diluting the overnight suspension to a density of 10^4 microorganisms per milliliter. The MIC determinations were performed by the agar dilution method; Mueller–Hinton agar (Oxoid) and Sabouraud dextrose agar (Oxoid) were used for bacterial and fungal strains, respectively, to prepare quadrant plates with serial dimethylsulfoxide twofold dilutions of the different chemicals tested. A 20 μl sample of each 10^4 ml^{-1} microbial suspension was inoculated on to each chemical-containing quadrant. Control plates consisted of Mueller–Hinton agar or Sabouraud dextrose agar alone, culture medium with dimethylsulfoxide and culture medium with known antimicrobial drugs, like ampicillin (10 μg/disk) for bacterial strain or econazole (10 μg/disk) for *C. albicans*. All the plates were then incubated at 37°C overnight.

Table 3
Compounds **3a**–**3m**



3 a-m

Comp.	R	Yield (%)	m.p. (°C)	Formula (C, H, N)
3a	H	52	186–9	$C_{20}H_{16}N_4O$
3b	2-Cl	69	181–3	$C_{20}H_{15}N_4Ocl$
3c	3-Cl	35 [a]	126–9	$C_{20}H_{15}N_4Ocl$
3d	4-Cl	65	218–220	$C_{20}H_{15}N_4Ocl$
3e	2-Br	60	162–4	$C_{20}H_{15}N_4Obr$
3f	3-Br	33 [a]	115–7	$C_{20}H_{15}N_4Obr$
3g	4-Br	62	234–6	$C_{20}H_{15}N_4Obr$
3h	2-F	40 [a]	174–7	$C_{20}H_{15}N_4OF$
3i	3-F	38 [a]	178–181	$C_{20}H_{15}N_4OF$
3j	4-F	69	194–7	$C_{20}H_{15}N_4OF$
3k	2-CH_3	42 [a]	156–8	$C_{20}H_{15}N_4O$
3l	3-CH_3	40 [a]	171–3	$C_{20}H_{15}N_4O$
3m	4-CH_3	41 [a]	213–6	$C_{21}H_{18}N_4O$

[a] The yield was calculated on the basis of the amount of α,β-unsaturated ketone employed in the synthesis (see Section 3).

4. Results and discussion

A series of 5-aryl-1-isonicotinoyl-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives **3a**–**3m** have been synthesized with the aim of evaluating their antimycobacterial activity toward a strain of *M. tuberculosis* $H_{37}Rv$ and a strain of *M. tuberculosis* H4, isolated from human bronchial aspirates. All the synthesized compounds exhibited an interesting in vitro antimycobacterial activity against the tested strains of *M. tuberculosis*, their MIC values ranging from 8 to 16 μg/ml (Table 5). However, none of the compounds exhibited any antimycobacterial activity against the strains of *M. bovis* and *M. gordonae* at the maximal employed concentration (64 μg/ml). Compounds **3a**–**3m** were inactive against the tested strains of *C. albicans* and *E. coli*, and exhibited a very low activity toward the strain of *S. epidermidis*. Since the substituents on the phenyl residue at the 5-position on the cycle do not exert any important modulatory role on the activity, pyrazoline derivatives, modified by the replacement of the substituted phenyl residue with heterocyclic rings, may lead to compounds with higher antimycobacterial activity. The presence of the 2-pyridinyl residue at 3-position on the pyrazoline cycle may exert an important role on the activity of the tested compounds because 3,5-diaryl-1-isonicotinoyl-4,5-dihydro-1*H*-pyrazole derivatives were found to be inactive against *M. tuberculosis* $H_{37}Rv$ [18]. However, it cannot be ruled out that the rather constant activity of compounds **3a**–**3m** depends on a release of isoniazid in the biological medium, even if no degradation of the compounds in solution was observed after several months. If the activity of compounds **3a**–**3m** is due to the release of isoniazid, the lack of antimycobacterial activity of the above 3,5-diaryl-1-isonicotinoyl-4,5-dihydro-1*H*-pyrazole derivatives [18] might be attributed to the inhibition of isoniazid release through a possible stabilizing effect of a hydrogen bond between the hydroxy

Table 4
Spectral data of compounds **3a**–**3m**

Comp.	R	IR (nujol, cm^{-1})	^1H NMR (DMSO, TMS) (δ)	Mass m/z [M^+]
3a	H	1647	3.46 (dd, 1H, H_A, upfield H of CH_2; $J_{AB}=18.68$ Hz, $J_{AX}=5.13$ Hz), 3.92 (dd, 1H, H_B, downfield H of CH_2; $J_{BA}=18.68$ Hz, $J_{BX}=11.72$ Hz), 5.80 (dd, 1H, H_X, CH; $J_{XA}=5.13$ Hz, $J_{XB}=11.72$ Hz), 7.25–8.75 (m, 13H, arom. and pyr.)	328
3b	2-Cl	1623	3.30 (dd, 1H, H_A; $J_{AB}=18.68$ Hz, $J_{AX}=5.13$ Hz), 3.95 (dd, 1H, H_B; $J_{BA}=18.68$ Hz, $J_{BX}=11.62$ Hz), 6.04 (dd, 1H, H_X; $J_{XA}=5.13$ Hz, $J_{XB}=11.62$ Hz), 7.13–8.69 (m, 12H, arom. and pyr.)	362, 364
3c	3-Cl	1650	3.40 (dd, 1H, H_A; $J_{AB}=18.62$ Hz, $J_{AX}=5.19$ Hz), 3.93 (dd, 1H, H_B; $J_{BA}=18.62$ Hz, $J_{BX}=11.60$ Hz), 5.74 (dd, 1H, H_X; $J_{XA}=5.19$ Hz, $J_{XB}=11.60$ Hz), 7.0–8.8 (m, 12H, arom. and pyr.)	362, 364
3d	4-Cl	1637	3.40 (dd, 1H, H_A; $J_{AB}=18.62$ Hz, $J_{AX}=5.19$ Hz), 3.90 (dd, 1H, H_B; $J_{BA}=18.62$ Hz, $J_{BX}=11.60$ Hz), 5.75 (dd, 1H, H_X; $J_{XA}=5.19$ Hz, $J_{XB}=11.60$ Hz), 7.05–8.8 (m, 12H, arom. and pyr.)	362, 364
3e	3-Br	1650	3.30 (dd, 1H, H_A; $J_{AB}=18.31$ Hz, $J_{AX}=4.39$ Hz), 4.00 (dd, 1H, H_B; $J_{BA}=18.31$ Hz, $J_{BX}=11.72$ Hz), 6.10 (dd, 1H, H_X; $J_{XA}=4.39$ Hz, $J_{XB}=11.72$ Hz), 6.85–8.85 (m, 12H, arom. and pyr.)	406, 408
3f	3-Br	1649	3.46 (dd, 1H, H_A; $J_{AB}=18.68$ Hz, $J_{AX}=4.76$ Hz), 3.95 (dd, 1H, H_B; $J_{BA}=18.68$ Hz, $J_{BX}=11.72$ Hz), 5.75 (dd, 1H, H_X; $J_{XA}=4.76$ Hz, $J_{XB}=11.72$ Hz), 7.10–8.8 (m, 12H, arom. and pyr.)	406, 408
3g	4-Br	1668	3.44 (dd, 1H, H_A; $J_{AB}=18.68$ Hz, $J_{AX}=5.13$ Hz), 3.94 (dd, 1H, H_B; $J_{BA}=18.68$ Hz, $J_{BX}=11.72$ Hz), 5.75 (dd, 1H, H_X; $J_{XA}=5.13$ Hz, $J_{XB}=11.72$ Hz), 7.15–8.80 (m, 12H, arom. and pyr.)	406, 408
3h	2-F	1644	3.47 (dd, 1H, H_A; $J_{AB}=18.68$ Hz, $J_{AX}=5.49$ Hz), 3.96 (dd, 1H, H_B; $J_{BA}=18.68$ Hz, $J_{BX}=12.09$ Hz), 5.98 (dd, 1H, H_X; $J_{XA}=5.49$ Hz, $J_{XB}=12.09$ Hz), 7.15–8.8 (m, 12H, arom. and pyr.)	346
3i	3-F	1646	3.45 (dd, 1H, H_A; $J_{AB}=18.68$ Hz, $J_{AX}=5.13$ Hz), 3.93 (dd, 1H, H_B; $J_{BA}=18.68$ Hz, $J_{BX}=12.09$ Hz), 5.77 (dd, 1H, H_X; $J_{XA}=5.13$ Hz, $J_{XB}=12.09$ Hz), 7.0–8.8 (m, 12H, arom. and pyr.)	346
3j	4-F	1638	3.42 (dd, 1H, H_A; $J_{AB}=18.68$ Hz, $J_{AX}=4.76$ Hz), 3.90 (dd, 1H, H_B; $J_{BA}=18.68$ Hz, $J_{BX}=11.72$ Hz), 5.75 (dd, 1H, H_X; $J_{XA}=4.76$ Hz, $J_{XB}=11.72$ Hz), 6.90–8.90 (m 12H, arom. and pyr.)	346
3k	2-CH_3	1645	2.50 (s, 3H, CH_3), 3.30 (dd, 1H, H_A; $J_{AB}=18.68$ Hz, $J_{AX}=4.76$ Hz), 3.95 (dd, 1H, H_B; $J_{BA}=18.68$ Hz, $J_{BX}=11.72$ Hz), 5.99 (dd, 1H, H_X; $J_{XA}=4.76$ Hz, $J_{XB}=11.72$ Hz), 7.0–8.94 (m, 12H arom. and pyr.)	342
3l	3-CH_3	1644	2.30 (s, 3H, CH_3), 3.43 (dd, 1H, H_A; $J_{AB}=18.92$ Hz, $J_{AX}=4.88$ Hz), 3.90 (dd, 1H, H_B; $J_{BA}=18.92$ Hz, $J_{BX}=11.60$ Hz), 5.74 (dd, 1H, H_X; $J_{XA}=4.88$ Hz, $J_{XB}=11.60$ Hz), 6.9–8.8 (m, 12H, arom. and pyr.)	342
3m	4-CH_3	1641	2.30 (s, 3H, CH_3), 3.45 (dd, 1H, H_A; $J_{AB}=18.68$ Hz, $J_{AX}=5.13$ Hz), 3.90 (dd, 1H, H_B; $J_{BA}=18.68$ Hz, $J_{BX}=11.72$ Hz), 5.72 (dd, 1H, H_X; $J_{XA}=5.13$ Hz, $J_{XB}=11.72$ Hz), 7.0–8.8 (m, 12H, arom. and pyr.)	342

group in the *ortho* position on the 3-aryl residue and the nitrogen atom at the 2-position in the pyrazoline cycle of these compounds. It will be of interest to verify if analogous 3,5-diaryl-pyrazoline derivatives without the *ortho*-hydroxy substituent on the phenyl ring at the 3-position on the cycle may exhibit antimycobacterial properties. On the other hand, compounds **3a**–**3m** are characterized by the presence in the 3-position of the 2-pyridinyl substituent, which can contribute to the activity. The replacement of the isonicotinoyl group in compounds **3a**–**3m** with other acyl derivatives may be important in order to establish the possible significance of the 2-pyridinyl residue with respect to the antimycobacterial activity. On the basis of these considerations, the synthesis and the antimycobacterial activity evaluation of new pyrazoline derivatives are now in progress.

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Table 5
Activity of the 5-aryl-1-isonicotinoil-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives **3a**–**3m** against *M. tuberculosis* $H_{37}Rv$ and *M. tuberculosis* H4 clinical isolate

Comp.	MIC (μg/ml)	
	M. tuberculosis $H_{37}Rv$	*M. tuberculosis* H4 clinical isolate [a]
3a	8	8
3b	8	8
3c	8	8
3d	8	8
3e	8	16
3f	8	16
3g	8	16
3h	16	16
3i	16	16
3j	16	16
3k	8	8
3l	8	16
3m	16	16

[a] *M. tuberculosis* strains resulted sensitive to isoniazid (5 μg/disk) and rifampicin (30 μg/disk).

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